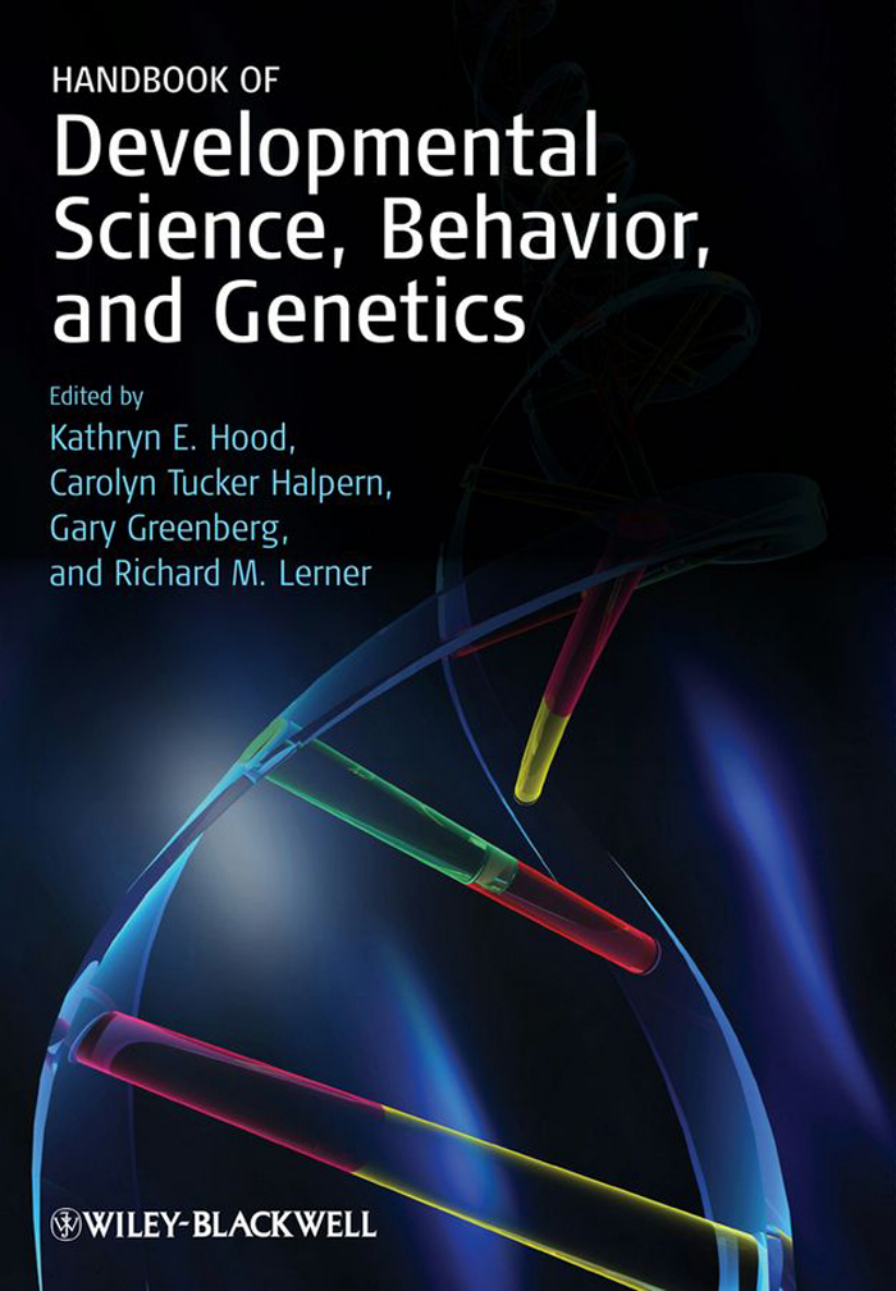
HANDBOOK OF

Developmental Science, Behavior, and Genetics

Edited by
Kathryn E. Hood,
Carolyn Tucker Halpern,
Gary Greenberg,
and Richard M. Lerner



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Foreword: Gilbert Gottlieb and the Developmental Point of View

Evelyn Fox Keller

Gilbert Gottlieb is widely known for his life-long struggle against the dichotomies between nature and nurture, and more specifically, between innate and acquired, that so hobble our thinking about biological and psychological development. Development, as he so clearly recognized, is an immensely complex process that depends on ongoing interactions between whatever makes up the organism at any given time and its environment; and it simply cannot be understood in terms of separate (or separable) forces, elements, or factors. Decades of his own research on the role of experience in the emergence of animal behavior taught him just how dire was the need for a different explanatory model, and indeed, much of his theoretical work was devoted to the articulation of such an alternative – of an explanatory framework that begins with what he liked to call the "developmental point of view."

A developmental point of view requires a "relational" ("coactive" and "bidirectional") view of causality; an appreciation of the continuity between prenatal and postnatal, innate and acquired; the recognition that epigenesis is ongoing, multifaceted, not predetermined but highly dependent on experience (or, to use the term that Gottlieb preferred for describing this process, "probabilistic"), and top-down as well as bottom up. Finally, a developmental point of view requires us to shift our focus from population statistics to the study of individual trajectories for it is only through the study of such trajectories that one can begin to understand the dynamics of developmental change.

Gottlieb devoted his entire career to fleshing out this perspective, and there is no denying his influence. He leaves behind an impressive body of both experimental results and conceptual proposals, and perhaps most important, a host of students who were deeply inspired by his example, and who, in their own labs, continue in his tradition and carry on his mission. And yet, notwithstanding the magnitude of his influence, shortly before his death, he confessed to a former student that "getting across the developmental point of view has been the largest failure of my

career" (Miller, 2007, p. 777). It is impossible for anyone who has struggled with these issues not to sympathize, or to fail to appreciate the magnitude of the obstacles facing any attempt to reconfigure the terms of our analyses.

As we know, Gottlieb was hardly the first to undertake this challenge, nor was he alone even in his own time. As he freely acknowledged, his debt to those who preceded him (especially, to Zing-Yang Kuo: (1898–1970), T. C. Schneirla (1902–1968), and Daniel S. Lehrman (1919–1972)) was immense; indeed, it was on their work that his own went on to build. He was equally appreciative of the contributions of like-minded contemporaries (e.g., Patrick Bateson, Susan Oyama, Richard M. Lerner), as he was of the contributions of a younger generation of colleagues. And I suspect that all of these authors have shared Gottlieb's frustration, for all of them have confronted the same obstacles, inevitably giving rise to the question of why the difficulties should be quite so intractable. Daniel Lehrman (1970, pp. 18–19) suggested we look to semantic problems for an understanding:

> When opposing groups of intelligent, highly educated, competent scientists continue over many years to disagree, and even to wrangle bitterly, about an issue which they regard as important, it must sooner or later become obvious that the disagreement is not a factual one, and that it cannot be resolved by calling to the attention of the members of one group . . . the existence of new data which will make them see the light . . . If this is, as I believe, the case, we ought to consider the roles played in this disagreement by semantic difficulties arising from concealed differences in the way different people use the same words, or in the way the same people use the same words at different times; [and] by differences in the concepts used by different workers. (1970, pp. 18–19)

I would go further. It is not just that we use the same words in different ways, that the language of behavioral genetics is hopelessly polysemic, but also that we seem to be trapped by the absence of adequate alternatives. Indeed, the lack of a vocabulary capable of doing justice to the developmental point of view constituted a formidable obstacle for Gottlieb, and his frequent coining of new terms suggests that he was well aware of the problem. The difficulty (as he himself clearly saw) is that introducing a new vocabulary is a far from simple task, and it requires a great deal more than the efforts of a few individuals. Language changes only when the felt need for a new vocabulary becomes truly widespread.

I am persuaded, however, that winds of change are in the air. New appreciation of many of Gottlieb's themes – of the agency of organisms in constructing their environments (see, e.g., Odling-Smee et al., 2003), of the plasticity of development (West-Eberhard, 2003), of the role of phenotypic plasticity in the genesis of evolutionary novelty (Kirschner & Gerhart, 2005), of the deeply contextual character of biological information -- has begun to penetrate the main corridors of contemporary biology. These themes not only both echo and support many of Gottlieb's own arguments, but also extend the "developmental point of view" into

new domains. Signs of change are also evident in studies of the most primitive molecular levels of life. Recent findings in genomics have brought fundamental new challenges to the very concept of a particulate gene, leading a number of molecular geneticists (and others) to call for a more dynamic and relational discourse of genetics for the 21st century (see, e.g., Fox Keller & Harel, 2007; Kapranov et al, 2007; Pearson, 2006; Silver, 2007). I only wish that Gottlieb could have lived to see the creation of the more accommodating home for his work that will, I believe, come with the realization of these signs of change.

References

Fox Keller, E., & Harel, D. (2007). Beyond the gene. *PLoS ONE*, **2**(11): e1231 doi:10.1371/journal.pone.0001231

Kapranov, P., Willingham, A. T., & Gingeras, T. R. (2007). Genome-wide transcription and the implications for genomic organization. *Nature Reviews Genetics*, **8**, 413–423.

Kirschner, M. W., & Gerhart, J. C. (2005). *The plausibility of life: Resolving Darwin's dilemma*. New Haven: Yale University Press.

Lehrman, D. S. (1970). Semantic and conceptual issues in the nature-nurture problem. In L. Aronson, E. Tobach, D. S. Lehrman, & J. S. Rosenblatt (Eds.), *Development and evolution of behavior* (pp. 17–52). New York: W. H. Freeman.

Miller, D. B. (2007). From nature to nurture, and back again. *Developmental Psychobiology*, **49**, 770–779.

Odling-Smee, F. J., Laland, K., & Feldman, M. W. (2003). *Niche construction: The neglected process in evolution*. Princeton, NJ: Princeton University Press.

Pearson, H. (2006). What is a gene? *Nature*, **441**, 399–401.

Silver, L. (2007). The year of miracles. *Newsweek*, October 15.

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. New York: Oxford University Press.

Preface and Acknowledgments

The Handbook of Developmental Science, Behavior, and Genetics commemorates the historically important and profound contributions made by Gilbert Gottlieb across a scholarly career spanning more than four decades. Gottlieb was preparing this handbook when his untimely death in 2006 brought his work on this project to a halt. However, with the permission and support of the Gottlieb Family, the editors of this work have decided to complete Gottlieb's "last book," which was designed to bring together in one place cutting-edge theory, research, and methodology affording a modern scientific understanding of the role of genes within the integrated and multi-level (or "fused") developmental system, that is, the system constituted by the levels of organization – ranging from the inner biological (e.g., genetic, hormonal, or neuronal) through the designed and natural physical ecological and historical – comprising the ecology of organism development.

Gottlieb's career was dedicated to providing rigorous experimental evidence to bear on such an integrative approach to understanding the dynamics of organism and context relations that provides the fundamental process of development. His work, – and those of other colleagues in comparative and developmental science – for instance, Z. Y. Kuo, T. C. Schneirla, Ethel Tobach, Jay Rosenblatt, Daniel Lehrman, Howard Moltz, and George Michel – was the major scientific basis for rejecting the reductionism and counterfactual, "split" conceptions (of variables purportedly linked alone to nature- or to nurture-related processes) used in other approaches to understanding the links among genes, behavior, and development, for example, as found in behavioral genetics (or in other reductionist accounts of the role of biology in development, for example, sociobiology or evolutionary psychology).

Accordingly, the scholarship that Gottlieb envisioned having in this handbook – and the scholarship we as editors who have tried to implement his vision hope we have presented – offers readers the cutting-edge of theory and research from developmental-systems-predicated scholarship in biological, comparative, and developmental science. Together, this work underscores the usefulness of the synthetic, developmental systems approach to understanding the mutually influential relations among genes, behavior, and context that propel the development of organisms across their life spans.

Our aspiration is that the scholarship that we present in this *Handbook* will constitute a watershed reference work documenting the current ways in which psychological, biological, comparative, and developmental science are framed and,

as well, advance a developmental systems approach to understanding the dynamics of mutually influential organism-environment relations. Represented as organism ↔ context relations, these relations constitute the basic unit of analysis in comparative and developmental science. In addition, from the theoretical and empirical approaches championed by Gottlieb, these organism ↔ context relations constitute the basis of change across the life spans of all organisms. We owe to Gilbert Gottlieb the clarity of theoretical vision and the standard for rigorous empirical work that has enabled this dynamic, developmental perspective to frame the cutting edge of contemporary scientific inquiry about the role of variables from all levels of organization, from genes through history, in constituting the fundamental, relational process involved in the development of all organisms across their respective life spans.

There are numerous other people to whom we owe enormous thanks for their contributions to this *Handbook*. Clearly, we are deeply grateful to the colleagues who contributed to this work, both for their superb scholarly contributions and for their commitment to working collaboratively to honor the work and memory of Gilbert Gottlieb. Without the excellent scholarship they contributed to this *Handbook* we could not honor the memory of Gilbert Gottlieb – as scientist, colleague, and friend – as thoughtfully, thoroughly, and richly as we are now able to do.

We also thank the two superb managing editors at the Institute for Applied Research in Youth Development – Leslie Dickinson and Jarrett Lerner – for their editorial work. Their commitment to quality and productivity, and their resilience in the face of the challenges of manuscript production, are greatly admired and deeply appreciated. Kathryn E. Hood is pleased to acknowledge the generous hospitality of the Center for Developmental Science at Chapel Hill, which long has welcomed visiting scholars such as Gilbert Gottlieb. Carolyn Halpern is grateful to her co-editors for their scholarship and insights, and to Gilbert Gottlieb for his mentorship and collaboration. Gary Greenberg is grateful to his wife Patricia Greenberg for her unstinting and continued support and encouragement and for understanding his long hours at the computer. Richard M. Lerner is grateful to the John Templeton Foundation, the National 4-H Council, the Philip Morris Smoking Prevention Department, and the Thrive Foundation for Youth for supporting his work during the development of this project.

Finally, we owe our deepest and most enduring debt to Gilbert Gottlieb, to whom we most obviously wish to dedicate this *Handbook*. Gilbert Gottlieb was one of the pillars of 20th century comparative psychology. His intellect, generosity, and kindness are warmly remembered and sorely missed.

Kathryn E. Hood
Carolyn Halpern
Gary Greenberg
Richard M. Lerner

Part I
Introduction

1

Developmental Systems, Nature-Nurture, and the Role of Genes in Behavior and Development

On the legacy of Gilbert Gottlieb

Kathryn E. Hood, Carolyn Tucker Halpern, Gary Greenberg and Richard M. Lerner

The histories of both developmental and comparative science during the 20th century attest unequivocally to the fact that the theory and research of Gilbert Gottlieb – along with the work of such eminent colleagues as T. C. Schneirla (1956, 1957), Zing-Yang Kuo (1967; Greenberg & Partridge, 2000), Jay Rosenblatt (e.g., this volume), Ethel Tobach (1971, 1981), Daniel Lehrman (1953, 1970), Howard Moltz (1965), and George Michel (e.g., this volume) – may be seen as the most creative, integrative, generative, and important scholarship in the field (cf. Gariépy, 1995). For more than a third of a century Gilbert Gottlieb (e.g., 1970, 1997; Gottlieb, Wahlsten, & Lickliter, 2006) provided an insightful theoretical frame, and an ingenious empirical voice, to the view that:

> an understanding of heredity and individual development will allow not only a clear picture of how an adult animal is formed but that such an understanding is indispensable for an appreciation of the processes of evolution as well [and that] the persistence of the nature-nurture dichotomy reflects an inadequate understanding of the relations among heredity, development, and evolution, or, more specifically, the relationship of genetics to embryology. (Gottlieb, 1992, p. 137)

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Gottlieb attempted to heal the Cartesian nature-nurture split between biological and social science (Overton, 2006) by developing an ingenious – and what would come to be seen as the cutting-edge – theoretical conception of the dynamic and mutually influential relations, or "coactions," among the levels of organization comprising the developmental system, that is, levels ranging from the genetic through the sociocultural and historical. In devising a developmental systems theoretical perspective about the sources of development, and bringing rigorous comparative developmental data to bear on the integrative concepts involved in his model of mutually influential, organism ↔ context relations, Gottlieb's theory and research (e.g., Gottlieb, 1991, 1992, 1997, 1998, 2004; Gottlieb et al., 2006) became the exemplar in the last decades of the 20th century and into the first portion of the initial decade of the 21st century of the postmodern, relational metatheory of developmental science (Overton, 1998, 2006).

Gottlieb presents an integrative, developmental systems theory of evolution, ontogenetic development, and – ultimately – causality. Gottlieb argued that "The cause of development – what makes development happen – is the relationship of the components, not the components themselves. Genes in themselves cannot cause development any more than stimulation in itself can cause development" (Gottlieb, 1997, p. 91). Similarly, he noted that "Because of the emergent nature of epigenetic development, another important feature of developmental systems is that causality is often not 'linear' or straightforward" (Gottlieb, 1997, p. 96).

Gottlieb offered, then, a probabilistic conception of epigenesis, one that constitutes a compelling alternative to views of development that rest on what he convincingly argued was a counterfactual, split, and reductionist nature-nurture conception (see Overton, 2006). His theory, and the elegant data he generated in support of it, integrate dynamically the developmental character of the links among genes, behavior, and the multiple levels of the extra-organism context – the social and physical ecology – of an individual's development (see too Bronfenbrenner, 1979, 2005; Bronfenbrenner & Morris, 2006; Ford & Lerner, 1992; Lerner, 2002). In sum, Gottlieb's work has influenced several generations of comparative and developmental scientists to eschew simplistic, conceptually reductionist, and split (i.e., nature as separate from nurture) conceptions of developmental process and to think, instead, systemically and, within the context of rigorous experimental and/or longitudinal studies, to attend to the dynamics of mutually influential organism ↔ context relations. His work has had and continues to have a profound impact on theory and research in diverse domains of science pertinent to the development of organisms.

Gottlieb's career was dedicated to providing rigorous experimental evidence to bear on this integrative approach to understanding these dynamics of organism and context relations. His work constitutes a major scientific basis for rejecting the reductionism and counterfactual approach to understanding the links among genes, behavior, and development, for example, as found in behavioral genetics, sociobiology or evolutionary psychology, and other reductionist approaches.

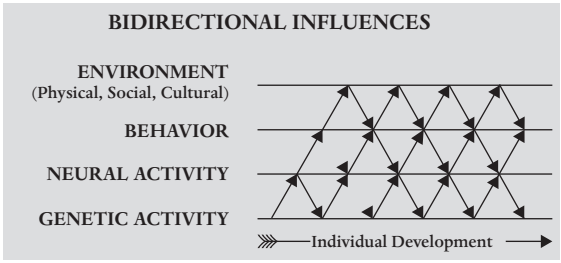


Figure 1.1. Gilbert Gottlieb's developmental systems theory: A developmental-psychobiological framework for understanding the character and evolution of individual development. *Source*: Gottlieb 1992.

Gottlieb was a preeminent developmental scientist and theoretician who, throughout his career, battled against scientific reductionism and advocated an open, holistic, multilevel systems approach for understanding development. His developmental systems theory grew from decades of his research, which covered the range of emerging and continuing issues in understanding the dynamic fusion of biology and ecology that constitutes the fundamental feature of the developmental process (e.g., Gottlieb, 1997, 1998). In particular, he challenged the deterministic concept of an innate instinct, and offered instead his generative conception of probabilistic epigenesis as a basis for shaping behavioral development as well as evolutionary change.

Gottlieb's contention is that development proceeds in concert with influences from all levels of the organism and the context. "A probabilistic view of epigenesis holds that the sequence and outcomes of development are probabilistically determined by the critical operation of various endogenous and exogenous stimulative events" (Gottlieb, 2004, p. 94). The bidirectional and coactional processes occurring within and across levels of a developmental system were succinctly captured in his figurative systems framework (Gottlieb, 1992), shown in Figure 1.1.

In addition to his own empirical research, Gottlieb avidly searched across disciplines for observations and research findings that exemplified his concepts, that is, the co-actions in the model depicted in Figure 1.1.

The Goals of the *Handbook*

The *Handbook of Developmental Science, Behavior, and Genetics* commemorates the historically important and profound contributions made by Gilbert Gottlieb across a scholarly career spanning more than four decades. Gottlieb was preparing this

Handbook when his untimely death in 2006 brought his work on this project to a halt. However, with the permission and support of the Gottlieb family, the editors of this work have decided to complete Gottlieb's "last book," which was designed to bring together in one place the cutting-edge theory, research, and methodology that provide the modern scientific understanding of the integration of levels of organization in the developmental system – ranging from genes through the most macro levels of the ecology of development. The dynamics of this integration constitute the fundamental, relational process of development.

Accordingly, the scholarship that Gottlieb arranged to have included in this *Handbook* will present to biological, comparative, and developmental scientists – both established and in training – the cutting-edge of contemporary theory and research underscoring the usefulness of the synthetic, developmental systems theory approach to understanding the mutually influential relations among genes, behavior, and context that propel the development of organisms across their life spans.

In sum, we hope that this *Handbook* will be a watershed reference for documenting the current status of comparative and developmental science and for providing the foundation from which future scientific progress will thrive. The organization and chapters of the *Handbook* actualize its contribution. It is useful, therefore, to explain how the structure and content of the *Handbook* instantiate and extend Gottlieb's scholarship and vision.

The Plan of this *Handbook*

We are grateful that Evelyn Fox Keller provides a foreword to this *Handbook*, one that so well frames its contribution to developmental and comparative science. Keller notes the importance for science of the innovative explanatory model devised by Gottlieb, what he termed the "developmental point of view." She explains how this conception requires a "relational" ("coactive" and "bidirectional") view of causality; an appreciation of the continuity between prenatal and postnatal, innate and acquired; the recognition that epigenesis is ongoing, multifaceted, not predetermined but, instead, highly dependent on experience (what Gottlieb described as constituting a probabilistic process), and involving a shift in focus from population statistics to the study of individual trajectories. Given the centrality in Gottlieb's work of refining this developmental point of view, after this opening chapter we reprint a key paper authored by Gottlieb, one that explains his conception of probabilistic epigenesis through discussing what are normally occurring environmental and behavioral influences on gene activity.

To place this view into its historical and theoretical contexts, Part II of the Handbook is devoted to discussions of the theoretical foundations for the developmental study of behavior and genetics. James Tabery and Paul E. Griffiths

provide a historical overview of traditional behavior genetics. They note that historical disputes between quantitative behavioral geneticists and developmental scientists stem largely from differences in methods and conceptualizations of key constructs, and in epistemological disagreement about the relevance of variation seen in populations. In turn, Mae Wan Ho revisits the links between development and evolution by discussing developmental and genetic change over generations. She reviews recent evidence in support of the idea that evolutionary novelties arise from non-random developmental changes defined by the dynamics of the epigenetic system; and shows how the organism participates in shaping its own development and adaptation of the lineage.

Douglas Wahlsten next discusses the assumptions and pitfalls of traditional behavior genetics. He notes that the concept of additivity of genes and environment, key to heritability analysis, is in conflict with contemporary views about how genes function as a part of a complex developmental system. Molecular genetic experiments indicate that genes act at the molecular level but do not specify phenotypic outcomes of development.

Next, George F. Michel discusses the connections between environment, experience, and learning in the development of behavior. He focuses on the concept of "Umwelt" and the meaning of gene–environment interaction in behavioral development.

The final chapter in this section of the book, by Ty Partridge and Gary Greenberg, discusses contemporary ideas in physics and biology in Gottlieb's psychology. The chapter reviews current ideas in biology, physiology, and physics and shows how they fit into Gottlieb's developmental systems perspective. The concepts of increasing complexity with evolution and that of emergence are discussed in detail and offer an alternative to reductionist genetic explanations of behavioral origins.

Framed by these discussions of the theoretical foundations of Gottlieb's view of how genes are part of the fused processes of organism ↔ context interactions that comprise the developmental system, Part III of the *Handbook* presents several empirical studies of behavioral development and genetics. Jay S. Rosenblatt discusses the mother as the developmental environment of the newborn among mammals and describes direct and indirect effects on newborn learning. His chapter provides a thorough, up-to-date discussion of maternal–young behavior among placental animals. The discussion is presented from both evolutionary and developmental perspectives. In the next chapter, Scott R. Robinson and Valerie Méndez-Gallardo provide data on fetal activity, amniotic fluid, and the epigenesis of behavior that, together, enable one to blur the "boundaries" of the organism.

Susan A. Brunelli, Betty Zimmerberg, and Myron A. Hofer discuss how family effects may be assessed through animal models of developmental systems. They provide data about the selective breeding of rats for differences in infant ultrasound vocalization related to separation stress. They find that later behaviors in each line

reflect active and passive coping styles. Similarly, Kathryn E. Hood demonstrates how early and later experience alters alcohol preference in selectively bred mice. She reports that the developmental emergence of behavior often shows increasing complexity over time. Philosophical and empirical sources suggest that emergent complexity entails specific internal developmental sources as well as external constraints and opportunities.

In turn, Allyson Bennett and Peter J. Pierre discuss the contribution of genetic, neural, behavioral, and environmental influences to phenotypic outcomes of development. They report that nonhuman primate studies model the interplay between genetic and environmental factors that contribute to complex disorders. Such translational research incorporating genetic, neurobiological, behavioral, and environmental factors allows insight into developmental risk pathways and ultimately contributes to the prevention and treatment of complex disorders.

Expanding on the discussion of gene-environment interactions, Lesley J. Rogers discusses the social and broader ecological context of the interactive contributions of genes, hormones, and early experience to behavioral development. Her presentation expands upon her earlier critical discussions of issues of genetic determinism in the treatment of neural lateralization. She offers empirical support for an experiential, developmental interpretation of lateralization in vertebrates.

Lawrence V. Harper discusses the idea of epigenetic inheritance by noting that multiple sources of change in environment and organism collaborate to provide coordinated changes in physiology and behavior over the course of development. Many of these factors are not obvious, but may be effective in producing a fit of organism and environment. Carolyn Tucker Halpern discusses the significance of non-replication of gene-phenotype associations. She notes that the failure to replicate gene-phenotype associations continues to be a problem in newer work testing gene-environment interactions, and may be exacerbated in genome-wide association studies. She argues that, given the many layers of regulation between the genome and phenotypes, and the probabilistic nature of development, criteria for replication merit renewed attention.

The next chapter, by Robert Lickliter and Christopher Harshaw, explains how the ideas of canalization and malleability enable elucidation of the regulatory and generative roles of development in evolution. They review evidence from birds and mammals demonstrating that the developmental processes involved in producing the reliable reoccurrence (*canalization*) of phenotypes under species-typical conditions are the same as those involved in producing novel phenotypic outcomes (*malleability*) under species-atypical circumstances. In other words, canalization and malleability are not distinct developmental phenomena – both are products of the organism's developmental system. As Gottlieb recognized, understanding the dynamics of canalization and malleability can contribute to a fuller understanding of phenotypic development and advance both developmental and evolutionary theory.

To document the breadth of the use of Gottlieb's ideas to developmental and comparative science, Part IV of the *Handbook* presents chapters that illustrate applications of his theory and research to human development. For instance, extending to humans the ideas discussed in Part III about gene-environment interactions within the developmental system, Cathi B. Propper, Ginger A. Moore, and W. Roger Mills-Koonce discuss child development, temperament, and changes in individual physiological functioning. They use a developmental systems approach to explore the reciprocal influences of parent-infant interactions and candidate genes on the development of infant physiological and behavioral reactivity and regulation. They emphasize that appreciating gene-environment coactions is paramount for understanding and accurately representing the complexities of infant temperament and emotion development.

In the following chapter, Jay Joseph discusses genetic research in psychiatry and psychology. He presents a critical analysis of the research most often put forward in support of the current consensus position in psychiatry and psychology that psychiatric disorders such as schizophrenia, ADHD, and bipolar disorder, and variation in normal psychological traits such as personality and IQ, are strongly influenced by genetic factors. Joseph argues that the evidence for this position, which consists mainly of family, twin, and adoption studies, provides little if any support for an important role for genetics. His analysis is especially relevant today in light of the ongoing failure, in some cases after decades of internationally coordinated gene-finding efforts, to discover the specific genes believed to underlie psychiatric disorders and psychological traits.

In turn, Peter C. M. Molenaar compares the developmental explanatory power of studies of inter-individual versus intra-individual variation. He presents a simulation of development to demonstrate how standard quantitative genetic analysis based on inter-individual variation yields biased results, especially in the context of nonlinear epigenetics. He outlines the use of a system-specific approach to obtain valid results about developmental processes.

Demonstrating the macro ecological breadth of the concepts associated with Gottlieb's integrative, developmental systems theory, Elaine L. Bearer discusses behavior as both an influence on and a result of the genetic program. She links the study of non-kin rejection, ethnic conflict, and issues in global health care within the frame of the theoretical ideas she proposes. Finally, a similarly broad discussion of the impact of Gottlieb's ideas is provided by Richard M. Lerner, Michelle J. Boyd, Megan K. Kiely, Christopher M. Napolitano, and Kristina L. Schmid. They discuss the contributions of Gilbert Gottlieb to promoting positive human development by pointing to applications of developmental systems theory to benefit individuals, families, and communities. They explain how the potential for plasticity of development that is part of Gottlieb's model affords an optimistic view about the potential of developmental science to optimize the course of human life. Accordingly, they discuss how Gottlieb's developmental systems model provides

a frame for the applications of developmental systems theory to policies and programs that can promote positive human development.

Conclusions

Throughout his career Gottlieb used his empirical work to support and further develop his theoretical approach to developmental systems and, with admirable persistence and high quality productivity, to convince the scientific community that the classic dualistic, nature-nurture split that focused on single causes of developmental change was a false one. The chapters in this *Handbook* illustrate convincingly the scope and power of his scholarship, an influence that integrated cutting-edge theoretical work across multiple disciplines and across numerous species, including humans.

Indeed, Gottlieb's developmental systems theoretical perspective leads us to recognize that, if we are to have an adequate and sufficient science of development, we must integratively study individual and contextual levels of organization in a relational and temporal manner (Bronfenbrenner, 2005). And if we are to serve both the scholarly community and our nation's and the world's individuals and families through our science, if we are to help develop successful policies and programs through our scholarly efforts, then we must make great use of the integrative temporal and relational model of the individual that is embodied in the developmental systems perspective Gottlieb forwarded.

Gottlieb would have been a bit surprised and, assuredly would have expressed great humility, by the extension of his theory and research to matters pertinent to enhancing the quality of human life. In addition to his accomplishments as a scientist, Gilbert Gottlieb displayed modesty, enormous interpersonal warmth, and wry humor. He will of course be remembered for his historically important innovations in comparative and developmental theory and research. But we believe he should also be remembered for his kindness and his generosity to junior colleagues and students, as well as his resoluteness, his consistently high level of intellectual integrity, his avid pursuit of historical precedents for his ideas, and his excitement about research, including field, laboratory, and library research. His enjoyment of convivial relationships with colleagues was tangible, and his maintenance of long-term relationships with intellectual companions was impressive, including some that were realized through email. He both shaped a science and built a community within it!

We hope that this *Handbook* will be of use to both senior scientists and, as well, younger scholars who may not be familiar with Gottlieb Gottlieb's work and who did not have the distinct honor and great privilege to have Gilbert Gottlieb as a colleague, mentor, and friend. We hope, also, that the *Handbook* will serve as an archival source for his theoretical and empirical discoveries, which together

advance the prospects for a thoroughly developmental science. We hope as well that the documentation of his influence will enable the memory of this extraordinary scientist and person to live on.

References

Bronfenbrenner, U. (1979). *The ecology of human development: Experiments by nature and design*. Cambridge, MA: Harvard University Press.

Bronfenbrenner, U. (Ed.) (2005). *Making human beings human: Bioecological perspectives on human development*. Thousand Oaks, CA: Sage.

Bronfenbrenner, U., & Morris, P. A. (2006). The bioecological model of human development. In W. Damon & R. M. Lerner (Eds. in Chief) & R. M. Lerner (Vol. Ed.), *Handbook of child psychology: Vol. 1. Theoretical models of human development* (6th ed., pp. 793–828). Hoboken, NJ: Wiley.

Ford, D. H., & Lerner, R. M. (1992). *Developmental systems theory: An integrative approach*. Thousand Oaks, CA: Sage.

Gariépy, J. (1995). The evolution of a developmental science: Early determinism, modern interactionism, and new systemic approach. In R. Vasta (Ed.). *Annals of child development: A research annual*, Vol. **11** (pp. 167–222). London, England: Jessica Kingsley.

Gottlieb, G. (1970). Conceptions of prenatal behavior. In L. R. Aronson, E. Tobach, D. S. Lehrman, & J. S. Rosenblatt (Eds.), *Development and evolution of behavior: Essays in memory of T. C. Schneirla* (pp. 111–137). San Francisco, CA: Freeman.

Gottlieb, G. (1991). The experiential canalization of behavioral development: Theory. *Developmental Psychology*, **27**, 4–13.

Gottlieb, G. (1992). *Individual development and evolution: The genesis of novel behavior*. New York: Oxford University Press.

Gottlieb, G. (1997). *Synthesizing nature-nurture: Prenatal roots of instinctive behavior*. Mahwah, NJ: Erlbaum.

Gottlieb, G. (1998). Normally occurring environmental and behavioral influences on gene activity: From central dogma to probabilistic epigenesis. *Psychological Review*, **105**, 792–802.

Gottlieb, G. (2004). Normally occurring environmental and behavioral influences on gene activity: From central dogma to probabilistic epigenesis. In C. Garcia Coll, E. Bearer, & R. M. Lerner (Eds.), *Nature and nurture: The complex interplay of genetic and environmental influences on human behavior and development* (pp. 85–106. Mahwah, NJ: Erlbaum.

Gottlieb, G., Wahlsten, D., & Lickliter, R. (2006). Biology and human development. In W. Damon & R. M. Lerner (Eds. in Chief) & R. M. Lerner (Vol. Ed.), *Handbook of child psychology: Vol. 1. Theoretical models of human development* (6th ed., pp. 210–257). Hoboken, NJ: Wiley.

Greenberg, G., & Partridge, G. (2000). Prologemena to Praxiology redux: The psychology of Zing-Yang Kuo. *From Past to Future: Clark Papers on the History of Psychology. Vol 2(2). From instinct to epigenesis: Lessons from Zing-Yang Kuo* (pp. 13–37).

Kuo, Z. Y. (1967). *The dynamics of behavior development: An epigenetic view*. New York: Plenum.

Lehrman, D. S. (1953). A critique of Konrad Lorenz's theory of instinctive behavior. *Quarterly Review of Biology*, **28**, 337–363.

Lehrman, D. S. (1970). Semantic and conceptual issues in the nature-nurture problem. In L. R. Aronson, E. Tobach, D. S. Lehrman, & J. S. Rosenblatt (Eds.). *Development and evolution of behavior: Essays in memory of T. C. Schneirla* (pp. 17–52). San Francisco: Freeman.

Lerner, R. M. (2002). *Concepts and theories of human development* (3rd ed.).Mahwah, NJ: Erlbaum.

Moltz, H. (1965). Contemporary instinct theory and the fixed action pattern. *Psychological Review*, **72**, 27–47.

Overton, W. F. (1998). Developmental psychology: Philosophy, concepts, and methodology. In W. Damon (Ed. In Chief) & R. M. Lerner (Vol. Ed.), *Handbook of child psychology: Vol. 1. Theoretical models of human development* (5th ed., pp. 107–187). Hoboken, NJ: Wiley.

Overton, W. F. (2006). Developmental psychology: Philosophy, concepts, and methodology. In W. Damon & R. M. Lerner (Eds. in Chief) & R. M. Lerner (Vol. Ed.), *Handbook of child psychology, Vol. 1. Theoretical models of human development* (6th ed., pp. 18–88). Hoboken, NJ: Wiley.

Schneirla, T. C. (1956). Interrelationships of the innate and the acquired in instinctive behavior. In P. P. Grassé (Ed.), *L'instinct dans le comportement des animaux et de l'homme*. Paris, France: Mason et Cie.

Schneirla, T. C. (1957). The concept of development in comparative psychology. In D. B. Harris (Ed.), *The concept of development: An issue in the study of human behavior* (pp. 78–108). Minneapolis: University of Minnesota Press.

Tobach, E. (1971). Some evolutionary aspects of human gender. *Journal of Orthopsychiatry*, **41**, 710–715.

Tobach, E. (1981). Evolutionary aspects of the activity of the organism and its development. In R. M. Lerner & N. A. Busch-Rossnagel (Eds.), *Individuals as producers of their development: A lifespan perspective* (pp. 37–68). New York: Academic Press.

2

Normally Occurring Environmental and Behavioral Influences on Gene Activity

From central dogma to probabilistic epigenesis

Gilbert Gottlieb

The central dogma of molecular biology holds that ''information'' flows from the genes to the structure of the proteins that the genes bring about through the formula DNA → RNA → Protein. In this view, a set of master genes activates the DNA necessary to produce the appropriate proteins that the organism needs during development. In contrast to this view, probabilistic epigenesis holds that necessarily there are signals from the internal and external environment that activate DNA to produce the appropriate proteins. To support this view, a substantial body of evidence is reviewed showing that external environmental influences on gene activation are normally occurring events in a large variety of organisms, including humans. This demonstrates how genes and environments work together to produce functional organisms, thus extending the author's model of probabilistic epigenesis.

> The new discipline of the genetics of behaviour, to judge by some recent books, is caught in the dogmas of Mendelian genetics without regard to developments in modem genetics during the last ten years, and to modern experimental approaches to the genetic roots of behaviour. Books on the subject usually begin with an account of the principles of Mendelian genetics. The material on behaviour deals mainly with mutated animals and their observed changes in behaviour. That is exactly what genetic principles predict. If an important mutation should not be followed by a change in behaviour – then geneticists would have to worry about the validity of the principles.

Handbook of Developmental Science, Behavior, and Genetics Edited by Kathryn E. Hood, Carolyn Tucker Halpern, Gary Greenberg, and Richard M. Lerner

> What these books fail to pay attention to is the trend in modern genetics which deals with the activation of gene areas, with the influence of external factors on the actualization of gene-potentials and their biochemical correlates in behaviour . . .. I would venture to guess that, apart from the dogma, the main reason for this silence is the fear of even the slightest suspicion that one might misinterpret such facts to mean that a Lamarckian mechanism were at work. (Hydén, 1969, pp. 114–115).

In the ensuing decades since Hydén made the above observation, things have not changed very much. A virtual revolution has taken place in our knowledge of environmental influences on gene expression that has not yet seeped into the social sciences in general and the behavioral sciences in particular. Aside from the feared misinterpretation of Lamarckian mechanisms at work, there is an explicit dogma, formulated as such that does not permit environmental influences on gene activity: the ''central dogma of molecular biology,'' first enunciated by Crick in 1958.

Although the central dogma may seem quite remote from psychology, I think it lies behind some psychological and behavioral theories that emphasize the sheerly endogenous construction of the nervous system and early behavior (e.g., Elman et al., 1996; Spelke & Newport, 1998) and the ''innate foundation of the psyche'' (e.g., Tooby & Cosmides, 1990), independent of experience or functional considerations: The essentially dichotomous view that genes and other endogenous factors construct part of the organism and environment determines other features of the organism. This article attempts to show how genes and environments necessarily cooperate in the construction of organisms, specifically, how genes require environmental and behavioral inputs to function appropriately during the normal course of individual development.

Predetermined and Probabilistic Epigenesis

In earlier articles, I described two concepts of epigenetic development: predetermined and probabilistic epigenesis (Gottlieb, 1970, 1976). In these early formulations, the difference between the two points of view hinged largely on how they conceived of the structure–function relationship. In predeterminism, it was unidirectional ($S \rightarrow F$), whereas in probabilism it was bidirectional ($S \leftrightarrow F$). Subsequently, I (Gottlieb, 1976, p. 218; 1983, p. 13; 1991, p. 13) extended the uni- and bidirectionality to include genetic activity:

Predetermined Epigenesis
Unidirectional Structure—Function Development
Genetic activity (DNA $\rightarrow$ RNA $\rightarrow$ Protein) $\rightarrow$
structural maturation $\rightarrow$ function, activity, or experience

Probabilistic Epigenesis
Bidirectional Structure—Functional Development
Genetic activity (DNA ↔ RNA ↔ Protein) ↔
structural maturation ↔ function, activity, or experience

As it applies to the nervous system, *structural maturation* refers to neurophysiological and neuroanatomical development, principally the structure and function of nerve cells and their synaptic interconnections. The unidirectional structure-function view assumes that genetic activity gives rise to structural maturation that then leads to function in a nonreciprocal fashion, whereas the bidirectional view holds that there are reciprocal influences among genetic activity, structural maturation, and function. In the unidirectional view, the activity of genes and the maturational process are pictured as relatively encapsulated or insulated, so that they are uninfluenced by feedback from the maturation process or function, whereas the bidirectional view assumes that genetic activity and maturation are affected by function, activity, or experience. The bidirectional or probabilistic view applied to the usual unidirectional formula calls for arrows going back to genetic activity to indicate feedback serving as signals for the turning on and off of genetic activity. The usual view, as is discussed below in the section on the central dogma of molecular biology, calls for genetic activity to be regulated by the genetic system itself in a strictly feed-forward manner. In this article, I (a) present the central dogma as a version of predetermined epigenesis, and (b) elaborate on the prior description of probabilistic epigenesis to bring it up to date on what is now known about the details of the bidirectional effects among genetic activity, structural maturation, neural and behavioral function, and experience.

The Central Dogma

The central dogma asserts that ''information'' flows in only one direction from the genes to the structure of the proteins that the genes bring about through the formula DNA → RNA → Protein. (Messenger RNA [mRNA] is the intermediary in the process of protein synthesis. In the lingo of molecular biology, DNA → RNA is called *transcription* and RNA → Protein is called *translation*.) After retroviruses (RNA → DNA) were discovered in the 1960s, Crick wrote a postscript to his 1958 report in which he congratulated himself for not claiming that reverse transcription was impossible: ''In looking back I am struck not only by the brashness which allowed us to venture powerful statements of a very general nature, but also by the rather delicate discrimination used in selecting what statements to make'' (Crick, 1970, p. 562). He then went on to consider the central dogma formula, DNA → RNA → Protein, in much more explicit detail than in his earlier paper.

In particular, he wrote, "These are the three [information] transfers which the central dogma postulates never occur:

Protein → Protein
Protein → DNA
Protein → RNA" (p. 562).

I suppose if one is going to be brash about making proposals in largely unchartered waters, it stands to reason one might err, even given the otherwise acknowledged insight of the author regarding other scientific issues. In the present case, Crick was wrong in two of the three central-dogmatic postulates described above. Regarding protein–protein interactions, it is now known that in neurodegenerative disorders such as Creutzfeldt–Jakob disease, prions (abnormally conformed proteins) can transfer their abnormal conformation to other proteins (meaning Protein → Protein transfer of information), without the benefit of nucleic acid participation (RNA or DNA) (Telling et al., 1996). The strength of the dogma that nucleic acids are required for "information transfer" is so compelling that some people believe there must be something like an RNA-transforming virus that brings about the changed protein conformation, even though there is no evidence for such a virus (Chesebro, 1998; Grady, 1996).

Regarding Protein → DNA transfer, there has long been recognized a class of regulative proteins that bind to DNA, serving to activate or inhibit DNA expression (i.e., turning genes on or off; reviews in Davidson, 1986; Pritchard, 1986).

With respect to the third prohibited information transfer (Protein → RNA), which would amount to reverse translation, to my knowledge, that phenomenon has not yet been observed.

Any ambiguity about the controlling factors in gene expression in the central dogma was removed in a later article by Crick, in which he specifically said that the genes of higher organisms are turned on and off by other genes (Crick, 1982, p. 515). Figure 2.1 shows the central dogma of molecular biology in the form of a diagram.

The Genome According to Central Dogma

The picture of the genome that emerges from the central dogma is (a) one of encapsulation, setting the genome off from supragenetic influences, and (b) a largely feed-forward informational process in which the genes contain a blueprint or master plan for the construction and determination of the organism. In this view, the genome is not seen as part of the development-physiological system of the organism, responsive to signals from internal cellular sources such as the cytoplasm of the cell, cellular adhesion molecules (CAMs), or to extracellular influences such

Figure Not Available

as hormones, and certainly not to extraorganismic influcences such as stimuli or signals from the external environment. Witness the well-known biologist Ernst Mayr's (1982) view "that the DNA of the genotype does not itself enter into the developmental pathway but simply serves as a set of instructions" (p. 824). Mae-Wan Ho (1984) characterized this view of the genes as the unmoved movers of development and the masters of the cellular slave machinery of the organism. Ho's work on the transgenerational effects of altered cytoplasmic influences seriously faults Mayr's view, as does the research reviewed by Jablonka and Lamb (1995).

Genes are conserved during evolution, therefore, some of the same genes are found in many different species. What this has demonstrated is that there is not an invariable association between the activity of a specific gene and the part of the body in which it is active. One of the best demonstrations is the activity of the so-called *Hox* genes that are found in a number of species (Grenier, Garber, Warren, Whitington, & Carroll, 1997). As shown in Figure 2.2, in fruit flies the *Hox* genes are

Figure Not Available

active only in the abdominal segment of the body, whereas in centipedes the same *Hox* genes are active in all segments of the body except the head. And, in a related wormlike creature, *Onychophora*, the *Hox* genes are active only in a single segment of the organism in its hindmost region. Because these are not homologous parts of these three species, this example demonstrates that the specific developmental contributions of the same genes vary as a consequence of the developmental system in which they find themselves. Genes that play a role in the abdominal segment of fruit flies are active in virtually all the bodily segments of centipedes, but only in a single segment in *Onychophora*.

The main point of this article is to extend the normally occurring influences on genetic activity to the external environment, thereby further demonstrating that a genome is not encapsulated and is in fact a part of an organism's general developmental-physiological adaptation to environmental stresses and signals: Genes express themselves appropriately only in responding to internally and externally generated stimulation. Further, in this view, although genes participate in the making of protein, protein is also subject to other influences (Davidson, 1986; Pritchard, 1986), and protein must be further stimulated and elaborated to become part of the nervous system (or other systems) of the organism, so that genes operate at the lowest level of organismic organization and they do not, in and of themselves, produce finished traits or features of the organism.[1]

Thus, there is no correlation between genome size and the structural complexity of organisms (reviewed in Gottlieb, 1992, pp. 154–157), nor is there a correlation between numbers of genes and numbers of neurons in the brains of a variety of organisms (see Table 2.1). The organism is a product of epigenetic development, which includes the genes as well as many other supragenetic influences. Since this latter point has been the subject of numerous contributions (reviewed in Gottlieb, 1992, 1997), I shall not deal with it further here, but, rather restrict this article to documenting that the activity of genes is regulated the same way as the

Table 2.1. Approximate number of genes and neurons in the brains of organisms in different lineages

Lineage and organism	*Genes*	*Neurons*
Chordates		
Mus musculus	70,000	40 million
Homo sapiens	70,000	85 billion
Nematodes		
Caenorhabdhitis elegans	14,000	302
Arthropods		
Drosophila melanogaster	12,000	250,000

Note. The exact number of neurons in the brain of *C. elegans* is known to be 302. From "Evolution and Modification of Brains and Sensory Systems," by G. L. Gabor Miklos, 1998, reprinted by permission of *Daedalus*, Journal of the American Academy of Arts and Sciences, from the issue titled "The Brain," Spring 1998, Vol. 127, No. 2, p. 200.

rest of the organism; the activity of genes is called forth by signals from the normally occurring external environment, as well as the internal environment (Nijhout, 1990; Pritchard, 1986). Although this fact is not well known in the social and behavioral sciences, it is surprising to find that it is also not widely appreciated in biology proper (Strohman, 1997). In biology, the external environment is seen as the agent of natural selection in promoting evolution, not as a crucial feature of individual development (van der Weele, 1995). Many biologists subscribe to the notion that "the genes are safely sequestered inside the nucleus of the cell and out of reach of ordinary environmental effects" (Wills, 1989, p. 19).

Normally Occurring Environmental Influences on Gene Activity

As can be seen in Table 2.2, a number of different naturally occurring environmental signals can stimulate gene expression in a large variety of organisms from nematodes to humans. The earliest demonstration of this regularly occurring phenomenon that I could find in intact organisms is in the work of H. Hydén (Hydén & Egyházi, 1962). In this rarely cited study, hungry rats had to learn to traverse a narrow rod from an elevated starting platform to an elevated feeding platform–a veritable balancing act. The nuclear base ratios in their vestibular nerve cells were then compared with an untrained control group and a control group given passive vestibular stimulation. The RNA base ratios in the experimental groups differed from both control groups. There was no difference between the control groups.

I think the Hydén and Egyházi (1962) study is rarely cited because the results not only do not fit into any existing paradigm, they also seem to raise the Lamarckian spectre mentioned by Hydén (1969) in the opening quotation.[2] If that is the case, there is an elementary misunderstanding. First, environmental stimulation of gene activity in the organ of balance does not mean the genes were necessarily altered in the process or, second, if they were altered, there is no reason to assume that the alteration was passed on to the progeny, as would be required by the way Lamarck used the notion of the inheritance of acquired characters in his theory of evolution.[3] In the Hydén and Egyházi study, the most conservative and acceptable explanation is that genes (DNA) were turned on in the experimental group in a way that they were not turned on in the control groups, resulting in an alteration of RNA base ratios in the experimental group.

To understand the findings summarized in Table 2.2, the nongeneticist will need to recall that the sequence of amino acids in proteins is determined by the sequence of nucleotides in the gene that "codes" for it, operating through the intermediary of mRNA. So there are three levels of evidence of genetic activity in Table 2.2: protein expression or synthesis, mRNA activity, and genetic activity itself. A difference in number of brain cells as a consequence of environmental

Table 2.2. Normally occurring environmental and behavioral influences on gene activity

Species	*Environmental signal or stimulus*	*Result (alteration in)*	*Study*
Nematodes	Absence or presence of food	Neuronal *daf-7* gene mRNA expression, inhibiting or provoking larval development	Ren et al. (1996)
Fruit flies	Transient elevated heat stress during larval development	Heat shock proteins and thermotolerance	Singh and Lakhotia (1988)
Fruit flies	Light-dark cycle	PER and TIM protein expression and circadian rhythms	Lee, Parikh, Itsukaichi, Bae, & Edery (1996); Myers et al. (1996)
Various reptiles	Incubation temperature	Sex determination	Reviewed in Bull (1983); Van der Weele (1995)
Songbirds (canaries, zebra finches)	Conspecific song	Forebrain mRNA	Mello, Vicario, & Clayton (1992)
Hamsters	Light-dark cycle	Pituitary hormone mRNA and reproductive behavior	Hegarty, Jonassent, & Bittman (1990)
Mice	Acoustic stimulation	c-*fos* expression, neuronal activity, and tonotopy in auditory system	Ehret and Fisher (1991)
Mice	Light-dark cycle	c-*fos* mRNA expression in suprachiasmatic nucleus of hypothalamus and circadian locomotor activity	Smeyne et al. (1992)

Rats	Tactile stimulation	c-*fos* expression and number of somatosensory cortical neurons	Mack & Mack (1992)
Rats	Learning task involving vestibular system	Nuclear RNA base ratios in vestibular nerve cells	Hydén & Egyházi (1962)
Rats	Visual stimulation	RNA and protein synthesis in visual cortex	Rose (1967)
Rats	Environmental complexity	Brain RNA diversity	Uphouse & Bonner (1975); review in Rosenzweig & Bennett (1978)
Rats	Prenatal nutrition	Cerebral DNA (cerebral cell number)	Zamenhof & van Marthens (1978)
Rats	Infantile handling; separation from mother	Hypothalmic mRNAs for corticotropin-releasing hormone throughout life	Meaney et al. (1996)
Cats	Visual stimulation	Visual cortex RNA complexity (diversity)	Grouse, Scheier, Letendre, & Nelson (1980)
Humans	Academic examinations taken by medical students (psychological stress)	Interleukin 2 receptor mRNA (immune system response)	Glaser et al. (1990)

Note. mRNA = messenger RNA; PER and TIM are proteins arising from *per* (*period*) and *tim* (*timeless*) gene activity.

influences, as in the Mack and Mack (1992), and Zamenhof and van Marthens (1978) studies, means that DNA activity has been turned on by the environmental stimulation. In the case of the more recent of these two studies, Mack and Mack were able to measure *fos* activity as well as count the number of cortical cells, whereas in the earlier study, Zamenhof & van Marthens were able only to count the number of cerebral cells as evidence of DNA activity.

As noted in Table 2.2, there are important neural and behavioral correlations to genetic activity, even though the activity of the genes is quite remote from these effects. The posttranslational expression of genes beyond the initial synthesis of protein involves the intervention of many factors before the end product of gene activity is realized (review in Pritchard, 1986, p. 179).

The fact that normally occurring environmental events stimulate gene activity during the usual course of development in a variety of organisms means that genes and genetic activity are part of the developmental-physiological system and do not reside outside of that system as some biologists and others have assumed on the basis of the central dogma. The mechanisms by which environmental signals turn on genetic activity during the normal course of development is being actively explored in a number of laboratories. The interested reader is referred to the reviews by Campbell and Zimmermann (1982), Curran, Smeyne, Robertson, Vendrell, and Morgan (1994), Holliday (1990), Jablonka and Lamb (1995), Morgan and Curran (1991), and Rosen and Greenberg (1994). Psychologists may be particularly interested in the fact that environmentally provoked gene expression is thought to be required for long-term memory (review in Goelet, Castellucci, Schacher, & Kandel, 1986).

From Central Dogma of Molecular Biology to Probabilistic Epigenesis

The main purpose of this article is to place genes and genetic activity firmly within a developmental-physiological framework, one in which genes not only affect each other and mRNA, but are affected by activities at other levels of the system up to and including the external environment. This developmental system of bidirectional, coactional influences is captured schematically in Figure 2.3. In contrast to the unidirectional and encapsulated genetic predeterminism of the central dogma, a probabilistic view of epigenesis holds that the sequence and outcomes of development are probabilistically determined by the critical operation of various endogenous and exogenous stimulative events (Gottlieb, 1970, p. 111; recent review in Gottlieb, 1997). The probabilistic-epigenetic framework presented in Figure 2.3 is based not only on what we now know about mechanisms of individual development at all levels of analysis, but the framework also derives from our understanding of evolution and natural selection. Natural selection serves as a

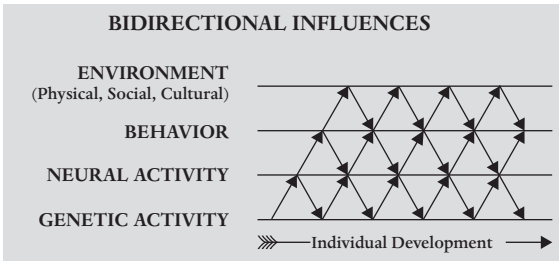


Figure 2.3. Probabilistic-epigenetic framework: Depiction of the completely bidirectional and coactional nature of genetic, neural, behavioral, and environmental influences over the course of individual development. From *Individual Development and Evolution: The Genesis of Novel Behavior* (p. 186) by Gilbert Gottlieb, 1992, New York: Oxford University Press. Copyright 1992 by Oxford University Press, Inc. Reprinted with permission.

filter and preserves reproductively successful phenotypes. These successful phenotypes are products of individual development and thus are a consequence of the adaptability of the organism to its developmental conditions. Therefore, natural selection has preserved (favored) organisms that are adaptably responsive both behaviorally and physiologically to their developmental conditions.

Organisms with the same genes can develop very different phenotypes under different ontogenetic conditions, as demonstrated by the two extreme variants of a single parasitic wasp species shown in Figure 2.4, and by identical twins reared apart in the human species (Figure 2.5; these twins were first described by Shields in 1962, pp. 43–44, 178–180, and later by Tanner, 1978, p. 119).[4]

Since the probabilistic-epigenetic view presented in Figure 2.3 does not portray enough detail at the level of genetic activity, it is useful to flesh that out in comparison to the previously described central dogma of molecular biology.

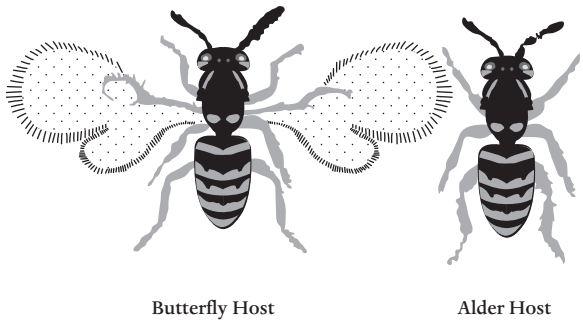


Figure 2.4. Two very different morphological outcomes of development in the minute parasitic wasp. The outcomes depended on the host (butterfly or alder fly) in which the eggs were laid. The insects are of the same species of parasitic wasp (*Trichogramma semblidis*). Adapted on the basis of Wigglesworth (1964).

Figure Not Available

As shown in Figure 2.6, the original central dogma explicitly posited one-way traffic from DNA → RNA → Protein and was silent about any other flows of information (Crick, 1958). Later, after the discovery of retroviruses (RNA → DNA information transfer), Crick (1970) did not claim to have predicted that phenomenon, but, rather that the original formulation did not expressly forbid it. In the bottom of Figure 2.6, probabilistic epigenesis, being inherently bidirectional in the horizontal and vertical levels (Figure 2.3), has information flowing not only from RNA → DNA but between Protein ↔ Protein and DNA ↔ DNA. The only relationship that is not yet supported is Protein → RNA, in the sense of reverse translation (protein altering the structure of RNA), but there are other influences of protein on RNA activity (not its structure) that would support such a directional flow. For example, a process known as *phosphorylation* can modify proteins such that they activate (or inactivate) other proteins (Protein → Protein), which, when activated, trigger rapid association of mRNA (Protein → RNA activity). When mRNAs are transcribed by DNA, they do not necessarily become imme-

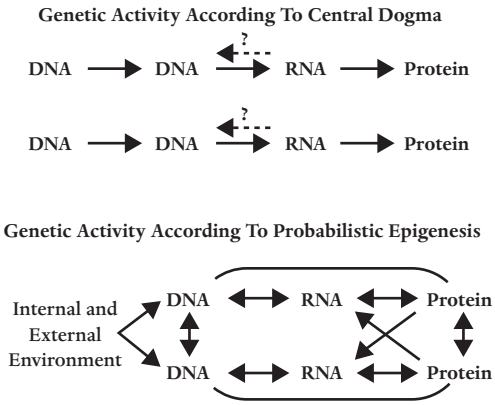
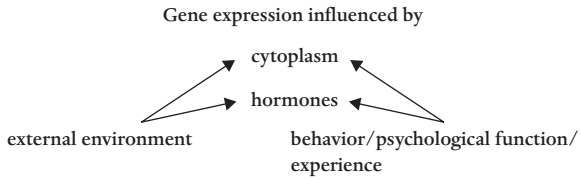


Figure 2.6. Different views of influences on genetic activity in the central dogma and probabilistic epigenesis. The filled arrows indicate documented sources of influence, whereas the open arrow from Protein back to RNA remains a theoretical possibility in probabilistic epigenesis and is prohibited in the central dogma (as are Protein ↔ Protein influences). Protein → Protein influences occur (a) when prions transfer their abnormal conformation to other proteins and (b) when, during normal development, proteins activate or inactivate other proteins as in the phosphorylation example described in text. The filled arrows from Protein to RNA represent the activation of mRNA by protein as a consequence of phosphorylation, for example. DNA ↔ DNA influences are termed "epistatic," referring to the modification of gene expression depending on the genetic background in which they are located. In the central dogma, genetic activity is dictated solely by genes (DNA → DNA), whereas in probabilistic epigenesis internal and external environmental events activate genetic expression through proteins (Protein → DNA), hormones, and other influences. To keep the diagram manageable, the fact that behavior and the external environment exert their effects on DNA through internal mediators (proteins, hormones, etc.) is not shown; nor is it shown that the protein products of some genes regulate the expression of other genes. (See text for further discussion.)

diately active but require a further signal to do so. The consequences of phosphorylation could provide that signal (Protein → Protein → mRNA activity → Protein), A process like this appears to be involved in the expression of "fragile X mental retardation protein" under normal conditions and proves disastrous to neural and psychological development when it does not occur (Weiler et al., 1997).[5] An excellent overview of the various roles of phosphorylation in the nervous system is provided by Hyman and Nestler (1993, Chapter 4).

Amplifying the left side of the bottom of Figure 2.6, it is known that gene expression is affected by events in the cytoplasm of the cell, which is the immediate environment of the nucleus and mitochondria of the cell wherein DNA resides, and by hormones that enter the cell and its nucleus. This feed-downward effect can be visualized thusly:



According to this view, different proteins are formed depending on the particular factors influencing gene expression. Concerning the effect of psychological functioning on gene expression, we have the evidence in Table 2.2 of heightened interleukin 2 receptor mRNA, an immune system response, in medical students taking academic examinations (Glaser et al., 1990). More recently, in an elegant study that traverses all levels from psychological functioning to neural activity to neural structure to gene expression, Cirelli, Pompeiano, and Tononi (1996) showed that genetic activity in certain areas of the brain is higher during waking than in sleeping in rats. In that study, the stimulation of gene expression was influenced by the hormone norepinephrine flowing from locus coeruleus neurons that fire at very low levels during sleep and at high levels during waking and when triggered by salient environmental events. Norepinephrine modifies neural activity and excitability, as well as the expression of certain genes. So, in this case, we have evidence for the interconnectedness of events relating the external environment and psychological functioning to genetic expression by a specifiable hormone emanating from the activity of a specific neural structure whose functioning waxes and wanes in relation to the psychological state of the organism.

Importance of Behavioral and Neural Activity in Determining Gene Expression, Anatomical Structure, and Physiological Function

Many, if not all, of the normally occurring environmental influences on genetic activity summarized in Table 2.2 involve behavioral and neural mediation. In the spirit of this article, I want to emphasize the contribution of events above the genetic level (the whole organism and environmental context) by way of redressing the balance to the way many think about the overriding importance of molecular biology. The earliest synaptic connections in the embryonic and fetal nervous system are created by spontaneous activity of nerve cells (reviews in Corner, 1994; Katz & Shatz, 1996). This early, "exuberant" phase produces a very large array of circuits that are then pared down by the organism's encounters with its prenatal and postnatal environments. In the absence of behavioral and neural activity (e.g., experimentally induced paralysis), cells do not die, and circuits do not become pruned in an adaptive way that fits the organism to the demands of its physical,

social, and cultural environments (Pittman & Oppenheim, 1979). A recent review of the development and evolution of brain plasticity may be found in Black and Greenough (1998).

Sometimes one reads the perfectly reasonable-sounding suggestion that, although genes do not make anatomical, physiological, or behavioral traits, the genes constrain the outer limits of variation in such traits. It is, of course, the developmental system, of which the genes are a part (Figure 2.3), and not solely the genes, that constrains development. It is not possible to predict in advance what the outcome of development will be when the developing organism is faced with novel environmental or behavioral challenges never before faced by a species or strain of animal. This has been known since 1909 when Woltereck did the first experiments that resulted in the open-ended concept of the norm of reaction, an idea that has been misunderstood by some behavior geneticists who think of genes as setting up a too-narrow range of reaction (reviews in Gottlieb, 1995; Piatt & Sanislow, 1988).

A very striking example of the role of novel behavior bringing about an entirely new anatomical structure can be seen in Slijper's (1942) goat in Figure 2.7. This animal was bom with undeveloped forelimbs and adopted a kangaroolike form of locomotion. As a result, its skeleton and musculature became modified, with a pelvis and lower spinal column like that of a biped instead of a quadruped (Figure 2.7). Thus, although there can be no doubt that genes and other factors place constraints on development, Slijper's goat shows that it is not possible to know the limits of these constraints in advance, even though it might seem quite reasonable to assume, in advance of empirical inquiry, that a quadruped is not capable of bipedality. Although an open-ended, empirically based norm of reaction

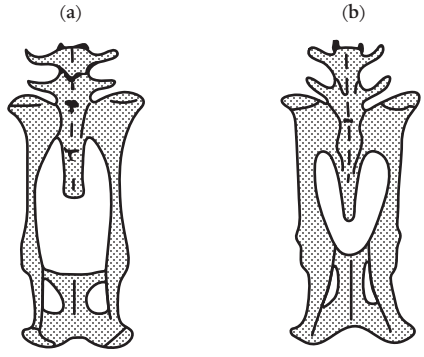


Figure 2.7. Modification of pelvic and spinal anatomy consequent to bipedalism. The figure shows (a) the pelvis and lower spine of a normal quadrupedal goat, and (b) the pelvis and lower spine of a goat born without forelimbs and that adopted a form of locomotion similar to a kangaroo. From *Foundation of Developmental Genetics* (p. 310) by D. J. Pritchard, 1986, London and Philadelphia: Taylor & Francis. Copyright 1986 by Dorian Pritchard. Reprinted with permission.

can accommodate Slijper's goat, a narrowly constrained, rationally based range of reaction cannot, no matter how reasonable it seems. It may very well be that all quadruped species cannot adapt bipedally, but we cannot know that without perturbing the developmental system.

Summary and Conclusions

It is tempting to show the nice link between probabilistic epigenesis and an epigenetic behavioral theory of evolution; however, that topic has been reviewed in depth in several recent publications (Gottlieb, 1992, 1997), so I will forego that temptation here in favor of sticking to the main point of this article. The central dogma lies behind the persistent trend in biology and psychology to view genes and environments as making identifiably separate contributions to the phenotypic outcomes of development. Quantitative behavior genetics is based on this erroneous assumption. It is erroneous because animal experiments have shown again and again that it is not possible to identify the genetic and environmental components of any phenotype, whether behavioral, anatomical, or physiological (extensive review in Wahlsten & Gottlieb, 1997).[6] Although genes no doubt play a constraining role in development, the actual limits of these constraints are quite wide and, most important, cannot be specified in advance of experimental manipulation or accidents of nature as documented in Figures 2.3, 2.4 and 2.7. (The prenatal environment also plays a constraining role that cannot be known in advance of experimental or manipulative inquiry; Gottlieb, 1971, 1997.) There is no doubt that not only genes and environments constrain development at all levels of the system (Figure 2.3).

The theoretical crux of this article is that the internal and external environments supply the necessary signals to the genes that instigate the eventual production of the requisite proteins under normal as well as unusual conditions of development. There is no genetic master plan or blueprint that is self-actualized during the course of development, as was assumed by the central dogma. Without doubt, there are unusual developmental conditions to which genes cannot respond adaptably, but the range of possible adaptable genetic responses to strange environmental conditions is truly astounding, as when bird oral epithelial cells mixed with mouse oral mesenchyme cells resulted in the production of a fully enameled molar tooth (Kollar & Fisher, 1980). The phrase "scarce as a hen's tooth" is based on the fact that bird oral epithelial cells never produce teeth when in conjunction with bird oral mesenchyme cells, as would be the case under normal conditions of development. If this finding is "clean" (no mouse oral epithelial cells accidentally contaminating the mix), it involves the appropriate reactivation of a genetic combination that had been latent for 80 million years when birds' last toothed ancestor existed (Pritchard, 1986, pp. 308–309). Also, the

finding that a crucial nutriment experimentally deleted from the environment of bacterial cells could lead to the production of that nutriment by a genetic recombination (*adaptive mutation*) caused a storm of disbelief in the biological community until it was shown that there was indeed a molecular basis for this "theoretically impossible" finding (Harris, Longerich, & Rosenberg, 1994; Thaler, 1994).

It will be interesting to see how probabilistic epigenesis becomes modified in the ensuing years as more information accrues through the necessarily interdisciplinary and multidisciplinary efforts of future researchers. The contrasting ideas of predetermined and probabilistic epigenesis were first put forward in Gottlieb (1970). Although the central dogma as depicted in Figure 2.6 is consistent with the formulation of predetermined epigenesis, it is too much to claim that the contrasting formulation of probabilistic epigenesis in 1970 predicted all the details of the relationships in the lower half of Figure 2.6. One can only say that those relationships are consistent with the bidirectional influences stated in the probabilistic formula Genetic Activity ↔ Structure ↔ Function presented in Gottlieb (1976, p. 218) and elaborated in Gottlieb (1991, see especially Appendix, p. 13). As I have described in detail elsewhere (e.g., Gottlieb, 1992, 1997), the formulation of probabilistic epigenesis was built on the writings of Kuo (1976), Lehrman (1970), Montagu (1977), and Schneirla (1960).

Finally, in response to a concern raised by colleagues who have read this article in manuscript form, I do hope that the emphasis on normally occurring environmental influences on gene activity does not raise the spectre of a new, subtle form of "environmentalism." If I were to say organisms are often adaptably responsive to their environments, I don't think that would label me as an environmentalist. So, by calling attention to genes being adaptably responsive to their internal and external environments, I am not being an environmentalist, but I am merely including genetic activity within the probabilistic-epigenetic framework that characterizes the organism and all of its constituent parts (Figure 2.3). The probabilistic-epigenetic view follows the open-systems view of development championed by the biologists Ludwig von Bertalanffy (1933/1962), Paul Weiss (1939/1969), and Sewall Wright (1968). Their writings were based on a highly interactive conception of embryology, and the central dogma simply overlooked this tradition of biological theorizing, resulting in an encapsulated formulation of genetic activity at odds with the facts of embryological development. (The current reductionist theoretical stance of molecular biology continues to disregard epigenetic considerations; Strohman, 1997.) Building on the insights of von Bertalanffy, Weiss, and Wright, the probabilistic-epigenetic view details the cooperative workings of the embryological open-systems view at the genetic and neural levels, prenatal and postnatal behavior, and the external environment. This view fleshes out at the prenatal and intraorganismic levels of analysis various other approaches in developmental psychology: ecological (Bronfenbrenner, 1979), transactional (Sameroff, 1983), contextual (Lerner & Kaufman, 1985), interactional or holistic (Johnston, 1987; Magnusson, 1988), individual-sociological (Valsiner, 1997),

structural-behavioral (Horowitz, 1987), dynamic systems (Thelen & Smith, 1994), and, most globally speaking, interdisciplinary developmental science (Cairns, Elder, & Costello, 1996).

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Notes

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1. Among the most scholarly early critiques to make this point was that of G. Stent (1981), who wrote:

   > For the viewpoint that the structure and function of the nervous system of an animal is specified by its genes provides too narrow a context for actually understanding developmental processes and thus sets a goal for the genetic approach that is unlikely to be reached. Here too ''narrow'' is not to mean that a belief in genetic specification of the nervous system necessarily implies a lack of awareness that in development there occurs an interaction between genes and environment, a fact of which all practitioners of the genetic approach are certainly aware. Rather, ''too narrow'' means that the role of the genes, which, thanks to the achievements of molecular biology, we now know to be the specification of the primary structure of protein molecules, is at too many removes from the processes that actually ''build nerve cells and specify neural circuits which underlie behavior'' to provide an appropriate conceptual framework for posing the developmental questions that need to be answered. (pp. 186–187)

   Stent's critique was taken a step further by Nijhout (1990), who wrote in a general way about the importance of interactions, above the genetic level, in the internal environment of the organism to bring about growth and differentiation (*morphogenesis*).

Nijhout's point was that "genes do not . . . 'cause' or 'control' morphogenesis; they enable it to take place" (p. 443). Even more pertinent to the theme of this article, Nijhout wrote that the genes whose products are necessary during development are activated by stimuli that arise from the cellular and chemical processes of development. Thus the network or pattern of gene activation does not constitute a program, it is both the consequence of, and contributor to, development. (pp. 443)

In this article, I extend this point of view to the external environment.

2. Due to the great advances in molecular techniques since 1962, some present-day researchers may question the results of Hydén and Egyházi on methodological grounds.
3. Although it is not a popular idea, and it is a separate question, genes can be altered by internal (reverse transcription, for example) and external events during development and, under certain conditions, the activities of these altered genes can persist across generations (Campbell & Perkins, 1983; Campbell & Zimmermann, 1982; Holliday, 1990; Jablonka & Lamb, 1995).
4. This great amount of phenotypic variation observed in identical twins (sharing the same genotype) coordinates well with the enormous degree of phenotypic variation in the human species, in which there is in fact only a very small degree of individual genetic variation at the level of DNA. DNA is composed of two base pairs of nucleotides. There is such a small amount of variation in these base pairs in the human population that any two individuals selected at random from anywhere on earth would exhibit differences in only three or four base pairs out of 1,000 base pairs (i.e., .3% or .4%!; Cann, 1988; Merriwether et al., 1991).
5. The label of "fragile X mental retardation protein" makes it sound as if there is a gene (or genes) that produces a protein that predisposes to mental retardation, whereas, in actual fact, it is this protein that is absent from the brain of fragile X mental retardates, and thus represents a failure of gene (or mRNA) expression rather than a positive genetic contribution to mental retardation. The same is likely true for other "genetic" disorders, whether mental or physical. Such disorders most often represent biochemical deficiencies of one sort or another due to the lack of expression of the requisite genes and mRNAs to produce the appropriate proteins necessary for normal development. Thus, the search for "candidate genes" in psychiatric or other disorders is most often a search for genes that are not being expressed, not for genes that are being expressed and causing the disorder. So-called "cystic fibrosis genes" and "manic-depression genes," among others, are in this category. The instances that I know of in which the presence of genes causes a problem are Edward's syndrome and Trisomy 21 (Down's syndrome), wherein the presence of an extra, otherwise normal, Chromosomes 18 and 21, respectively, cause problems because the genetic system is adapted for two, not three, chromosomes at each location. In some cases, it is of course possible that the expression of mutated genes can be involved in a disorder, but, in my opinion, it is most often the lack of expression of normal genes that is the culprit.
6. This is not the same as saying one cannot pinpoint the participation of specific genes and specific environments in *contributing* to phenotypic outcomes. However, because genes and environments always collaborate in the production of any phenotype, it is not possible to say that a certain component of the phenotype was caused exclusively by genes (independent of environmental considerations) and that some other component was caused exclusively by environment (independent of a genetic contribution).

An understanding of developmental phenomena demands a relational or coactive concept of causality as opposed to singular causes acting in supposed isolation (discussed at length in Gottlieb, 1991, 1997). Overton (1998) has presented a historical overview on the topic of dualistic conceptions of causality versus the more recent relational or coactive concept of causality. Further, with respect to the erroneous separation of hereditary and environmental contributions to the phenotype by quantitative behavior geneticists, Wahlsten (1990) has shown that the absence of heredity–environment interaction is a statistical artifact stemming from the insufficient power of the analysis of variance to detect such interactions, not the empirical absence of such gene–environment interactions.

References

Black, J. E., & Greenough, W. T. (1998). Developmental approaches to the memory process. In: J. L. Martinez & R. P. Kesner (Eds.), *Learning and memory: A biological view* (3rd ed., pp. 55–88). New York: Academic Press.

Bronfenbrenner, U. (1979). *The ecology of human development: Experiments by nature and design*. Harvard University Press,. Cambridge, MA.

Bull, J. J. (1983). *Evolution of sex determining mechanisms*. Menlo Park, CA: Benjamin/Cummings.

Cairns, R. B., Elder, G. H., & Costello, E. J. (Eds.). (1996). *Developmental science*. New York: Cambridge University Press.

Campbell, J. H., & Perkins, P. (1983). Transgenerational effects of drug and hormonal treatments in mammals: A review of observations and ideas. *Progress in Brain Research*, **73**, 535–553.

Campbell, J. H., & Zimmermann, E. G. (1982). Automodulation of genes: A proposed mechanism for persisting effects of drugs and hormones in mammals. *Neurobehavioral Toxicology and Teratology*, **4**, 435–439.

Cann, R. L. (1988). DNA and human origins. *Annual Review of Anthropology*, **17**, 127–143.

Chesebro, B. (1998). BSE and prions: Uncertainties about the agent. *Science*, **279**, 42–43.

Cirelli, C., Pompeiano, M., & Tononi, G. (1996). Neuronal gene expression in the waking state: A role for locus coeruleus. *Science*, **274**, 1211–1215.

Corner, M. A. (1994). Reciprocity of structure-function relations in developing neural networks: The odyssey of a self-organizing brain through research fads, fallacies, and prospects. *Progress in Brain Research*, **102**, 3–31.

Crick, F. H. C. (1958). On protein synthesis. *Symposia of the Society for Experimental Biology, No. 12. The biological replication of macro-molecules* (pp. 138–163). Cambridge, England: Cambridge University Press.

Crick, F. (1970). Central dogma of molecular biology. *Nature*, **227**, 561–563.

Crick, F. (1982). DNA today. *Perspectives in Biology and Medicine*, **25**, 512–517.

Curran, T., Smeyne, R., Robertson, L., Vendrell, M., & Morgan, J. I. (1994). FoslacZ expression: A transgenic approach to gene activation in the brain. In S. Zalcman,

R. Scheller, & R. Tsien (Eds.), *Molecular neurobiology: Proceedings of the Second NIMH Conference* (pp. 165–172). Rockville, MD: National Institute of Mental Health.

Davidson, E. H. (1986). *Gene activity in early development*. Orlando, FL: Academic Press.

Ehret, G., & Fisher, R. (1991). Neuronal activity and tonotopy in the auditory system visualized by c-*fos* gene expression. *Brain Research*, **567**, 350–354.

Elman, J. L., Bates, E. A., Johnson, M. H., Karmiloff-Smith, A., Parisi, D., & Plunkett, K. (1996). *Rethinking innateness: A connectionist perspective on development*. Cambridge, MA: MIT Press.

Glaser, R., Kennedy, S., Lafuse, W. P., Bonneau, R. H., Speicher, C., Hillhouse, J., & Kiecolt-Glaser, J. K. (1990). Psychological-stress-induced modulation of interleukin 2 receptor gene expression and interleukin 2 production in peripheral blood leukocytes. *Archives of General Psychiatry*, **47**, 707–712.

Goelet, P., Castelluci, V. F., Schacher, S., & Kandel, E. R. (1986). The long and short of long-term memory—A molecular framework. *Nature*, **322**, 419–422.

Gottlieb, G. (1970). Conceptions of prenatal behavior. In L. R. Aronson, E. Tobach, D. S. Lehrman, & J. S. Rosenblatt (Eds.), *Development and evolution of behavior: Essays in memory of T. C. Schneirla* (pp. 111–137). San Francisco: W. H. Freeman.

Gottlieb, G. (1971). *Development of species identification in birds: An inquiry into the prenatal determinants of perception*. Chicago: University of Chicago Press.

Gottlieb, G. (1976). Conceptions of prenatal development: Behavioral embryology. *Psychological Review*, **83**, 215–234.

Gottlieb, G. (1983). *The psychobiological approach to developmental issues*. In M. M. Haith & J. J. Campos (Eds.), *Handbook of child psychology: Infancy and developmental psychobiology* (4th ed., Vol. **2;** pp. 1–26). New York: Wiley.

Gottlieb, G. (1991). Experiential canalization of behavioral development: Theory. *Developmental Psychology*, **27**, 4–13.

Gottlieb, G. (1992). *Individual development and evolution: The genesis of novel behavior*. New York: Oxford University Press.

Gottlieb, G. (1995). Some conceptual deficiencies in 'developmental' behavior genetics. *Human Development*, **38**, 131–141.

Gottlieb, G. (1997). *Synthesizing nature–nurture: Prenatal roots of instinctive behavior*. Mahwah, NJ: Erlbaum.

Grady, D. (1996). Ironing out the wrinkles in the prion strain problem. *Science*, **274**, 2010.

Grenier, J. K., Garber, T. L., Warren R., Whitington, P. M., & Carroll, S. (1997). Evolution of the entire arthropod *Hox* gene set predated the origin and radiation of the onychophoran/arthropod clade. *Current Biology*, **7**, 547–553.

Grouse, L. D., Schrier, B. K., Letendre, C. H., & Nelson, P. G. (1980). RNA sequence complexity in central nervous system development and plasticity. *Current Topics in Developmental Biology*, **16**, 381–397.

Harris, R. S., Longerich, S., & Rosenberg, S. M. (1994). Recombination in adaptive mutation. *Science*, **264**, 258–260.

Hegarty, C. M., Jonassent, J. A., & Bittman, E. L. (1990). Pituitary hormone gene expression in male golden hamsters: Interactions between photoperiod and testosterone. *Journal of Neuroendocrinology*, **2**, 567–573.

Ho, M. -W. (1984). Environment and heredity in development and evolution. In M.-W. Ho, & P. T. Saunders (Eds.), *Beyond neo-Darwinism: An introduction to the new evolutionary paradigm* (pp. 267–289). San Diego, CA: Academic Press.

Holliday, R. (1990). Mechanisms for the control of gene activity during development. *Biological Reviews*, **65**, 431–471.

Horowitz, F. D. (1987). *Exploring developmental theories: Toward a structural/behavioral model of development*. Hillsdale, NJ: Erlbaum.

Hydén, H. (1969). Afterthoughts. In A. Koestler & J. R. Smythies (Eds.), *Beyond reductionism: New perspectives in the life sciences* (pp. 114–117). London: Hutchinson.

Hydén, H., & Egyházi, E. (1962). Nuclear RNA changes of nerve cells during a learning experiment in rats. *Proceedings of the National Academy of Sciences, USA*, **48**, 1366–1373.

Hyman, S. E., & Nestler, E. J. (1993). *The molecular foundations of psychiatry*. Washington, DC: American Psychiatric Press.

Jablonka, E., & Lamb, M. J. (1995). *Epigenetic inheritance and evolution: The Lamarckian dimension*. Oxford, England: Oxford University Press.

Johnston, T. D. (1987). The persistence of dichotomies in the study of behavioral development. *Developmental Review*, **7**, 149–182.

Katz, L. C., & Shatz, C. J. (1996). Synaptic activity and the construction of cortical circuits. *Science*, **274**, 1133–1138.

Kollar, E. J., & Fisher, C. (1980). Tooth induction in chick epithelium: Expression of quiescent genes for enamel synthesis. *Science*, **207**, 993–995.

Kuo, Z. -Y. (1976). *The dynamics of behavior development* (enlarged ed). New York: Plenum.

Lee, C., Parikh, V., Itsukaichi, T., Bae, K., & Edery, I. (1996). Resetting the *Drosophila* clock by photic regulation of PER and a PER-TIM complex. *Science*, **271**, 1740–1744.

Lehrman, D. S. (1970). Semantic and conceptual issues in the nature-nurture problem. In L. R. Aronson, E. Tobach, D. S. Lehrman, & J. S. Rosenblatt (Eds,), *Development and evolution of behavior: Essays in memory of T. C. Schneirla* (pp. 17–52). San Francisco: W. H. Freeman.

Lerner, R. M., & Kaufman, M. B. (1985). The concept of development in contextualism. *Developmental Review*, **5**, 309–333.

Mack, K. J., & Mack, P. A. (1992). Induction of transcription factors in somatosensory cortex after tactile stimulation. *Molecular Brain Research*, **12**, 141–147.

Magnusson, D. (1988). *Individual development from an interactional perspective: A longitudinal study*. Hillsdale, NJ: Erlbaum.

Mayr, E. (1982). *The growth of biological thought*. Harvard University Press: Cambridge, MA.

Meaney, M. J., Diorio, J., Francis, D., Widdowson, J., LaPlante, P., Caldji, C., Sharma, S., Secki, J. P., & Plotsky, P. M. (1996). Early environmental regulation of forebrain glucocorticoid receptor gene expression: Implications for adrenocortical responses to stress. *Developmental Neuroscience*, **18**, 49–72.

Mello, C. V., Vicario, D. S., & Clayton, D. F. (1992). Song presentation induces gene expression in the songbird forebrain. *Proceedings of the National Academy of Sciences, USA*, **89**, 6818–6822.

Merriwether, D. A., Clark, A. G., Ballinger, S. W., Schurr, T. G., Soodyall, H., Jenkins, T., Sherry, S. T., & Wallace, D. C. (1991). The structure of human mitochondrial DNA variation. *Journal of Molecular Evolution*, **33**, 543–555.

Miklos, G. L..Gabor (in press). Evolution and modification of brains and sensory systems. *Daedalus*.

Montagu, A. (1977). Sociogenic brain damage. In S. Arieti & G. Chrzanowski (Eds.), *New dimensions in psychiatry: A world view* (Vol. **2**, pp. 4–25). New York: Wiley.

Morgan, J. I., & Curran, T. (1991). Stimulus-transcription coupling in the nervous system: Involvement of the inducible proto-oncogenes *fos* and *jun*. *Annual Review of Neurosciences*, **14**, 421–451.

Myers, M. P., Wager-Smith, K., Rothenfluh-Hilfiker, A., & Young, M. W. (1996). Light-induced degradation of TIMELESS and entertainment of the *Drosophila* circadian clock. *Science*, **271**, 1736–1740.

Nijhout, H. F. (1990). Metaphors and the role of genes in development. *BioEssays*, **12**, 441–446.

Overton, W. F. (1998). Developmental psychology: Philosophy, concepts, and methodology. In R. M. Lerner (Ed.), *Handbook of child psychology: Theoretical models of human development* (5th ed., vol. **1**, pp. 107–189). New York: Wiley.

Pittman, R., & Oppenheim, R. W. (1979). Cell death of motoneurons in the chick embryo spinal cord. IV: Evidence that a functional neuromuscular interaction is involved in the regulation of naturally occurring cell death and the stabilization of synapses, *Journal of Comparative Neurology*, **187**, 425–446.

Platt, S. A., & Sanislow, C. A. (1988). Norm-of-reaction: Definition and misinterpretation of animal research. *Journal of Comparative Psychology*, **102**, 254–261.

Pritchard, D. J. (1986). *Foundations of developmental genetics*. London and Philadelphia: Taylor and Francis.

Ren, P., Lin, C.-S., Johnson, R., Albert, P. S., Pilgrim, D., & Riddle, D. L. (1996). Control of *C. elegans* larval development by neuronal expression of a TGFβ homolog. *Science*, **274**, 1389–1391.

Rose, S. P. R. (1967). Changes in visual cortex on first exposure of rats to light: Effect on incorporation of tritiated lysine into protein. *Nature*, **215**, 253–255.

Rosen, L. B., & Greenberg, M. E. (1994). Regulation of *c-fos* and other immediate-early genes in PC-12 cells as a model for studying specificity in neuronal signaling. In S. Zalcman, R. Scheller, & R. Tsein (Eds.), *Molecular neurobiology: Proceedings of the Second NIMH Conference* (pp. 203–216). Rockville, MD: National Institute of Mental Health.

Rosensweig, M. R., & Bennett, E. L. (1978). Experiential influences on brain anatomy and brain chemistry in rodents. In G. Gottleib (Ed.), *Early influences* (pp. 289–327). New York: Academic Press.

Sameroff, A. J. (1983). Developmental systems: Contexts and evolution. In W. Kessen (Ed.), *Handbook of child psychology: History, theory, and methods*. (4th ed., Vol. **1**, pp. 237–294). New York: Wiley.

Schneirla, T. C. (1960). Instinctive behavior, maturation—Experience and development. In B. Kaplan & S. Wapner (Eds.), *Perspectives in psychological theory—Essays in honor of Heinz Werner* (pp. 303–334). New York: International Universities Press.

Shields, J. (1962). *Monozygotic twins*. London: Oxford University Press.

Singh, A. K., & Lakhotia, S. C. (1988). Effect of low-temperature rearing on heat shock protein synthesis and heat sensitivity in *Drosophila melanogaster*. *Developmental Genetics*, **9**, 193–201.

Slijper, E. J. (1942). Biologic-anatomical investigations on the bipedal gait and upright posture in mammals, with special reference to a little goat, bom without forelegs. II. *Proceedings Series C Biological and Medical Sciences: Koninklijke Nederlandse Akademie vim Wetenschappen Amsterdam*, **45**, 407–415.

Smeyne, R. J., Schilling, K., Robertson, L., Luk, D., Oberdick, J., Curran, T., & Morgan, J. I. (1992). Fos-lacZ transgenic mice: Mapping sites of gene induction in the central nervous system. *Neuron*, **8**, 13–23.

Spelke, E. S., & Newport, E. L. (1998). Nativism, empiricism, and the development of knowledge. In R. M. Lerner (Ed.), *Handbook of child psychology: Theoretical models of human development* (5th ed., Vol. **1**, pp. 275–340). New York: Wiley.

Stent, G. (1981). Strength and weakness of the genetic approach to the development of the nervous system. *Annual Review of Neuroscience*, **4**, 163–194.

Strohman, R. C. (1997). The coming Kuhnian revolution in biology. *Nature Biotechnology*, **15**, 194–200.

Tanner, J. M. (1978). *Foetus into man: Physical growth from conception to maturity*. Cambridge, MA: Harvard University Press.

Telling, G. C., Parchi, P., DeArmond, S. J., Cortelli, P., Montagna, P., Gabizon, R., Mastrianni, J., Lugaresi, E., Gambetti, P., & Pruisner, S. B. (1996). Evidence for the conformation of the pathologic isoform of the prion protein enciphering and propagating prion diversity. *Science*, **274**, 2079–2082.

Thaler, D. S. (1994). The evolution of genetic intelligence. *Science*, **264**, 224–225.

Thelen, E., & Smith, L. B. (1994). *A dynamic systems approach to cognition and action*. Cambridge, MA: MIT Press.

Tooby, J., & Cosmides, L. (1990). The past explains the present: Emotional adaptations and the structure of ancestral environments. *Ethology and Sociobiology*, **11**, 375–424.

Uphouse, L. L., & Bonner, J. (1975). Preliminary evidence for the effects of environmental complexity on hybridization of rat brain RNA to rat unique DNA. *Developmental Psychobiology*, **8**, 171–178.

Valsiner, J. (1997). *Culture and the development of children's action: A theory of human development*. (2nd ed.). New York: Wiley.

Van der Weele, C. (1995). Images of development: Environmental causes in ontogeny. Unpublished doctoral dissertation, Vrije University, Amsterdam.

von Bertalanffy, L. (1962). *Modern theories of development: An introduction to theoretical biology*. New York: Harper. (Original work published 1933)

Wahlsten, D. (1990). Insensitivity of the analysis of variance to heredity-environment interaction. *Behavioral and Brain Sciences*, **13**, 109–120; open peer commentary, 121–161.

Wahlsten, D., & Gottlieb, G. (1997). The invalid separation of nature and nurture: lessons from animal experimentation. In R. J. Sternberg & E. Grigorenko (Eds.), *Intelligence, heredity, and environment* (pp. 163–192). New York: Cambridge University Press.

Weiler, I. J., Irwin, S. A., Klintsova, A. Y., Spencer, C. M., Brazelton, A. D., Miyashiro, K., Comery, T., Patel, B., Eberwine, J., & Greenough, W. T. (1997). Fragile X mental retardation protein is translated near synapses in response to neurotransmitter activation. *Proceedings of the National Academy of Sciences USA*, **94**, 5395–5400.

Weiss, P. (1939). *Principles of development: A text in experimental embryology*. New York: Holt, Rinehart and Winston. (Reprinted in 1969 by Hafner.)

Wigglesworth, V. B. (1964). *The life of insects*. Cleveland, OH: World Publishing.

Wills, C. (1989). *The wisdom of the genes: New pathways in evolution*. New York: Basic Books.

Woltereck, R. (1909). Weitere experimentelle Untersuchungen über Artveränderung, speziell über das Wesen quantitativer Artunterschiede bei Daphniden. [Further experimental investigations of species modification, particularly the nature of quantitative species differences in daphnia]. *Verhandlungen der Deutschen Zoologischen Gesellschaft*, **19**, 110–173.

Wright, S. (1968). *Evolution and the genetics of population: Vol. 1: Genetic and biometric foundations*. Chicago: University of Chicago Press.

Zamenhof, S., & van Marthens, E. (1978). Nutritional influences on prenatal brain development. In G. Gottlieb (Ed.), *Early influences* (pp. 149–186). New York: Academic Press.

Part II

Theoretical Foundations for the Developmental Study of Behavior and Genetics

3

Historical and Philosophical Perspectives on Behavioral Genetics and Developmental Science

James Tabery and Paul E. Griffiths

Why is Behavioral Genetics so Controversial?

Disputes over the scientific validity of behavioral genetics are as old as the field itself. Often these disputes have been politicized, with less-than-rational motivations attributed by disputants to their opponents. In early 20th-century Britain when Ronald A. Fisher developed many of the now-classical statistical methodologies of behavioral genetics (Fisher, 1924, 1925, 1930), critics such as J. B. S. Haldane and Lancelot Hogben alleged that these methods were being employed in a scientifically invalid manner to support eugenics (Haldane, 1938, 1946; Hogben, 1933). These criticisms were met with reciprocal criticism of the "communists and fellow-travelers" who questioned these statistical tools (Mazumdar, 1992). Decades later, in the late 1960s and 1970s, behavioral genetics came under fire again as a result of claims about the genetic basis for the gap in IQ scores between white and black populations. In this "IQ Controversy" critics of hereditarianism, such as Richard Lewontin, Leon Kamin, and Noam Chomsky, made no secret of their belief that the research they attacked was certainly racist in substance and perhaps even carried out with a racist agenda. Hereditarians like Arthur Jensen, Richard Herrnstein, and Hans Eysenck responded that their critics were not genuinely concerned with the validity of their scientific research, but merely seeking to censor science in the pursuit of their progressive political agendas. Comparisons with Nazi race-science were met with comparisons to Stalin's suppression of Mendelism (Kevles, 1995).

As a result of this heated history, it has become difficult to address methodological and conceptual issues in behavioral genetics without being aligned with one or other side of what is taken to be primarily a political and not an intellectual

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dispute (Tabery, 2009b). Even the strictly scientific literature on behavioral genetics is littered with references to the ideological motivations of the researcher's opponents. Turning to historical and philosophical studies of behavioral genetics, Jonathan Kaplan has chronicled what he called the "limits and lies" of human genetics, while Neven Sesardic has claimed that the entire discipline of philosophy of science has a structural bias against hereditarian explanations (Kaplan, 2000; Sesardic, 2005). Charges of ignorance have been met with charges of blind dogmatism (Chase, 1980; Levin, 1997).

It is certainly true that many participants in the disputes over behavioral genetics have their eyes on the social, ethical, and political consequences of the research. However, the ongoing disputes are not simply the result of the unshakeable, environmentalist bias of critics, nor of the dogmatic, hereditarian bias of proponents. The tendency to reduce the disputes to such political motivations belies intellectual disagreements that are both deep and important for behavioral genetics, but which have been marred by a lack of conceptual clarity.

The purpose of this chapter is to redirect attention to and then disentangle some of these cases of conceptual confusion within the disputes over behavioral genetics. Thus, the goal is to bring to the forefront genuinely intellectual disagreements which can be legitimately debated in the place of the more commonly referenced political disagreements which tend to defy rational discourse. We begin with a brief history of traditional behavioral genetics and of the emergence of conflicting aspirations to create a "developmental behavioral genetics," With this history in place, we then take up three concepts that have generated much debate within the field: *norm of reaction*, *genotype-environment interaction*, and *gene*. Rather than offer a political explanation of these debates, we instead address some conceptual issues that have made it difficult for the disputants to engage in productive discussion. Moreover, we argue that a common thread underlies these separate debates: a disagreement over the epistemological relevance of potential variation that is not manifest in actual populations to the understanding of development.

A Brief History of Traditional Behavioral Genetics and of Aspirations for a "Developmental" Behavioral Genetics

A discipline such as traditional, quantitative behavioral genetics may be defined either methodologically or sociologically (Hull, 1988). Methodologically, traditional behavioral genetics consists of the application of quantitative genetic methods to behavioral phenotypes. These methods were initially developed by figures such as Fisher, Haldane, Sewall Wright, and George Udny Yule in order to integrate the Mendelian model of inheritance with the existing, biometrical tradition in the study of natural selection (Provine, 2001; Tabery, 2004). In 1918 Fisher published his seminal "The Correlation between Relatives on the Supposi-

tion of Mendelian Inheritance." In the process of demonstrating the compatibility of Mendelian and biometrical models, Fisher also introduced a new statistical concept – *variance* (Box, 1978). The concept was of interest to Fisher because it seemed to offer him a means of quantifying genetic differences and environmental differences and establishing *how much* each cause of variation contributed to total phenotypic variation for a trait in a population. Much of Fisher's career was spent developing statistical methods such as the analysis of variance (ANOVA), tests of statistical significance, and the design of experiments, mostly with the goal of answering this how-much question about the *relative contributions of nature and nurture*. This focus on relative contributions became a defining methodological feature of quantitative behavioral genetics.

From a more sociological perspective, the emergence of behavioral genetics can be dated to around 1960 (Fuller & Simmel, 1986; Plomin & McClearn, 1993; Whitney, 1990). The date is chosen to recognize the publication of John Fuller and William Thompson's *Behavior Genetics*. "The time seems ripe," the authors stated in the preface, "for a modern treatment of the division of knowledge we have called 'behavior genetics'" (Fuller & Thompson, 1960, p. v). A textbook-style treatment, *Behavior Genetics* introduced the basics of cellular, genetic, and population biology; the methods of inbreeding, ANOVA, and twin studies; and their applications to personality, intelligence, and mental disorders. The pivotal disciplinary event occurred a decade later, in 1970, with the creation of the journal *Behavior Genetics*, and the founding of the Behavior Genetics Association with Ukranian-American geneticist Theodosius Dobzhansky as its first president.

In later years, advances in molecular biology facilitated the investigation of the role that genes played in the development of a phenotype at the molecular level. When the focus was on humans, however, ethical considerations largely confined behavioral geneticists to traditional quantitative genetic methods. Thus, classic twin and adoption studies were employed to evaluate the relative contributions of different sources of variation, along with gene-hunting studies, which track the distribution of genetic markers in families (linkage studies) or populations (association studies) in an attempt to seek out candidate genes associated with behavioral traits (Kendler, 2005).

While traditional, quantitative behavioral genetics is a clearly defined field, both intellectually and institutionally, *developmental* behavioral genetics is less a field than an aspiration. Understanding the role of genes in behavioral development is clearly amongst the most important desiderata for contemporary life and social science, but it is far from clear what kinds of studies will yield this understanding, whether this issue defines a single field with a distinctive set of methods, and how such a field would relate to traditional behavioral genetics. The most straightforward vision of a developmental behavioral genetics involves the application of the traditional behavioral genetic methods to developmental data, that is, to repeated observations of the same phenotype at different stages of development – the study of "distributions of individuals developing across time" as Sandra Scarr has char-

acterized the field (Scarr, 1995, p. 158; see also Plomin, 1983). Scarr, following in the Fisherian tradition of focusing on the relative contributions of various sources of variation, argued that developmental behavioral genetics should seek the causes of phenotypic variation, rather than the causes of phenotypes, and ask *how much* phenotypes depend on certain causes, rather than *how* they depend on them (see also Plomin, 1983, p. 254; Scarr, 1992, 1993, 1995). These methodological stipulations have been used for over 50 years to defend traditional behavioral genetics against the accusations that (1) it does not yield causal explanations and (2) it cannot explain phenomena at the individual, as opposed to the population, level. It was Scarr's aim to insulate the proposed new discipline from the same accusations, and to insist that within the stipulated limits it will yield genuine answers to genuine questions about behavioral development.

This vision of a developmental behavioral genetics has been fiercely rejected by scientists from developmentally-oriented disciplines, including experimental embryologists, developmental psychobiologists and developmental geneticists. Some of the strongest criticism has come from developmental psychobiology, a research tradition which emerged in the 1960s from earlier work on behavioral development by comparative psychologists such as Theodore C. Schneirla, his student Daniel S. Lehrman, and more recently in the work of Gilbert Gottlieb. The International Society for Developmental Psychobiology was founded in 1967 and a journal of the same name followed in 1968. The term "psychobiology" has been used in diverse ways in the past century, but usually with the intention of preventing some psychological phenomenon from getting lost in the enthusiasm for single, currently productive reductionistic research strategy (Dewsbury, 1991). "Developmental psychobiology" denotes just such an integrative, multi-disciplinary approach to behavioral development, seeking to integrate genetic analysis with behavioral embryology and the evolutionary study of animal behavior (e.g., Michel & Moore, 1995). The inclusion of animal behavior research in this synthesis points to another of the field's historical roots, in the rapprochement between ethology and comparative psychology in the 1960s (e.g., Hinde, 1966). Developmental psychobiology can thus be interpreted as the study of behavioral development in the spirit of Niko Tinbergen's (1963) program for the biology of behavior, according to which the "four questions" of the mechanistic causes of behavior, of developmental causation, of the ecological role of behavior, and of its evolution are to be answered (and, indeed, posed) in the light of one another.

According to the critics of traditional behavioral genetics, the prefix "developmental" can only be meaningfully attached to behavioral genetics if behavioral genetics abandons the traditional methodological restrictions discussed above. The new discipline must set out to elucidate the *causal mechanisms* of behavioral development rather than quantify *causes of variation* in behavioral development, and must ask *how* genes cause development rather than *how much* development genes cause. Traditional, quantitative genetic methods are fundamentally unsuited to the study of the causal role of genes in development, the

argument goes, because they analyze and explain phenomena at the level of the population and not the individual organism, and because they explain the differences between individuals, rather than how those individuals came to have the phenotypes that they do (Ford & Lerner, 1992; Gottlieb, 1995, 2003). But to advocates of the statistical vision of developmental behavioral genetics, these criticisms simply confuse different scientific questions that can, and should, be kept apart; they confuse different *levels of analysis* (Plomin, 1990; Scarr, 1995; Surbey, 1994). Instances of this dispute can be found in disagreements over concepts such as norm of reaction, gene-environment interaction, and gene. It is to these more specific disagreements that we now turn.

Two Conceptions of the Norm of Reaction

Wilhelm Johanssen's 1911 paper introducing the terms "gene," "genotype," and "phenotype" made extensive use of the norm of reaction concept introduced a few years earlier by German biologist Richard Woltereck (Johannsen, 1911; Woltereck, 1909; for a history see Sarkar, 1999). The norm of reaction depicts the effect on the phenotype of rearing one or more genotypes in a range of environments (see Figure 3.1). It is a powerful visual embodiment of Johannsen's new, Mendelian

Figure Not Available

conception of heredity. Earlier discussions of heredity, he argued, had tried to establish a direct relationship between the phenotypes of ancestor and descendant. The new "Genotype Conception of Heredity" recognized that this direct, hereditary relationship only exists at the level of the gene. Similarities between ancestral and descendant phenotypes depend not just on heredity, but also on shared environmental conditions.

The concept of a norm of reaction has been a locus of disagreement between traditional behavior geneticists and developmental scientists. This disagreement has been marked by a terminological distinction. Developmental scientists have tended to employ the term *reaction norm* (or *norm of reaction*), while traditional behavioral geneticists have often preferred the term *reaction range*. As we have shown in detail elsewhere (Griffiths & Tabery, 2008), these two terms were initially synonyms: "The diverse phenotypes that may arise from the interplay between a given genotype and various environments in which this genotype may live constitute *the norm, or range, of reaction* of that genotype" (Sinnott, Dunn, & Dobzhansky, 1950, p. 24, emphasis added). The use of the term "reaction range" in behavioral genetics can ultimately be traced back to Dobzhansky, but the most influential immediate source was the work of Irving Gottesman, in whose work the term took on a different, although closely related, meaning. In his earliest presentation of the concept Gottesman explained that "For our purposes the best way to conceptualize the contribution of heredity to intelligence is to think of heredity as determining a norm of reaction (Dobzhansky, 1955) or as fixing a reaction range" (Gottesman, 1963, p. 254) and provided a hypothetical example of such norms or ranges of reaction (Figure 3.1). In this figure Gottesman used the term reaction range ("RR") to label the range of phenotypic variation actually exhibited by each genotype in the range of environments to which they were reared. The reaction range was thus the portion of the reaction norm realized in the actual environments for which data were available. In his later work Gottesman emphasized the idea of a reaction range, and this became the standard concept in the behavioral genetics and psychological literature of subsequent decades.

Gottesman's notion of a reaction range has been criticized by scientists in the developmental research tradition. One prominent criticism is that the reaction range concept implies that the phenotype is deterministically restricted within a certain range by the genotype; whereas the reaction norm places no such genotypic limits on development. "Gottesman's notion of reaction range sets strict and predictable upper and lower limits for a genotype. . . . The norm of reaction, in contrast, holds that a knowledge of phenotypic outcome under one or many rearing conditions does not allow one to predict the outcome when novel rearing conditions are encountered" (Gottlieb, 1995, pp. 134–135; see also Gottlieb, 2003). This criticism originated in a paper by Steve Anderson Platt and Charles A. Sanislow III (1988) who criticized the conflation of the concepts of reaction range and reaction norm by behavioral geneticists and argued that the deterministic reaction range should be replaced by the reaction norm.

The idea that the reaction range, and not the reaction norm, implies the existence of fixed limits to phenotypic plasticity is, however, mistaken. Gottesman certainly did speak of heredity as "fixing" and "determining" the reaction range. But Dobzhansky too claimed that the genotype "determines" the reactions of the organism to the environment, and that the reaction norm was "circumscribed by the genotype" (Dobzhansky, 1951; Sinnott et al., 1950). Dobzhansky emphasized that such determination or circumscription was specific to the particular environments tested, and that other environments might be encountered or interventions might be developed, which could lead to phenotypic outcomes outside the range previously observed. But Gottesman did not deny this when he gave the term "reaction range" its current meaning. The reaction range is the difference between minimum and maximum phenotypic values in the range of environments for which data are actually available. It is an empirical characteristic of a reaction norm and, thus, no more indicative of fixed upper and lower limits than the reaction norm (Turkheimer, Goldsmith, & Gottesman, 1995, p. 143).

But this does not mean that there was no substance to the disagreement between developmental scientists like Gottlieb and behavioral geneticists like Gottesman. The norm of reaction is a theoretical entity which encompasses not only actual variation, but also potential variation in untested environments. Behavioral geneticists saw this abstractness as limiting the scientific utility of the reaction norm concept: it is impractical to expose each genotype to the full range of possible environments and so we never know the full reaction norm. In contrast, reaction range is an operational concept. It is often reasonable to extrapolate an observed linear relationship between gene and phene, and so within some range of actual environments we can be reasonably sure what can and cannot be achieved. This limited but valuable understanding of the genetic potential of the system, they argued, should not be thrown out like the baby with the bathwater merely because it is not total understanding (Turkheimer et al., 1995, pp. 147–152).

Developmental scientists like Gottlieb took a very different view, which reflected the very different scientific work in which they were engaged. Whereas traditional behavioral genetics was based on observational datasets, Gottlieb and his ilk were engaged in experimental investigations of the causal basis of behavioral development. From this point of view it seems far less utopian to seek to determine the full reaction norm. A causal model will both narrow down the class of environmental variables that affect the trait and also allow us to plug possible values into the model. In effect, a well-confirmed causal model of the development of a trait *embodies* the full norm of reaction of that trait. Moreover, abnormal and even unnatural environments are a powerful experimental tool with which to confirm such a causal model.

Thus, we suggest that the dispute over the concepts of reaction norm and reaction range was fundamentally about the epistemological relevance of merely potential variation, variation which is part of the reaction norm, but not part of the reaction range. For behavioral geneticists potential outcomes in unobserved

environments were a distraction from the task of drawing what conclusions we validly can from the observations we actually have. Reaction range was the useful scientific concept here, while the reaction norm merely embodied a distractingly utopian ideal of total knowledge. For developmental scientists, however, understanding behavioral development meant understanding the causal structure that responded with different outcomes to different environments. All parts of the full reaction norm, including those parts that may never be realized in nature, were epistemologically relevant in the sense that they were all potential tests of a causal model. In contrast, the realized reaction range was a superficial measure that confounded the causal structure of the system with the particular parameter settings found in extant populations. The next section will show that the same difference in perspective was at the heart of the better-known dispute about the meaning of "interaction."

Two Conceptions of Gene-Environment Interaction

It is a truism that genes interact with the environment during development. But this truism has been understood in very different ways by developmental scientists and traditional, quantitative behavioral geneticists. Traditional behavioral geneticists study the relative contributions of genotypic and environmental differences to total phenotypic variation in a trait in a population. One standard method for investigating these relative contributions is still Fisher's ANOVA. In its simplest form, ANOVA partitions total phenotypic variation for a trait (V_P) into a contribution attributable to genotypic variation (V_G) and a contribution attributable to environmental variation (V_E):

$$V_P = V_G + V_E \tag{3.1}$$

In this simple case, the two sources of variation are additive, meaning just that V_G and V_E together fully account for the total phenotypic variation. However, when the genotypic variation is dependent on the environmental distribution, and the environmental variation is dependent on the genotypic distribution, V_G and V_E become interdependent. This is *gene-environment interaction*, or G × E; it creates a potential problem for ANOVA because G × E generates its own source of variation ($V_{G\times E}$) ensuring the breakdown of the additivity in Equation (3.1) and requiring a modification that results in Equation (3.2):

$$V_P = V_G + V_E + V_{G\times E} \tag{3.2}$$

Another way to understand the difference between Equations (3.1) and (3.2) is with reference to the norm of reaction concept discussed in the last section. When V_G and V_E are additive as in Equation (3.1), then the norms of reaction (G1 and G2) will

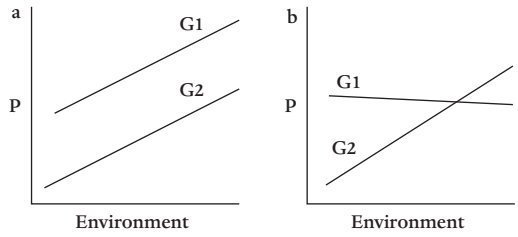

Figure 3.2. Hypothetical phenotypic curves: (a) Parallel phenotypic curves with no gene-environment interaction; (b) Non-parallel phenotypic curves with gene-environment interaction.

be parallel across the environment, as is the case in Figure 3.2a; however, when there is $V_{G\times E}$ as in Equation (3.2), then the norms of reaction will not be parallel across the environment, as is the case in Figure 3.2b.

In the context of traditional, quantitative behavioral genetics, interaction is understood as this statistical phenomenon: the interaction between genotypic and environmental sources of variation in a population, which results in the breakdown in additivity between main effects. But in the context of the experimental study of behavioral development interaction is a causal-mechanical phenomenon, not just a statistical one. Genetic and environmental factors causally interact in the processes that give rise to phenotypes.

Tabery (2007, 2008; see also Griffiths & Tabery, 2008) has labeled these two concepts of gene-environment interaction the "biometric" concept ($G \times E_B$) and the "developmental" concept ($G \times E_D$). The biometric concept of $G \times E_B$ can be traced back all the way to Fisher, who was the first to wrestle with the complications posed by $G \times E$ for his ANOVA. Through the work of Fisher and others $G \times E_B$ became part of the basic conceptual toolkit of population genetics, and then when the tools of population genetics were appropriated for behavioral genetics, $G \times E_B$ became part of that discipline as well (Tabery, 2007, 2008).

But $G \times E_B$ was never the only concept of gene-environment interaction. Fisher's formulation of ANOVA was immediately criticized by contemporaries like Haldane and especially Hogben for embodying a "false antithesis of heredity and environment" (Hogben, 1932, p. 201). Hogben understood gene-environment interaction not merely as a statistical phenomenon produced by the interaction of two sources of variation, G and E, but rather as the result of a third causal factor. This third factor consisted of the actual, physical combinations of genes and the environment found in the individuals that make up the population. This produced a "third class of variability," which "arises from the combination of a particular hereditary constitution with a particular kind of environment" (Hogben, p. 98). On this understanding, gene-environment interaction is *manifested* in statistical interaction between measured G and E, but it is *not constituted* by it. Even if no statistical interaction is present in the data, our causal models of development imply that genetic and environmental factors are nevertheless causally interacting within each

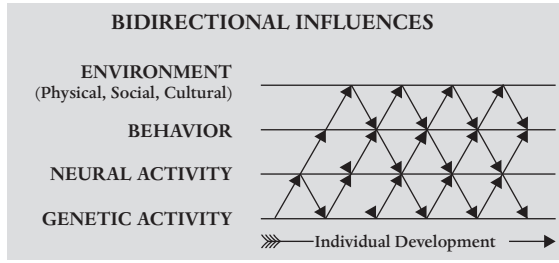

Figure 3.3. "Coaction" of genes and environment. *Source*: Gottlieb (1992, p. 186).

organism to produce the phenotype. The failure to observe any variance resulting from this causal interaction is something that needs to be explained through an appropriate causal model, such as, for example, Conrad H. Waddington's model of "developmental canalization" in which many different combinations of developmental factors converge on the same developmental outcome (Waddington, 1957). Like $G \times E_B$, the developmental concept of $G \times E_D$ persisted throughout the 20th century. But it was *developmental* geneticists, such as Waddington, rather than *population* geneticists, who kept this concept in currency (Tabery, 2007, 2008).

Introducing these two different senses of "interaction" allows us to make sense of the longstanding dispute between behavior geneticists and developmental scientists over $G \times E$. Developmental scientists like Gilbert Gottlieb made the idea of gene-environment interaction central to their understanding of behavioral development, introducing for example the idea of gene-environment *co-action* in individual development (Figure 3.3).

This developmental interconnectedness, Gottlieb claimed, ensured that $G \times E$ was the rule, not the exception (Gottlieb, 2003, p. 343). Traditional behavioral geneticists, however, have argued that it is a conceptual error to introduce considerations about the causal mechanisms of development into discussions of $G \times E$ in the study of individual differences. During the IQ controversy of the 1970s Arthur Jensen asserted that only the statistical sense of interaction was relevant to the understanding of individual differences. Criticism of behavioral genetics resulted from "a failure to understand the real meaning of the term 'interaction' as it is used in population genetics; but even more it is the result of failure to distinguish between (a) the *development* of the individual organism, on the one hand, and (b) *differences* among individuals in the population" (Jensen, 1973, p. 49). This *defense-by-distinction* quickly became the standard response of behavioral geneticists to their critics (Tabery, 2007):

> Unfortunately, discussions of genotype-environment interaction have often confused the population concept with that of individual development. It is important at the outset to distinguish genotype-environment interaction from what we shall call *interactionism*, the view that environmental and genetic threads in the fabric of behavior are so tightly interwoven that they are indistinguishable.
>
> (Plomin, DeFries, & Loehlin, 1977, p. 309).[1]

The defense-by-distinction contrasts the real meaning of the term "interaction" with muddle-headed "interactionism." It does admit that there is an alternative, coherent concept of interaction, but claims that this cannot be applied to the study of individual differences in a population. The idea seems to be that critics become "muddle-headed interactionists" when they insist on applying the causal sense of interaction, whose proper domain is the study of individual development, to the study of individual differences in a population.

We are not convinced by the defense-by-distinction. Why do some people develop a complex trait such as major depression, whilst others do not? This question incorporates a question about the causal mechanisms responsible for the *individual development* of depression, as well as a question about the causes of variation responsible for *individual differences* in depression. The causal-mechanical study of behavioral development has traditionally been concerned with the development of species-typical phenotypes, a feature it shares with most traditional developmental biology, but this is a contingent, historical fact, not an essential feature of this type of scientific inquiry. Individual differences are as much in need of causal mechanical explanation as species-typical phenotypes and in recent years such explanations have started to appear. For example, Michael Meaney and collaborators' well-known work on the molecular basis of individual differences in stress-reactivity gives a causal mechanical explanation of the distribution of such differences in populations (Meaney, 2001; Weaver et al., 2004). This explanation fits the prescription given by Hogben – it documents how different combinations of gene and environment are distributed in the population. Tabery has termed this kind of causal-mechanical explanation of population-level variation the study of "difference mechanisms" (Tabery, 2009a).

If we admit that the desire to provide causal-mechanical explanations of individual differences in populations is legitimate, but that it involves the application of a different sense of gene-environment interaction, we can neatly explain the ongoing disagreement between behavioral geneticists and developmental scientists about the data on $G \times E$. Behavioral geneticists regularly detect large main effects for genes and fail to identify a high level of statistical interaction between genes and environment ($G \times E_B$). One possible explanation is that behavioral genetic methods have a systematic tendency to underestimate interaction effects, as Douglas Wahlsten has alleged (Wahlsten, 2000, see also chapter 5 this volume). But let us grant that the study of individual differences in natural populations reveals surprisingly low levels of $G \times E_B$. For a traditional behavior geneticist $G \times E_B$ *is* interaction, and if this element of the variance is low there is little interaction. Period. The continued effort to document interaction in the face of this evidence, and particularly the search for abnormal environments that will generate interaction, seems merely perverse from this perspective. But for developmental scientists like Gottlieb, interaction is fundamentally a property of causal networks of material entities, and $G \times E_B$ is only the statistical manifestation of actual differences in these networks. Since we know on direct, biological grounds that

development is interactive, the failure to detect statistical interaction simply tells us that the developmental system is structured so as to render those developmental outcomes insensitive to variation in certain parameters (via mechanisms such as redundancy and feedback). Instead of concluding that there is no interaction, developmental scientists argue that we need to find interventions that will reveal it, such as using experimental interventions to drive parameters to values that would not be encountered in nature. Only by finding such interventions can we decipher the very causal pathways that explain the *lack* of interaction in normal conditions.

As we saw in the previous section, traditional behavioral genetics emphasizes the practical value of the conclusions we can derive from the variation we actually observe. In contrast, developmental scientists insist that the ultimate practical goal must be causal understanding; for this purpose, potential variation – even to extents not normally encountered in nature – is as important as actual variation. The difference between the traditional behavior genetic perspective and that of developmental scientists once again comes down to the epistemological relevance of variation which is possible but not actually observed under natural conditions.

Two Conceptions of Genes and Gene Action

Previous sections have highlighted two conceptual issues that help to explain the persistent tendency of advocates and critics of traditional behavioral genetics to talk past one another. In this section we consider a third source of miscommunication: different ways of conceptualizing genes and gene action. Agreement about the fundamental, ontological grounding of genetics in DNA is not enough to create a shared conceptualization of the gene. Traditional behavioral genetics (following quantitative genetics) conceptualizes genes in classical, Mendelian terms as intervening variables in the genetic analysis of phenotypes. In contrast, developmental psychobiologists standardly conceptualize genes as determinants of the value of a developmental parameter in the context of a larger developmental system (we will refer to these constructs as "developmental genes").

Let us examine these two conceptualizations of the gene in more detail. From its earliest days the gene was always a postulated physical unit of heredity. At a practical level, however, the genes of classical, Mendelian genetics were intervening variables that allowed prediction of the phenotypes of offspring from the phenotypes of parents. The aim of genetic analysis was not to test the theory of the gene, but to answer other biological questions using that theory (see the detailed reconstructions in Waters, 2004). Quantitative characters, like height and weight, which vary continuously between individuals, posed a significant problem for early geneticists, since only a character with discrete values can appear in Mendelian

ratios in offspring. However, as early as 1918, Fisher showed that statistical procedures for studying correlations between phenotypes could be interpreted in Mendelian terms (Fisher, 1918). In the simplest models of this kind, quantitative traits are treated as if they were the effect of a large number of genes each of which makes an equal, small contribution to variation in the character. The attitude of the geneticist to these postulated Mendelian "genes" is like the attitude we have toward "centers of mass" in physical theory. Centers of mass are mathematical devices. It would be foolish to look for them as additional constituents of matter alongside protons, neutrons, and the rest. Nevertheless, what we know about how matter is actually constituted justifies us introducing these entities into our calculations. In just the same way, whether we can identify specific DNA segments corresponding to the "genes" discussed in Fisher's proofs is simply not germane to the validity of the quantitative genetic results he derived. If the Mendelian framework is broadly correct then results derived by postulating these "genes" will be reliable.

The identification of DNA as the genetic material and the ongoing elucidation of its structure and function have had the result that the dominant meaning of "gene" in contemporary scientific usage is a DNA sequence which is transcribed to produce a messenger RNA molecule that in turn is processed to produce a protein or a functional RNA. But this does not mean that the classical, Mendelian conception of the gene has been or can be replaced by this molecular conception (Griffiths & Stotz, 2006, 2007). Geneticists continue to make use of classical genetic techniques to identify regions on chromosomes in which molecular genes may be located. Even when the explicit aim of this work is to identify molecular genes, the conceptualization of the gene that is actually used to do the work is the classical, Mendelian, conception. Some abnormalities in human limb development, for example, are associated with mutations in a gene on human chromosome 7. But research suggests that the gene in which the mutation is located plays no role in the development of these abnormalities (Lettice et al., 2002). Instead, embedded in that gene is a sequence of DNA that acts to regulate sonic hedgehog, a gene located about one million nucleotides away on the same chromosome. It is likely that sonic hedgehog is involved in the relevant aspects of limb development. But it is no criticism of the original research which found the "gene for" these abnormalities that what it found was, in another sense, not a gene (i.e., not a sequence of DNA transcribed to produce a messenger RNA molecule). Biologists have no difficulty thinking in Mendelian terms when applying traditional genetic techniques, and switching seamlessly to the molecular conception of the gene when examining the DNA sequences they have located. The traditional, Mendelian gene concept is alive and well and it would be intellectually crippling to insist on using only the molecular concept in genetic research (Griffiths & Stotz, 2006, 2007; Weber, 2004).

If traditional behavior genetics conceives genes primarily as Mendelian alleles, how have its critics such as developmental psychobiologists conceived them? While recent work in developmental psychobiology has begun to link the parameters of developmental models to the expression of specific coding sequences

in the genome (e.g., Meaney, 2001; Suomi, 2003), for most of the history of this research tradition such genes have been purely hypothetical. It has not been possible to manipulate specific genetic parameters of the developmental system in the same way as specific environmental parameters. This may have produced an environmentalist bias in empirical results, if not in conceptual framework. However, any such bias is rapidly being corrected now that it is practical to intervene at the molecular level, for example by unmethylating genes which were methylated as a result of earlier life-experience (Weaver et al., 2005). But until very recently, although developmental psychobiologists conceived of genes as mechanistic causes of development, the lack of direct, manipulable access to these causes led them to appear in representations such as Figure 3.3 as purely hypothetical determinants of the value of certain parameters of a developmental model.

Mendelian alleles and hypothesized developmental genes are both legitimate ways to introduce DNA sequences into two, very different theoretical contexts.[2] It is important to recognize, however, that the explanatory role which the "gene" plays in those two contexts is different. The presence of a developmental gene, by its very nature, explains a particular phenotype via the mediation of many other developmental parameters. In contrast, the Mendelian allele for a phenotypic *difference*, by its very nature, explains that *difference* without reference to other developmental parameters. The developmental gene is defined as the factor which plays such-and-such a role in relation to the other parameters of the developmental model. The introduction of a specific gene into a developmental model is justified by reference to the ability of the model as a whole to explain the effects of manipulations of its various parameters. In this context, explaining the presence of a phenotype by reference to the presence of a particular gene means drawing attention to how that particular genetic parameter interacts with the other parameters. The same point applies to explanations of phenotypic *differences*, which in this context draw attention to how a particular *change* in the genetic parameter ramifies through the system. But explanations of phenotypes in terms of the presence of Mendelian alleles do not share these features. The presence of a Mendelian allele explains the presence of the associated phenotypic difference because of the statistical association between alleles and phenotypes in a pedigree or a population. The epistemological value of this relationship derives precisely from the fact that it is robust across the actual distributions of other developmental parameter settings in the population from which it is derived and in which it can be legitimately extrapolated.

Thus, the developmental gene explains by reference to the developmental system as a whole, while the Mendelian allele explains by importing statistical information about specific alleles and phenotypes from some reference class, much as the reaction norm concept incorporates information about a developmental system as a whole while the reaction range concept limits itself to consideration of an actual population. We do not think that this is just another way of stating the

truism that Mendelian genetics explains phenotypic differences and not phenotypic states in themselves. Although the presence of a Mendelian allele can only explain a phenotypic difference, the state of a hypothetical developmental gene can explain a phenotypic difference *as well as* explaining an individual phenotype. In the context of a suitable developmental model, the distribution of values of a genetic parameter can explain the distribution of phenotypic states in a population, and thus the differences between one individual and another (see the section headed "Two Conceptions of Gene-Environment Interaction" above). But unlike an explanation in terms of which Mendelian alleles each individual possesses, an explanation that references the state of a developmental gene is a causal, developmental explanation which works by laying out the interaction of this specific genetic parameter with the system as a whole.

If we conceive of genes as parameters in a developmental model, then it will seem unsatisfactory to explain the presence of a phenotype or a phenotypic difference by alluding to the presence of a particular gene in the absence of any understanding of its role in development. With this conception of a gene, the fact that a gene has a specific phenotypic effect immediately raises the question why it has had that effect rather than other effects it might have had if other parameters were different, and thus directs attention to those other parameters.

Conversely, if we conceive of genes as Mendelian alleles, then it will seem unreasonable to demand knowledge about how a gene interacts with other genes and with the environment before accepting an explanation of a phenotypic difference which simply cites the presence of this allele. If the organism or organisms whose phenotypes are to be explained have been drawn from a suitable reference class, then the facts that caused the gene to be cited as an explanation imply that those other parameters will not make a difference. How, the traditional behavioral geneticist then asks, can they be explanatorily relevant?

Our suggestion is that claims by developmental scientists that "the mere presence of a gene" cannot in itself explain a phenotypic difference reflects their conception of the gene as something other than a Mendelian allele. If the explanation which is subject to this criticism is one that makes use of the traditional, Mendelian conception of the gene and which is strictly targeted at explaining a difference between two individuals in a specified population, then the criticism is unfair. Conversely, the claim that developmental parameters which do not account for any of the actual variance seen in a population are irrelevant to an explanation of trait differences seems to us to misunderstand the developmental conception of the gene and the nature of the explanations which feature genes so conceived. The "silent"' parameters are relevant because they confer on the other parameters the causal powers by virtue of which they account for some proportion of the variance. Thus, as in the previous two sections, questions about the epistemological relevance of possible but non-actual variation seem to underlie some of the disagreements between developmental scientists and behavioral geneticists.

Conclusion

Behavioral genetics has had a long and troubled relationship with other areas of developmental science. While there has been an undeniable political element to disputes between behavioral geneticists and developmental scientists, in this chapter we have tried to highlight some genuine intellectual issues that separate the disputants. We suggest that one reason people have become so ready to accuse one another of covert political motives in this arena is that behind some of the apparently merely semantic disagreements over the use of words like "interaction" are the genuine, conceptual issues highlighted above. Opponents who appear to be using terms ambiguously and refusing to accept what are intended to be clarifying distinctions, like that between statistical interaction and "interactionism," may instead be trying to use language in a way that reflects their own way of conceptualizing the subject. Unable to get their opponents to accept what seems to them an unarguable point, or to use a distinction that will clarify the debate as they see it, the competing discussants are reduced to seeking a non-intellectual explanation for their resistance. If our analysis is correct, it may be possible to take some of the heat out of these debates by elucidating the different starting points that lead workers from different traditions to see one another as misguided or confused on certain issues.

In this chapter we have examined three such issues: conceptions of norm of reaction, conceptions of gene-environment interaction, and conceptions of gene and gene-action. In the first case, reaction norm and reaction range, we rejected the criticism leveled by developmental psychobiologists against behavioral genetics that the concept of a reaction range implies the existence of fixed limits to phenotypic plasticity. But we argued that behavioral geneticists' preference for the concept of reaction range does reflect an emphasis on descriptive data about actual populations, an emphasis which developmental scientists have legitimate grounds to question. In the second case, biometric and developmental gene-environment interaction ($G \times E_B$ and $G \times E_D$), we rejected the criticism leveled by behavioral geneticists, that the introduction of a causal developmental sense of gene-environment interaction into discussions of individual differences is conceptually confused. We suggested that this sense of interaction can play a role in the explanation of individual differences, but only if attention is shifted from the actual variation seen in natural populations to the potential variation that can be revealed by experimental intervention. Third, and finally, we examined two ways in which genes can explain behavior and behavioral differences, one characteristic of explanations offered by behavioral geneticists and one characteristic of explanations offered by developmental scientists. We argued that interpreting an explanation of either kind with the conception of the gene characteristic of the other would rob that explanation of its force. We suggested that misinterpretations of this kind underlie some of the disputes between the two schools. In all three cases, we have

argued, the issue resolves to one of the relevance of potential variation that is not seen in actual populations but which could be produced by experimental intervention. This in turn reflects the different methods characteristic of these two scientific fields.

If correct, our diagnosis of the traditional hostility between behavioral genetics and other areas of developmental science gives grounds for considerable optimism about the future. Traditional quantitative genetic methods in behavioral genetics are rapidly giving way to molecular methods (Hamer, 2002; Kendler, 2005; Schaffner, 2006), and the effects of environmental interventions in developmental science are increasingly being analyzed at the level of gene expression (Meaney, 2004; Suomi, 2003). These developments suggest that we are on the brink of the emergence of a genuinely developmental behavior genetics that will meet the aspirations of both sides.[3]

Notes

1. For more recent uses of the defense-by-distinction, see Bouchard and Segal (1985, p. 393), Sesardic (2005, p. 49) and Surbey (1994, pp. 263–264).
2. These two ways of thinking have obvious similarities to Lenny Moss's (2003) distinction between Gene-P (statistical predictor of phenotype) and Gene-D (material gene with intrinsic template capacity). However, Moss contrasts a *concrete* Gene-D with an *abstract* Gene-P, and suggests that the phenotypic multi-potentiality of a Gene-D results from its being defined by its intrinsic nature and not by its contextually mediated effects as is Gene-P. In contrast, both the conceptualizations of the gene outlined here are abstractions from the molecular detail, and the phenotypic multi-potentiality of the "hypothetical developmental gene" results from the structure of the developmental system of which it is a part.
3. This chapter is a revised version of Griffiths and Tabery (2008), which first appeared in *New Ideas in Psychology*. Portions are reprinted with the permission of Elsevier.

References

Bouchard, T. J., & Segal, N. L. (1985). Environment and IQ. In B. B. Wolman (Ed.), *Handbook of intelligence: Theories, measurements, and applications* (pp. 391–464). New York: Wiley.

Box, J. F. (1978). *R.A. Fisher: The life of a scientist*. New York: Wiley.

Chase, A. (1980). *The legacy of Malthus: The social costs of the new scientific racism*. Urbana: University of Illinois Press.

Dewsbury, D. A. (1991). Psychobiology. *American Psychologist*, **46**, 198–205.

Dobzhansky, T. (1951). *Genetics and the origins of species* (3rd ed., Vol. **11**).New York: Columbia University Press.

Dobzhansky, T. (1955). *Evolution, genetics and man*. New York: Wiley.

Fisher, R. A. (1918). The correlation between relatives on the supposition of Mendelian inheritance. *Transactions of the Royal Society of Edinburgh*, **52**, 399–433.

Fisher, R. A. (1924). The biometrical study of heredity. *Eugenics Review*, **16**, 189–210.

Fisher, R. A. (1925). *Statistical methods for research workers*. Edinburgh: Oliver and Boyd Ltd.

Fisher, R. A. (1930). *The genetical theory of natural selection*. Oxford: Clarendon Press.

Ford, D. H., & Lerner, R. M. (1992). *Developmental systems theory: An integrative approach*. Newbury Park, CA: Sage.

Fuller, J. L., & Simmel, E. C. (1986). Trends in behavior genetics: 1960–1985. In J. L. Fuller & E. C. Simmel (Eds.), *Perspectives in behavior genetics* (pp. 1–27). Hillsdale, NJ: Erlbaum.

Fuller, J. L., & Thompson, W. R. (1960). *Behavior genetics*. New York: Wiley.

Gottesman, I. I. (1963). Genetic aspects of intelligent behavior. In N. R.Ellis (Ed.), *Handbook of mental deficiency* (pp. 253–296). New York: McGraw-Hill.

Gottlieb, G. (1992). *Individual development and evolution*. Oxford, New York: Oxford University Press.

Gottlieb, G. (1995). Some conceptual deficiencies in "developmental" behavior genetics. *Human Development*, **38**, 131–141.

Gottlieb, G. (2003). On making behavioral genetics truly developmental. *Human Development*, **46**(6), 337–355.

Griffiths, P. E., & Stotz, K. (2006). Genes in the Postgenomic era. *Theoretical Medicine and Bioethics*, **27**(6), 499–521.

Griffiths, P. E., & Stotz, K. (2007). Gene. In M. Ruse & D. Hull (Eds.), *Cambridge companion to philosophy of biology* (pp. 85–102). Cambridge: Cambridge University Press.

Griffiths, P. E., & Tabery, J. G. (2008). Behavioral genetics and development. *New Ideas in Psychology*: **26**:332–352.

Haldane, J. B. S. (1938). *Heredity and politics*. London: Allen and Unwin.

Haldane, J. B. S. (1946). The interaction of nature and nurture. *Annals of Eugenics*, **13**:197–205

Hamer, D. (2002). Rethinking behavior genetics. *Science*, **298**, 71–72.

Hinde, R. A. (1966). *Animal behaviour: A synthesis of ethology and comparative psychology*. New York: McGraw-Hill.

Hogben, L. (1932). *Genetic principles in medicine and social science*. New York: Alfred A. Knopf.

Hogben, L. (1933). *Nature and nurture: Being the William Withering memorial lectures*. London: George Allen and Unwin.

Hull, D. L. (1988). *Science as a process: An evolutionary account of the social and conceptual development of science*. Chicago: University of Chicago Press.

Jensen, A. R. (1973). *Educability and group differences*. New York: Harper and Row.

Johannsen, W. (1911). The genotype conception of heredity. *American Naturalist*, **45**(531), 129–159.

Kaplan, J. M. (2000). *The limits and lies of human genetic research*. New York: Routledge.

Kendler, K. S. (2005). Psychiatric genetics: A methodologic critique. *American Journal of Psychiatry*, **162**(1), 3–11.

Kevles, D. J. (1995). *In the name of eugenics: Genetics and the uses of human heredity* (2nd ed.). Cambridge, MA: Harvard University Press.

Lettice, L. A., Horikoshi, T., Heaney, S. J. H., van Baren, M. J., van der Linde, H. C., Breedveld, G. J., et al. (2002). Disruption of a long-range cis-acting regulator for Shh causes preaxial polydactyly. *Proceedings of the National Academy of Sciences*, **99**(11), 7548–7553.

Levin, M. (1997). *Why race matters: Race differences and what they mean*. Westport, CT: Praeger.

Mazumdar, P. M. H. (1992). *Eugenics, human genetics, and human failings: The Eugenics Society, its sources and its critics in Britain*. London: Routledge.

Meaney, M. J. (2001). Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annual Review Neuroscience*, **24**, 1161–1192.

Meaney, M. J. (2004). The nature of nurture: Maternal effects and chromatin remodelling. In J. T. Cacioppo & G. G. Berntson (Eds.), *Essays in social neuroscience* (pp. 1–14). Cambridge, MA: MIT Press.

Michel, G. F., & Moore, C. L. (1995). *Developmental psychobiology: An interdisciplinary science*. Cambridge, MA: MIT Press.

Moss, L. (2003). *What genes can't do*. Cambridge, MA: MIT Press.

Platt, S. A., & Sanislow, C. A., III (1988). Norm-of-reaction: Definition and misinterpretation of animal research. *Journal of Comparative Psychology*, **102**, 254–261.

Plomin, R. (1983). Introduction: Developmental behavioral genetics. *Child Development*, **54** (2), 253–259.

Plomin, R. (1990). *Nature and nurture: An introduction to human behavioral genetics*. Pacific Grove, CA: Brooks/Cole.

Plomin, R., DeFries, J. C., & Loehlin, J. (1977). Genotype-environment interaction and correlation in the analysis of human behavior. *Psychological Bulletin*, **84**, 309–322.

Plomin, R., & McClearn, G. E.(Eds.). (1993). *Nature, nurture, and psychology*. Washington, DC: American Psychological Association.

Provine, W. B. (2001). *The origins of theoretical population genetics* (2nd ed.).Chicago: University of Chicago Press.

Sarkar, S. (1999). From the reaktionsnorm to the adaptive norm: The norm of reaction, 1909–1960. *Biology and Philosophy*, **14**, 235–252.

Scarr, S. (1992). Developmental theories for the 1990s: Development and individual differences. *Child Development*, **63**(1), 1–19.

Scarr, S. (1993). Biological and cultural diversity: The legacy of Darwin for development. *Child Development*, **64**(5), 1333–1353.

Scarr, S. (1995). Commentary on Gottlieb's "Some conceptual deficiencies in 'developmental' behavior genetics". *Human Development*, **38**, 154–158.

Schaffner, K. F. (2006). Behaving: Its nature and nurture Part 2. In E. Parens, A. Chapman, & N. Press (Eds.), *Wrestling with behavioral genetics: Implications for understanding selves and society* (pp. 40–73). Baltimore, MD: Johns Hopkins University Press.

Sesardic, N. (2005). *Making sense of heritability*. Cambridge: Cambridge University Press.

Sinnott, E. W., Dunn, L. C., & Dobzhansky, T. (1950). *Principles of genetics*. New York: McGraw-Hill.

Suomi, S. J. (2003). How gene-environment interactions can influence emotional development in rhesus monkeys. In C. Garcia-Coll, E. L. Bearer, & R. M. Lerner (Eds.), *Nature and nurture: The complex interplay of genetic and environmental influences on human behavior and development*. Mahwah, NJ: Erlbaum.

Surbey, M. K. (1994). Discussion: Why expect a horse to fly?, reply to Wahlsten. *Canadian Psychology*, **35**(3), 261–264.

Tabery, J. (2004). The 'evolutionary synthesis' of George Udny Yule. *Journal of the History of Biology*, **37**, 73–107.

Tabery, J. (2007). Biometric and developmental gene-environment interactions: Looking back, moving forward. *Development and Psychopathology*, **19**, 961–976.

Tabery, J. (2008). R. A. Fisher, Lancelot Hogben, and the origin(s) of genotype-environment interaction. *Journal of the History of Biology*: **41**:717–761.

Tabery, J. (2009a). Difference mechanisms: Explaining variation with mechanisms. *Biology and Philosophy*, **24**(5), 645–664.

Tabery, J. (2009b). Making sense of the nature-nurture debate (Review of Neven Sesardic's *Making sense of heritability*). *Biology and Philosophy*, **24**(5), 711–723.

Tinbergen, N. (1963). On the aims and methods of ethology. *Zietschrift für Tierpsychologie*, **20**, 410–433.

Turkheimer, E., Goldsmith, H. H., & Gottesman, I. I. (1995). Commentary. *Human Development*, **38**, 142–153.

Waddington, C. H. (1957). *The Strategy of the Genes: A Discussion of Some Aspects of Theoretical Biology*. London: George Allen and Unwin.

Wahlsten, D. (2000). Analysis of variance in the service of interactionism. *Human Development*, **43**(1), 46.

Waters, C. K. (2004). What was classical genetics? *Studies in History and Philosophy of Science*, **35**(4), 783–809.

Weaver, I. C. G., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., et al. (2004). Epigenetic programming by maternal behavior. *Nature Neuroscience*, **7**(8), 847–854.

Weaver, I. C., Champagne, F. A., Brown, S. E., Dymov, S., Sharma, S., Meaney, M. J., et al. (2005). Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: Altering epigenetic marking later in life. *Journal of Neuroscience*, **25**(47), 11045–11054.

Weber, M. (2004). *Philosophy of experimental biology*. Cambridge, New York: Cambridge University Press.

Whitney, G. (1990). A contextual history of behavior genetics. In M. E. Hahn, J. K. Hewitt, N. D. Henderson & R. H. Benno (Eds.), *Developmental behavior genetics: Neural, biometrical, and evolutionary approaches* (pp. 7–24). New York: Oxford University Press.

Woltereck, R. (1909). Weitere Experimentelle Untersuchungen uber Artveranderung, speziel uber das Wesen Quantitativer Artunterschiede bei Daphnien. *Verhandlungen der Deutschen Zoologischen Gesellschaft*, **19**, 110–173.

4

Development and Evolution Revisited

Mae Wan Ho

In the new holistic perspective, epigenetics mediates between the psychosocial and biological realms, and holds the key to how we can shape our own development and future evolution.

The Grandparent Effect and Epigenetic Inheritance

The experience of young boys could affect not just their own health in later life, but also the health of their sons and grandsons. The finding, published in the *European Journal of Human Genetics* (Pembrey et al., 2006), was accompanied by a Commentary, "Sins of the Fathers, and Their Fathers" (Whitelaw, 2006).

Two years later, it was featured in *Prospect Magazine* in an article entitled "What Genes Remember" (Hunter, 2008), in which the author stated: "Many geneticists now think that the behaviour of our genes can be altered by experience – and even that these changes can be passed on to future generations. This finding may transform our understanding of inheritance and evolution."

The finding departs from well-known and generally accepted environmental effects on the unborn fetus in the mother's womb or other maternal effects, mediated by the many provisions in the egg cell during embryogenesis, and after birth, in mother's milk. Influences passed on through the *paternal* line, however, are associated with sperm cells that contain very little apart from the father's genes.

Apparently, the paternal grandfathers' food supply during mid childhood was linked to the risk of death in grandsons, but not in grand-daughters. Poor availability of food was associated with *reduced* risk of death in grandsons by

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35% while good availability of food was associated with increased risk of death by 67% compared with controls. In contrast, the nutritional status of the paternal grandmother had no influence on the grandsons but affected the granddaughters instead in a similar way. Good food availability increased the risk of death for granddaughters by 113%, while poor food availability decreased the risk of death by 49%.

The results imply that experience during a crucial period of an individual's life could influence more than one generation, and in a sex-specific way. Although the mechanisms behind such "grandparent effects" in humans are not yet known, they are being taken more seriously because similar cases of "epigenetic inheritance" have been documented in a substantial number of animal studies (Ho, 2009a). They are instances of Lamarckian inheritance of acquired characters that still get many biologists hot under the collar.

"Epigenetic" Then and Now

The term "epigenetic" as used today, generally refers to effects that do not involve DNA base sequence changes, but only the chemical modifications of DNA, or histone proteins in chromatin (complex of DNA and protein that make up chromosomes in the nucleus of cells), which alter gene expression states. Epigenetic inheritance has been defined (Bird, 2007) as "the structural adaptation of chromosomal regions so as to register signal or perpetuate altered activity states." But such definitions are rapidly becoming obsolete. In reality, epigenetic modifications encompass a great variety of mechanisms. They act during transcription, after transcription, and at translation of genetic messages; and can even rewrite genomic DNA (Ho, 2009a). Hence the distinction between genetic and epigenetic is increasingly blurred.

"Epigenetic" as originally used, was derived from *epigenesis*, the theory that organisms are not *preformed* in the germ cells, but come into being through a process of development in which the environment plays a formative role. It will become clear that most evolutionists have used epigenetic to mean both development *and* hereditary influences arising from environmental effects.

I confess to having very mixed feelings about the recent rise of epigenetics. Over 30 years ago, I published a paper with Peter Saunders entitled, "Beyond Neo-Darwinism, An Epigenetic Approach to Evolution" (Ho & Saunders, 1979), and became instantly tagged a "neo-Lamarckian." To get that paper in print, we had to wrangle with John Maynard-Smith (1920–2004), the then ruling neo-Darwinian, who later wrote a very hostile review of a volume we edited, *Beyond Neo-Darwinism: Introduction to a New Paradigm for Evolution* (Ho & Saunders, 1984). That book was also condemned by Ernst Mayr (1904–2005), grand doyen of neo-Darwinism. Mayr (1984) wrote a two-page tirade in the *Times Literary Supplement*

to put the record straight on his own role as "architect of the grand neo-Darwinian synthesis." For decades after, we were treated like pariahs by the neo-Darwinian establishment, as we continued to work towards the "new paradigm." At some point, a couple we met recalled how as students, they only dared read our papers "at night, under the blanket," and certainly would never admit to having done so.

This is a time for celebration, not so much a vindication of individuals ahead of their time, as a triumph of reason over obfuscation, of free, disinterested thinking over suppression. It is also the occasion to revisit and throw further light on those perennial issues that had preoccupied us.

Gilbert Gottlieb's Legacy

I dedicate this paper to the memory of Gilbert Gottlieb (1929–2006), who was an inspiration and role model. He was one of the earliest proponents of the epigenetic approach in animal behavior (see Greenberg, 2007); and had never allowed orthodox opinion to cloud his vision. He devised the most brilliant and sensitive experiments to test his hypotheses, taking great care not to inflict suffering on the animals he loved, always insisting on observing them as nearly as possible in their natural state. And he was tireless and brilliant in engaging critics throughout his life.

In an interview conducted by ex-student David Miller (2006), Professor of Psychology at the University of Connecticut, Gottlieb described the two people who influenced him the most. The first was Kuo Zing-Yan, a Chinese scientist who studied at the University of California Berkeley in the 1920s, had gone back to China, and became the head of several universities. Kuo was known for not accepting the idea of instinct as an inborn (genetically determined) fixed pattern of behavior characteristic of a species that only needed to be "released" by an environmental stimulus. Instead, he thought that all behavior *develops*. Gottlieb invited Kuo to work with him in his lab in Dorothea Dix Hospital in Raleigh, North Carolina. Together, they studied the perinatal development of ducks through a transparent window made on the egg shell.

The other person who influenced Gottlieb was T. C. Schneirla, who established the school of comparative psychology at the American Museum of Natural History in New York, and became Gottlieb's friend and mentor.

Towards the end of the interview, Gottlieb said with characteristic modesty: "I find that getting across the developmental point of view [as opposed to genetic determinist one] has been the largest failure of my career."

So, let's try to get the developmental (epigenetic) point of view across, just once more, beginning with a brief history of significant ideas and findings to take us to the present day.

Lamarck's Theory of Evolution

Evolution refers to the natural (as opposed to supernatural) origin and transformation of organisms on earth throughout geological history to the present day. There has been much speculation on evolution since historical records began, but the ideas that have come down to us originate in the European Enlightenment (see Wright, 1964). This period saw the beginning of Newtonian mechanics, mathematics and other modern scientific developments, including John Ray's concept of species and Carl Linnaeus´ system for classifying organisms. The power of rational thought to explain the material universe presented a deep challenge to received wisdom, especially the biblical account of creation according to the Christian Church. Evolution by natural processes, as opposed to special creation by God, was already on the mind of most educated people. Linnaeus came to accept a limited transformation of species later in his life; other prominent figures who wrote on evolution include the naturalist, G. L. Buffon and Charles Darwin's grandfather, Erasmus Darwin.

The first *comprehensive* theory of evolution was due to Jean Baptiste de Lamarck (1809). Lamarck (1744–1829) was also credited with having invented the discipline of biology. He was very much a product of the Enlightenment, both in his determination to offer a naturalistic explanation of evolution and in his systems approach (Barthelemy-Madaule, 1982; Burkhardt, 1977). Thus, he dealt at length with physics, chemistry, and geology before embarking on presenting evidence that biological evolution has occurred. And although "transmutation" was in the air, associated with Maupertuis, Buffon, Geoffroy St. Hilaire and others, it was generally thought to be limited in scope to within permanent types. Moreover, the evidence then accumulating on successive waves of extinction in the fossil record gave considerable support to Cuvier's "catastrophism," the idea that sudden worldwide catastrophes caused species extinction, which was consistent both with the fixity of species and with special creation. It was against this background that Lamarck postulated a uniformitarian theory (that causes operating in the past are the same as those that can be observed operating at present) based on extended time, the spontaneous generation of the living from the nonliving, and unlimited transmutation which gave rise to whole kingdoms of organisms beginning from a single origin of life. Lamarck had effectively transferred the power of creation from God to nature.

In addition, Lamarck proposed special mechanisms whereby new species could arise through changes in the relationship between the organism and its environment in the *pursuance of its basic needs*, which produce new characteristics that become inherited after many successive generations. These special mechanisms are "use and disuse." Use enhances and reinforces the development of the organs or tissues while disuse results in atrophy; and the "inheritance of acquired characters,"

the transmission to subsequent generations the tendency to develop certain new characteristics that the organism has acquired in its own development.

These proposals are all of a piece with Lamark's naturalistic, uniformitarian approach (Ho, 1984a). In a world without God, there is but reason; without divine intervention, there is but mechanical causation; and without ideal types, the only recourse is to search for that particular "order of things," the physical chemical forces responsible for the origin and evolution of life. *That* was Lamarck's project in which teleology (or Darwinian "selective advantage") is explicitly excluded. As consistent with his philosophy, Lamarck never used the term "adaptation" in his writings, even though he was referring to the phenomenon recognized as such by the school of Natural Theology, and hence also by Darwin and neo-Darwinians, the fit between biological form and function.

Lamarck was also responsible for the first epigenetic theory of evolution, in which development plays a key role in initiating the evolutionary change while specific epigenetic mechanisms transmit the change and reinforce it in subsequent generations (see Ho, 1983, 1984a, 1984b). The theory was incomplete, however, in not addressing the origin of biological form.

Lamarck's theory had been widely misrepresented as merely the inheritance of acquired characters, or caricatured as changes resulting from the "wish fulfillment" of the organism. Half a century later, Charles Darwin (1809–1882) was to include Lamarck's mechanisms as adjuncts to his own theory of evolution by natural selection.

Darwin and the Neo-Darwinian Synthesis

Contrary to popular confusion, natural selection is *not* evolution. Charles Darwin's theory is *not* that evolution had occurred; it is a (special) theory of a mechanism for evolution – natural selection. Rejecting natural selection as an explanation of evolution does not make one an anti-evolutionist or creationist. Lamarck, not Darwin, was responsible for the general theory that evolution had occurred; and neither Lamarck nor Darwin's grandfather Erasmus Darwin (1731–1802) who anticipated some of Lamarck's ideas, was a creationist, nor were the many critics of Darwin such as Samuel Butler (1835–1902) and Mivart St. George (1827–1900).

Darwin's (1859) theory of evolution by natural selection states that, given the organisms' capability to reproduce more of their numbers than the environment can support, and there are variations that can be inherited, then, within a population, individuals with the more favorable variations would survive to reproduce their kind at the expense of those with less favorable variations. The ensuing competition and "struggle for life" result in the "survival of the fittest," so the species will become better adapted to its environment. And if the environment itself changes in time there will be a gradual but definite "transmutation" of species.

Thus, nature effectively "selects" the fittest in the same way that artificial selection by plant and animal breeders ensures that the best or the most desirable characters are bred and preserved. In both cases, new varieties are created after some generations.

In *addition* to natural selection, Darwin invoked the effects of use and disuse, and the inheritance of acquired characters in the transmutation of species. It is clear, however, that those Lamarckian ideas do not fit into the theory of natural selection, and Darwin's followers all regard the lack of a theory of heredity and variation as the weakest link in the argument for natural selection. When Mendelian genetics was rediscovered at the turn of the past century and August Weismann (1834–1914) identified the material basis of heredity as the "germplasm" in germ cells that become separate from the rest of the animal's body in the course of early development, it seemed to offer a perfect explanation of how Mendelian genes could be passed on unchanged from one generation to the next. Darwinism was promptly reinterpreted according to the gene theory in the "neo-Darwinian synthesis" from the 1930s up to the 1950s and 1960s. This coincided with an extremely productive and exciting period in the history of biology as the gene theory itself continued to inspire a series of discoveries that culminated in the DNA double helix and the genetic code (see Ho, 1998).

The neo-Darwinian synthesis began with the mathematical representation of genes in populations and in plant breeding (biometrical genetics), which, together provided a rigorous theory of Darwinian natural selection in terms of genes for both discontinuous and continuously varying characters. Systematics and paleontology for their part, defined phylogenetic relationships and "adaptive radiations" of the major groups in accordance with Darwin's dictum of "descent with modification." At the same time, the detailed study of chromosomes together with mutational and other cytogenetic analyses eventually clarified the molecular basis of Mendelian genes, which are located to linear arrays on chromosomes. Heritable variations are generated by random mutations in these genes, different forms (alleles) of which are subject to natural selection via the different characters they determine, As the genes, according to Weismann, are insulated from environmental influences, they are passed on unchanged to the next generation, except for rare random mutations.

With the identification of DNA as the genetic material and the cracking of the genetic code in the 1950s and 1960s, the "Central Dogma" of molecular biology, propounded by Nobel Laureate Francis Crick (1916–2004), came to be accepted by most biologists. It states that the sequence of bases in each DNA is faithfully transcribed into RNA, and the RNA translated into a specific sequence of amino-acids of a protein in a one-way information flow; and no reverse information-flow is possible. This strengthened the "Weismann's barrier," supposed to strictly forbid environmental influences, or any experience in the life-time of the organism to directly, that is, predictably, affect its genes. In the new orthodoxy which reigned over the next 20 years, the organism tended to be seen as no more than a collection

of genes, its development, the unfolding of a "genetic program" encoded in the genome. Random mutations give rise to mutant characters and natural selection allows the fittest mutants to survive and reproduce. Environmental changes give new selective forces and evolution is thereby guaranteed.

Richard Dawkins (1976) pushed the neo-Darwinian theory to its logical conclusion in proposing that organisms are automatons controlled by "selfish genes," whose only imperative is to replicate at the expense of other "selfish genes." E. O. Wilson (1975) extended neo-Darwinian theory to animal and human societies to create the discipline of sociobiology, which posed the "paradoxical" question (i.e., paradoxical *within* neo-Darwinism): how could altruistic behavior evolve (given that genes and the behavior they control are fundamentally selfish)?

This paradox disappears, of course, when one rejects the ungrounded assumption, as we do, that selfishness or competitiveness is fundamental to the living world. Animals engage in competitive or aggressive acts, but that does not mean there are inherent qualities of competitiveness and aggressiveness, which can account for those acts. This assumption is behind the fruitless search for genes "predisposing" individuals to aggressive behavior, while copious evidence accumulated that environmental influences during childhood play the major determining role (see Ho, 2009b).

Examples of cooperation among animals far outstrip those of competition. Kropotkin (1914) gave abundant examples of the natural sociality of all animals, independent of genetic relatedness. Thus, one could invert E. O. Wilson's question and ask, why do animals compete, given their natural sociality? This highlights the socio-political underpinnings of all scientific theories. Darwinism is all of a piece with the Victorian English society preoccupied with competition and the free market, with capitalist and imperialist exploitation (see Ho, 1998 for further discussions).

Evolution, Development, and Heredity

The theories of evolution, development, and heredity are closely intertwined. Just as evolutionists needed a theory of heredity, so plant breeders in the 18th century, who inspired Mendel's discovery of genetics, were motivated by the question as to whether new species could evolve from existing ones. In accounting for change or transformation, it is also necessary to locate where constancy or stability resides, which constitutes heredity. But in order to explain the evolution of form and function, development (epigenesist) is central, as Lamarck clearly saw. In contrast, Darwin, and neo-Darwinists saw new variations arising at *random* in the sense that they bear no direct relationship to the environment, those that happen to be adaptive are selected, while the rest are eliminated. The theory of natural selection is essentially preformist, development playing little or no role in determining evolutionary change.

History has the habit of creating heroes and anti-heroes, and so Darwin triumphed while Lamarck bore the brunt of ridicule and obscurity. The main reason is that their theories are *logically* diametrically opposed. Darwin's theory is natural *selection*, and selection entails a separation of the organism from its environment. The organism is conceptually closed off from its experience, leading logically to Weismann's *barrier* and the Central Dogma of the genetic paradigm, which is reductionist in intent and in actuality. Lamarck's theory, on the other hand, is of *transformation* arising from the organism's own experience of the environment. It *requires* a conception of the organism as *open* to the environment, which it is, and invites us to examine the dynamics of transformation, as well as mechanisms whereby the transformation could become "internalized." Hence it leads logically to the epigenetic approach, which embraces the same holistic, systems thinking that Lamarck exemplified (Burkhardt, 1977).

The Genetic Paradigm and Neo-Darwinism

Neo-Darwinism is a theory based on genes, real or more often the case, fictitious. G. C. Williams (1966) stated explicitly (p. 4): "In explaining adaptation, one should assume the adequacy of the simplest form of natural selection, that of alternative alleles in Mendelian populations." Natural selection of alternative alleles can only be a valid description of reality when the following abstractions of the genetic paradigm are assumed to be true: (a) genes determine characters in a straightfor ward, additive way; (b) they are stable and, except for rare random mutations, are passed on unchanged to the next generation; and (c) there is no feedback from the environment to the organism's genes. All three assumptions have been falsified since the 1980s, if not before.

Assumption (a) was known to be false since the beginning of the neo-Darwinian synthesis, and to some of the most prominent "architects" of the grand synthesis such as Sewell Wright (Wright, 1969, 1978) and Ernst Mayr (1963). Wright argued that *selection relates to the organism as a whole, or to the social group, not to single genes except as a net resultant*. He saw that the major source of variability is in the recombination of already existing genes into a great number of different genotypes, many of which would occupy equivalent "adaptive peaks" in a "fitness landscape." Mayr, for his part, insisted that natural selection acts on "co-adapted gene complexes," and remained highly critical of "beanbag [population] genetics" such as that of R. A. Fisher (1930) and J. B. S. Haldane (1932), which dealt with selection of single genes. However, that still left both the "fitness landscape" and the "co-adaptive gene complex" undefined, and with little impact on the study of evolution in the mainstream, where it has been customary to identify a character, then assume there is a hypothetical gene (or set of genes) responsible for it, which may be selected in isolation from everything else (Ho, 2003).

Many critics pointed out that the mapping between genes and the organisms' characters (phenotype) in development is nonlinear and non-additive (see later), as it would already be if one were to take Wright and Mayr seriously. But "beanbag" genetics remained the explicit and implicit basis of all neo-Darwinian just-so stories in sociobiology that later became the hybrid discipline of "evolutionary psychology" (see Ho, 2009b), and continued its pernicious speculations to this day, when molecular geneticists have spectacularly failed to find gene variants predisposing individuals to even the most common diseases, let alone behavior (Ho, 2009c).

Assumptions (b) and (c) effectively isolated the organism from the environment, which therefore assumes the role solely as "selector." Of course, most people accepted that the environment also *interacts* with the organism, causing changes in its characteristics. However, it is supposed that the environment as "interactor" can be neatly separated from the environment that selects, for so long as the germline genes are stable, and do not change with the environment; and is protected from the rest of the body by "Weismann's barrier," it is irrelevant how the rest of the body is affected. This fits neatly with the separation between "instinctive" and "learned" behavior in ethology (see later). As only the genes are passed on in evolution, it also means that evolution is separate from development. Maynard Smith and Holliday (1979) have indeed declared that the gift of Weismannism to evolutionary (i.e., neo-Darwinian) theory is that "development can be safely ignored." Those assumptions had become untenable almost as soon as genetic engineering began in the mid-1970s.

Genes are not at all stable and unchanging, but fluid and dynamic, and in constant traffic with the environment. Feedback from the environment not only determines the moment to moment expression of particular genes in individual cells; it can end up rewriting the genes themselves, in violation of the Central Dogma. I shall go into more details later.

The Revival of Epigenetic Approaches Since Darwin and the Neo-Darwinian Synthesis

There are a number of different epigenetic theories of evolution since Lamarck; some predating the neo-Darwinian synthesis. One common starting point for all epigenetic theories is the developmental flexibility of all organisms. In particular, it has been observed that artificially induced developmental modifications often resemble (*phenocopy*) those existing naturally in related geographical races or species, that appear to be genetically determined. Thus, it seemed reasonable to assume that evolutionary novelties first arose as developmental modifications, which somehow became stably inherited (or not, as the case may be) in subsequent generations.

An early proponent of an epigenetic theory was James Mark Baldwin (1896), who suggested that modifications arising in organisms developing in a new environment produce "organic selection" forces internal to the organism, which act to stabilize the modification in subsequent generations. Another notable figure was Richard Goldschmidt (1940), who questioned the orthodox neo-Darwinian account that new species originate as the result of the accumulation, by natural selection, of small single gene effects over geological time, for he saw abundant evidence of "unbridgeable [genetic] gaps" between natural species. He proposed, therefore, that evolutionary novelties arise from time to time through *macromutations*, producing "hopeful monsters" that can initiate new species. In his defense, he was at pains to point out that monsters could be hopeful because of the inherent *organization* of the biological system that tends to "make sense" of the mutation. But, "macromutations" (involving changes in the DNA) that generate such hopeful monsters are hopelessly rare.

Søren Løvtrup (1974) advocated a similar theory of evolutionary novelties by macromutations for the origin of major taxonomic groups of organisms at the level of phyla. Theories invoking macromutations also suffer from the difficulty that major taxonomic groups have a habit of appearing suddenly in clusters, rather than being isolated at different geological times.

The extraordinarily rich fossil finds of the Cambrian "explosion" responsible for most of the major animal phyla have provided abundant evidence that evolution occurs in bursts of "adaptive radiation" followed by relatively long periods of stasis (Eldredge & Gould, 1972). And evolution does seem to proceed top-down, from phyla, to subphyla, classes, orders and so on (Valentine, 2004), rather than the converse, as Darwin and neo-Darwinian natural selection of small random mutations would predict. This suggests that "adaptive radiations" involve major novelties resulting from epigenetic reorganization from large environmental changes, which also seem to be associated with adaptive radiations.

There has been a revival of interest in studying development in an evolutionary framework ("evo-devo") (Blumberg, 2009; Brakefield, 2006; Carroll, 2005; Coyne, 2009; Gilbert, 2003). The field is still dominated by the idea that "genes control development" (Coyne, 2009), and large evolutionary changes in body pattern are the result of changes in gene regulation due to natural selection. There is no recognition that the *patterns* themselves, and biological *form* need to be explained in their own right, independently of whether natural selection operates or not, and independently of the action of specific genes (Ho & Saunders, 1979; Saunders, 1984). In a brilliant critique of the genetic determinist approach to behavior, Gottlieb (1998) also deconstructed the idea that genes determine body pattern by pointing to the very different patterns of *Hox* genes expression in the fruitfly, centipede, and Onychophora. *Hox* (homeotic) genes are supposed to control segmental patterning during development; instead, the same genes appear to be simply responding to different patterning processes in the different animals. There is decidedly no homology of genes corresponding to homology of biological structures.

One important motivation for focusing on development is that developmental changes are far from random or arbitrary (Ho & Saunders, 1979, 1982, 1984; Webster & Goodwin, 1982), and independent of specific genes.

Waddington's Theory of Canalization and Genetic Assimilation

The most influential figure among the "epigenetic evolutionists" was Conrad H. Waddington (1905–1975), who attempted to accommodate "pseudo-Lamarckian" phenomena within neo-Darwinism in his theory of genetic assimilation. Like all Darwinian and neo-Darwinian evolutionists, he wanted to explain the origin of *adaptive* characters, that is, characters that seem to fit the functions they serve.

Waddington (1957) conceptualized the flexibility and plasticity of development, as well as its capacity for regulating against disturbances, in his famous "epigenetic landscape," a general metaphor for the non-linear dynamics of the developmental process (Saunders, 1990). The developmental paths of tissues and cells are seen to be constrained or *canalized* to "flow" along certain valleys due to the "pull" or force exerted on the landscape by the various gene products that define the fluid topography of the landscape (Figure 4.1). Thus, certain paths along valley floors will branch off from one another to be separated by hills (thresholds) so that different developmental results (alternative attractors) can be reached from the same starting point. However, some branches may rejoin further on, so that different paths will nevertheless lead to the same developmental result. Genetic or environmental disturbances tend to "push" development from its normal pathway

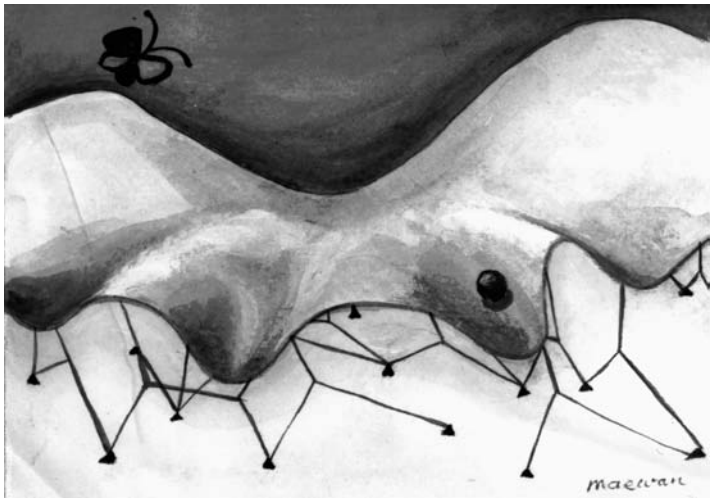

Figure 4.1. Waddington's epigenetic landscape.

across the threshold to another pathway. Alternatively, other valleys (developmental pathways) or hills (thresholds) may be formed due to changes in the topography of the epigenetic landscape itself.

The significance of the conceptual epigenetic landscape is that its topography is determined by *all* of the genes whose actions are inextricably interlinked, and is not immediately dependent on specific alleles of particular genes (Ho & Saunders, 1979). This is in accord with what we know about metabolism and the epigenetic system, particularly as revealed by the new genetics (see later). This also effectively decouples the evolution of the organism, of form and function, from alleles of specific genes, and explains the notable lack of correlation between morphological and genetic differences between species.

Waddington proposed that a new phenotype arises when the environment changes so that development proceeds to a new pathway in the epigenetic landscape, or else a remodeling of the epigenetic landscape itself takes place (both of which are possible from what we now know about epigenetic processes at the molecular level). Thereafter, the new phenotype becomes reinforced or "canalized," so that a more or less uniform phenotype results from a range of environmental stimuli, and later, the phenotype is "genetically assimilated," so it occurs in the absence of the original environmental stimulus.

Waddington was not specific as to the mechanisms involved in either canalization or genetic assimilation, except to argue that because they are advantageous (adaptive) there would be selection for the new phenotype presumably through appropriate "modifier" genes, that is, genes which modify the expression of the character (or the topography of the epigenetic landscape). He and colleagues carried out experiments showing that artificial selection for the bithorax phenocopy in *Drosophila* induced by ether exposure during early embryogenesis resulted in canalization and genetic assimilation.

Ho and Saunders' Epigenetic Theory of Evolution

The first distinctive feature of our epigenetic theory of evolution (Ho & Saunders, 1979, 1982, 1984) is that neo-Darwinian natural selection plays little or no role, based on evidence suggesting on the one hand that most genetic changes are irrelevant to the evolution of organisms, and on the other, that a relative *lack* of natural selection may be the prerequisite for major evolutionary change.

The second feature is that the intrinsic dynamics of the epigenetic system are determined not so much by gene interactions as by *physical and chemical forces and flows* of nonlinear complex systems in general, which are amenable to mathematical description (Saunders, 1984, 1992). That is why, contrary to the neo-Darwinian view, variations of the phenotype that arise during development in response to new environments are *non-random* and *repeatable*.

We proposed, therefore, that the intrinsic dynamical structure of the epigenetic system is the source of non-random variations that *direct* evolutionary change in the face of new environmental challenges. These evolutionary novelties are reinforced (canalized) in subsequent generations through cytoplasmic/epigenetic mechanisms, *independently of natural selection*.

When a population of organisms experience a new environment, *or adopt a new behavior*, the following sequence of events is envisaged.

1. A novel response arises during development; this could occur in *a large proportion, if not all of* the organisms in a population that are exposed to a new environmental stimulus. In the case of a new behavior initiated by a single individual in a social group, the behavior can also spread quite rapidly. (Kawai (1962) found that the new habit of washing sweet potatoes in the sea initiated by a young female had spread to the entire troop of wild monkeys on Koshima Island in Japan within 9 years.)
2. This response is "canalized," that is, deepened in intensity in successive generations exposed to the same environmental stimulus, and becomes regulated so that a more or less uniform response results from a range of intensity of the environmental stimulus. As distinct from Waddington's proposal that canalization is due to natural selection, it is independent of natural selection in our scheme, and this has been demonstrated in experiments in our laboratory subsequently (see later).
3. After some generations, the response *may* become "genetically assimilated," in that it arises even in the absence of the stimulus. As in Waddington's epigenetic landscape, this could entail a change in the topography to bias the original branch point in favor of the new pathway, so that the new phenotype will persist in the absence of the environmental stimulus. Random genetic mutations could be also be involved.

Since this theory was proposed, we have obtained important empirical and theoretical corroboration. We questioned Waddington's assumption that selection of (modifier) genes is necessary for canalization and genetic assimilation, and in a series of experiments, Ho, Tucker, Keeley, and Saunders (1983) demonstrated that canalization occurred in the *absence* of selection *for* the new character. We showed that successive generations of ether treatment during early embryonic development in *Drosophila* increased the frequency of the bithorax phenocopy in the adults, without selecting *for* the phenocopy. If anything, the phenocopy was almost certainly selected *against*, as it obviously interfered with flight and other normal functions. We had identified a case of 2epigenetic inheritance" of a maladaptive character, consistent with recent findings in "epigenetic toxicology," in which toxic effects of exposure to environmental pollutants are transmitted to grandchildren (Ho, 2009d).

We stipulated that genetic assimilation is not a necessary part of the response to change (Ho & Saunders, 1979), as it would preserve the important property of

developmental flexibility or "adaptability." In retrospect, this has proved correct. We now know that maternal behavior, long regarded as genetically inherited and instinctive, is actually associated with epigenetic gene markings that are erased at every generation, yet perpetuated indefinitely from mother to daughter (Ho, 2009b) (see later).

The complex nonlinear dynamics of the developmental process have been explored mathematically in greater detail (Saunders, 1984), and the evolutionary consequences made explicit (Saunders, 1992). For example, it accounts for "punctuated equilibria" (Eldredge & Gould, 1972), the observation in the fossil record of evolutionary stasis over long geological periods punctuated by the sudden appearance of new species or of rapid morphological change. It also shows how large organized changes can occur with a relatively small disturbance, or how continuously varying environmental parameters can nevertheless precipitate discontinuous phenotypic change.

As Saunders (1990) stated:

> From an evolutionary point of view, the most significant property of the epigenetic system is almost certainly its stability against both environmental perturbation and mutations. . .. Major changes might occur by long sequences of small ones. . . but the complicated interconnections [between genes] make this difficult to achieve. . .. On the other hand, a relatively small change in the landscape, if it occurs near a bifurcation point where one valley splits into two, could divert [development] down a different valley, and so to a very different end position than before. . .. In evolutionary terms, the first possibility corresponds to microevolution, and the second to macroevolution. The model suggests that. . . large evolutionary changes are not usually the result of long sequences of small ones. . .. Large changes are more likely to occur fairly rapidly, when a system is diverted into a new development pathway. This implies that we should expect to observe in evolution long periods of time in which only minor changes takes place. Occasionally, however, major changes could occur, often not related in any obvious way to the sequence of minor ones. Thus the model of evolution suggested by the epigenetic landscape is precisely that of punctuated equilibria. . .

The physical and chemical forces and flows that organize living systems were the subject of a book, *The Rainbow and the Worm: The Physics of Organisms* (Ho, 1993, 1998, 2008), now in its 3rd, much enlarged, edition, though it has been almost completely ignored by developmental biologists or evolutionists alike.

At least one study of the fossil record (Palmer, 2004) provided evidence that left-right asymmetry in animals and plants may have originated as phenotypic novelties that became genetically assimilated subsequently.

In our theory, natural selection plays little or no role in evolution (except in the negative sense of eliminating deleterious mutations with large effects) for the following reasons:

1. The epigenetic (developmental and non-genetic) novelties produced in response to new environments are common to most, if not all, individuals in a population, and would swamp out residual effects due to genetic variation.
2. The fluidity of the genome – the constant interaction between genome and environment, the epigenetic markings of genes, and the blurring between genetic and epigenetic – makes clear that organism and environment are inseparable; hence there can be no selection of any static, preformed variant that's independent, or random, with respect to the selective environment.
3. The physical and chemical forces and flows that *generate* biological patterns and forms are independent of natural selection, and require their own explanations.

Neo-Darwinists seem unable to recognize the logical incoherence of applying natural selection to organisms that are changed and changing in non-random ways under the selective regime. Nor do they accept that the generative dynamical forces, which both create and constrain biological patterns and forms, are *independent* of natural selection, relegating natural selection to a negative role of eliminating the unfit.

Instead, they insist that the generative dynamics only provide "developmental constraints" that limit the action of natural selection to some extent, but natural selection still plays the "creative" role in evolution (see Bonner, 1982).

I shall show why the dynamics that generate patterns and forms are much more than weak "developmental constraints" to natural selection; and then address the "neutral mutation hypothesis," the proposal that most, if not all, DNA base changes during evolution are due to random genetic drift decoupled from the evolution of organisms

Rational Taxonomy Based on the Generative Dynamics of Biological Form

The dynamics of developmental (epigenetic) processes, being amenable to mathematical description, provides a powerful perspective for understanding both the development and the evolution of form. That is the basis of "structuralism in biology" (Goodwin, Webster, & Sibatani, 1989, Webster & Goodwin, 1982); or more accurately in our view, "process structuralism" (Ho, 1984a, 1988a; Ho & Saunders, 1984; Saunders, 1984, 1989, 1992).

The developmental dynamics define a set of possible transformations that is highly constrained, so that particular transformations may be *predictably* linked to specific environmental stimuli. This provides a "rational taxonomy" of biological forms and a natural system of classification explicitly based on the dynamics of processes that generate the forms. I shall describe two examples, the first on segmentation defects in

Drosophila larva produced by exposing early embryos to ether vapor. The second deals with phyllotaxis, the arrangement of leaves around the stem.

The segmentation pattern of the first instar *Drosophila* larva is determined during early embryogenesis, when brief exposures to ether vapor produced characteristic defects in the segmental pattern reflecting a dynamic process arrested at different stages (Ho, Matheson, Saunders, Goodwin, & Smallcombe, 1987). We showed that a model of successive bifurcation was capable of producing all the observed defects as well as predict the existence of ones not yet observed (Ho, 1990).

Figure 4.2 (Ho, 1990) is a transformational "tree" of the range of segmental patterns obtained *during development*. The main sequence, going up the trunk of the tree, is the normal transformational pathway, which progressively divides up the body into domains, ending up with 16 body segments of the normal larva. All the rest (with solid outlines) are transformations in which the process of dividing up the body has been arrested at different positions in the body. The patterns with dotted outlines are hypothetical forms, not yet observed, connecting actual transformations.

This transformational tree reveals how different forms are related to one another; how superficially similar forms are far apart on the tree, while forms that look most different are neighbors. The transformational tree represents the forms that can be obtained during development (ontogeny), according to a model of successive bifurcation depicted in Figure 4.3

More importantly, the ontogenetic transformation tree also predicts the possible forms that can be obtained in evolution (phylogeny), mostly likely by going up the sequence of successive bifurcations, but occasional reversals to simpler forms could also take place. This is why phylogeny appears to recapitulate ontogeny (Gould, 1977). Though in actuality, it does not; ontogeny and phylogeny are simply related through the dynamics of the generic processes generating form.

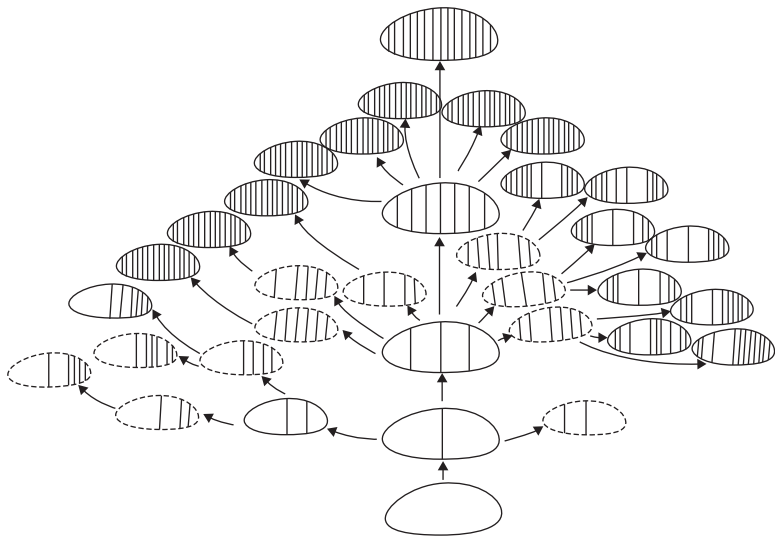

Figure 4.2. Transformation tree of body patterns in fruit fly larvae based on a model of successive bifurcation.

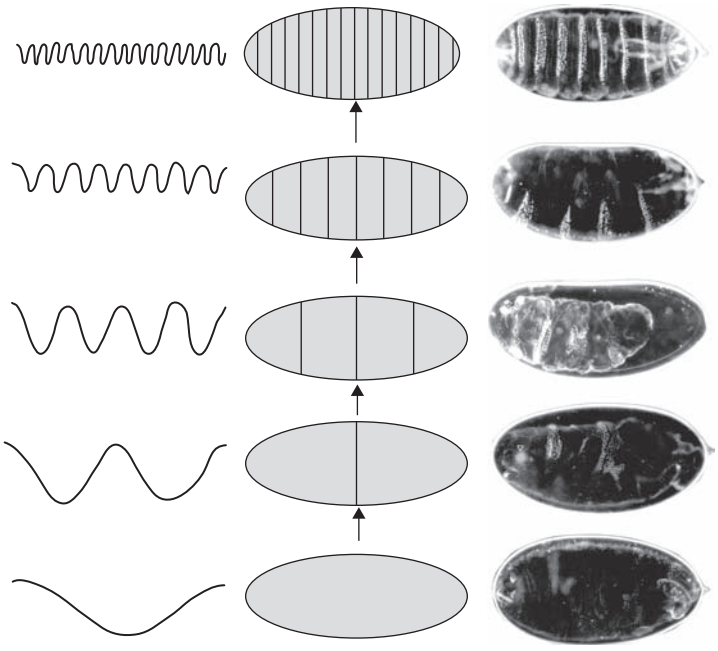

Figure 4.3. Model of successive bifurcation and actual embryos arrested in the main sequence.

A *natural* system of classification – one that reflects the natural transformational relationships – results from the tree. The 24 actual forms or species are classified hierarchically into one "Family" with two "Orders," the first Order containing three Genera, and the second Order, eight Genera. The forms not yet found (depicted in dotted lines in Figure 4.2), would also fit neatly in the natural system of classification should they be discovered in future. There are 676 possible forms according to the dynamic model of successive bifurcation. If all the body segments were free to vary independently, the number of possible forms would have been 2^{16}, or more than 60,000. This demonstrates how highly the generative dynamics can constrain the possible forms, and why, incidentally parallelisms are rife in evolution (Ho, 1984b; Ho & Saunders, 1982).

In the second example, we produced a transformation tree for all possible ways leaves are arranged around the stem in plants (Figure 4.4) (Ho & Saunders, 1994), based on the generic and robust dynamics that generate the patterns, discovered by French mathematical physicists Douady and Couder (1992). The discovery caused quite a stir in France, as leaf arrangement, or *phyllotaxis,* has been a long-standing problem in biology, ever since the brilliant mathematician and code-breaker Alan Turing (1912–1954) drew attention to how the spiral patterns of leaves around the stem conform to the Fibonacci sequence (Saunders, 1984, 1989). Many neo-Darwinian "just-so stories" have been invented over the years to account for different leaf arrangements in terms of "selective advantage"; all of which have

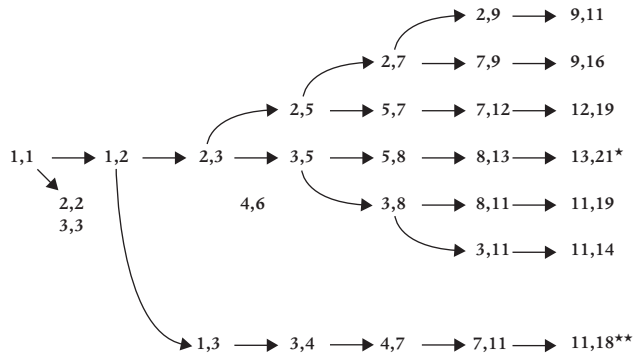

Figure 4.4. The transformation tree of possible phillotaxis patterns.

been proven irrelevant in one stroke. The power of dynamics – the syntax of form – is that it predicts the set of possible transformations, *excluding all others*. It also tells us how the possible forms are related by transformation (Ho, 2008a).

It is not known if all the possible forms in Figure 4.4 actually exist in nature. The main Fibonacci sequence with divergence angle of 137.5° is in the middle row (marked with *). At the bottom is an alternative Fibonacci sequence with divergence angle of 99.5 ° (marked with **). Like the transformation tree in Figure 4.2, it makes very definite predictions concerning neighboring transformations. Thus, parastichies 8,11 and 9,11 (secondary spirals, the numbers indicate spirals to the right and left respectively starting from the centre of the flower or top of the cone), despite their apparent similarity, are quite far apart on the tree, whereas the neighboring parastichies 8,13 and 13,21 appear superficially very different. As the tree is also an ontogenetic tree, it predicts that plants such as the Canadian pine (*Pinus resinosia*) with parastichies 8,13 in the cone, goes through all of the main sequence in development. We do not know if that is true, but we did find that the leaf shoot bearing the cone has 3,5 parastichies.

For the same reasons, we would predict that the decussate arrangement 2,2 is the earliest divergence from the main Fibonacci sequence, followed by the alternative Fibonacci sequence beginning with 1,3. Phylogenetic transformations are strictly predicted. For example, one would not expect an ancestor of a plant with parastichies 8,13 to have had parastichies 7,12, or even 2,5, but most likely, 5,8.

The dynamics of the processes are subject to contingent "complexification" (or simplification) in the course of evolution, by virtue of the lived experience of the organisms themselves. Nevertheless, it is highly constrained, when it comes to pattern formation.

It has become clear that directed genetic changes in given environments are just as non-random as morphological changes, and hence, possibly subject to comparable systemic constraints (Ho, 1987) (see later).

Natural Selection and Molecular Evolution

Molecular evolution, the study of how proteins and nucleic acids in different species evolve, has been dominated by the neutralist/selectionist controversy that continues to the present day.

Motoo Kimura (1924–1994) was best known for his neutral theory of molecular evolution (Kimura, 1968), which proposed that most of the amino acid and base changes in evolution resulted from random genetic drift of neutral mutations, that is, mutations that did not influence the "fitness" of the organisms. In fact, he did not deny that natural selection could be operating; only that it was not reflected in the evolution of molecules. In effect, molecular evolution appears decoupled from the evolution of organisms, which, at least, is consistent with all other observations indicating the lack of simple translations between genes and phenotype, and is an independent corroboration of Waddington's (1957) concept of the epigenetic landscape. Even neo-Darwinists like Sewell Wright and Ernst Mayr had insisted that natural selection acts on whole organisms or co-adapted gene complexes, and not single genes (see earlier).

The neutral mutation theory was inspired by earlier discoveries that gave rise to the idea of the "molecular clock" by comparing the number of amino acid differences in proteins from different species. Two kinds of sequence comparisons can be made using the same set of sequence data. The first compares the protein from a recently evolved organism such as a mammal with those from simpler or less complex species that evolved earlier such as amphibians and fishes. The second compares the protein from a simpler out group species as fishes against the more complex "sister species" that appeared later such as amphibians and mammals. The first kind of comparison indicates a near linear correlation between genetic distance and time of divergence, implying indirectly a constant mutation rate among different species. For example, human is closer to mouse, less to bird, still less to frog and least to fish. The second kind of comparison, significantly, gives the "genetic equidistance" result, where *all* sister species are approximately equidistant to the simpler out group. For example, human, mouse, bird and frog are all equidistant to fish in any protein. As all sister species are also equidistant in time to the out group fish, this was regarded as a direct confirmation of a constant, or similar mutation rate among different species.

The molecular clock hypothesis was first proposed by Emil Zuckerkandl and Linus Pauling (1962) based on the first kind of sequence comparison, but it was Emanuel Margoliash (1963) who performed both kinds of comparisons and discovered the "genetic equidistance" result.

According to the molecular clock hypothesis, the rate of amino acid or nucleotide substitution is approximately constant per year over evolutionary time and among different species. Two different species are thought to gradually accumulate mutations over time since their most recent common ancestor. Their

genetic distance would therefore be smaller in ancient times than today. As more and more data became available, the molecular clock hypothesis ran into trouble. Although there is a correlation between genetic distance and time of divergence, such correlation is not universal, and is often violated.

Numerous studies on extant organisms show that mutation rates are far from constant (Huang, 2009). For example, genetic differences between two subpopulations of medaka fish that had diverged for ~4 million years is 3 times that between two primate species, humans and chimpanzees, that are thought to have split 5–7 million years ago. Genetic distances measured on genealogical timescales of less than one million years are often an order of magnitude *larger* than those on geological timescales of more than a million years. Huang (2009) concluded therefore that genetic distance measured in evolutionary time has nothing to do with mutation rate measured in real time. The constant mutation rate is an "over-interpretation" of the genetic equidistance observation (Huang, 2008a).

A study on DNA and protein sequences of ancient fossils (Neanderthals, dinosaurs, and mastodons) (Huang, 2008b) showed that genetic distance had not always increased with time in the past history of life on earth. Neanderthals are *more* distant than modern humans to the out group chimpanzees in non-neutral DNA sequences, contrary to expectations from the molecular clock. This unexpected observation has been independently corroborated by analysis of Neanderthal mitochrondrial protein sequences (Green et al., 2008).

To illustrate the paradox, the percent identities between species for four randomly selected genes are compared. All four genes behave as good clocks in macroevolution from fish (*D. rerio*, zebrafish), to frog (*X. laevis*, African clawed toad), to bird (*G. gallus*, red jungle fowl), to mouse (*M. musculus*), and human (*H. sapiens*).

However, they give wildly contradictory timing when used for evolution at lower levels (see Table 4.1).When different species of fish are compared with each

Table 4.1. Genetic distance and estimated divergence time

	Percent identity				
	Prdm2	*BTK*	*CytC*	*GCA1A*	*Div. time (MyBP)*
H. sapiens vs D. rerio	39	61	80	66	450
H. sapiens vs X. laevis		55-	85	75	360
H. sapiens vs G. gallus	71	85	87	81	310
H. sapiens vs M. musculus	91	98	91	91	91
F. rubripes vs *D. rerio*	45				420
		71			400
			81		200
				91	91

Source: Huang (2009).

other, *F. rubripes* (puffer fish) vs *D. rerio*, divergence time ranged from 91 to 420 myBP.

Epigenetic Complexity *vs* Genetic Diversity, Macroevolution *vs* Microevolution

Huang (2009) proposed an ingenious theory that an inverse relationship exists between genetic diversity and epigenetic complexity: multicellular organisms differentiated into tissues and cells are epigenetically complex and can tolerate less genetic variation (germline DNA mutation), whereas single celled organisms, being epigenetically simple, can tolerate more. Consequently, each level of epigenetic complexity will reach its maximum level of variations. This simple theory was found to deduce or explain all the major features of evolution, including the genetic equidistance result and the paradox of different estimates of divergence times depending on the species compared (Table 4.1).

Humans are undoubtedly the most epigenetically complex species; but in terms of the number of genes, *H. sapiens* has only roughly 1.6 times that of a fruitfly and about the same as the mouse or fish. However, the number of certain enzymes responsible for epigenetic gene organization, such as the PRDM subfamily of histone methyltransferases, increases dramatically during metazoan evolution from 0 in bacteria yeasts and plants, to 2 in worms, 3 in insects, 7 in sea urchins, 15 in fishes, 16 in rodents and 17 in primates. Also, the core histone genes H2A, H2B, H3 and H4 have been duplicated in humans but not chimpanzees, and the number of genes for microRNA (which play key regulatory functions) correlates well with organismal complexity. Complex organisms also show complex gene expression patterns: 94% of human genes have alternative products or alternative splicing compared to only 10% in the nematode *C. elegans*.

For any organism of a certain epigenetic complexity, it can undergo epigenetic changes or genetic mutations in a certain range allowed by the epigenetic complexity. More significantly, epigenetic complexity change is almost by definition, macroevolution, whereas genetic changes due to mutations causing minor variations in phenotypes and not affecting the epigenetic programs are microevolution. Microevolution, says Huang (2009), is a continuous process of accumulating mutations.

For two distinctly different kinds of organisms over long evolutionary time, their genetic distance is independent of mutations rates and time but is *determined by the maximum genetic diversity of the simpler organisms of the two*. The gradual but stepwise increase in epigenetic complexity with time during macroevolution of distinct organisms results in the near linear correlation between *maximum* genetic distance and time of species divergence. Such a correlation holds only for macroevolution, and is not related to actual mutation rates. It is distinct from the correlation

between genetic distance (prior to reaching maximum) and time of divergence during short time scales or before reaching maximum in genetic distance. Actual mutation rates are usually fast enough for maximum genetic distance to be reached in evolutionary time, especially for fast evolving genes.

Macroevolution from simple to complex organisms is associated with a punctuational increase in epigenetic complexity and in turn a punctuational loss in genetic diversity. From a common ancestor, the genetic distance between two splitting descendants may gradually increase with time until a maximum is reached, remaining constant thereafter. This, Huang claims, explains top-down evolution (which is also consistent with the epigenetic origin of evolutionary novelties, see earlier).

The maximum genetic diversity hypothesis predicts that if time is long enough for genetic distance to reach the cap, the maximum genetic distance between two genera of the same family should be similar to that between two families, or orders, or phyla. That should be true for a very old group such as fungi. In contrast, the molecular clock hypothesis predicts that the genetic distance between two fungi genera of the same family should be *smaller* than that between families, and still smaller than that between orders, and so on. A random sampling of three proteins showed that the first prediction is correct.

Huang's theory does explain a lot, and resolves all the paradoxes in molecular evolution. But there is an important feature of epigenetically complex organisms that has been left out of consideration. It may not be so much that epigenetially complex organisms can tolerate less germline DNA variation, but rather, they have become more efficient at generating the sequence diversity required at the precise local somatic level; and incidentally, also more efficient at reducing it at the germline level through mechanisms such as gene conversion and concerted evolution (Ho, 2004d).

Epigenetic processes such as RNA editing, alternative and trans-splicing, exonization, and somatic hypermutations can generate huge sequence diversity wherever and whenever required. Some of those processes, coupled with reverse-translation, are powerful mechanisms for generating sequence diversity that can be tested by function within the individual organism, and then used to overwrite the germline sequence(s) (see Ho, 2009e, 2009f). Furthermore, there is now a range of evidence (see later) indicating that mutations are far from random, with the organism choosing when and how to mutate, or not to mutate at all (Ho, 2004c)

The significant lesson from molecular evolution is that macroevolution involves epigenetic changes independently of microevolution, which consist predominantly of the accumulation of random neutral mutations. This is just what has been predicted from the nonlinear dynamics of the epigenetic system described earlier. Epigenetically complex organisms are also much more effective at generating somatic sequence diversity, and possibly, at rewriting somatic and even germline genomes with sequences that have been tested for function (more on this later).

The Demise of the Genetic Paradigm and Mechanistic Biology

The assumptions that genes are stable and insulated from environmental influences are pivotal to the genetic paradigm and neo-Darwinian theory. They were inspired by Weismann's theory of the germplasm which, however, has been flawed from the start. Plants do not have separate germ cells at all, for every somatic cell is potentially capable of becoming a germ cell, which is why plants can be propagated from cuttings. Most animals also do not have germ cells that separate from the rest of the body early in development (Buss, 1987). Furthermore, there is no evidence that the genes in germ cells are stable or immune from environmental influences once they become differentiated from the rest of the body. We now know that the environment can impact directly on the germ cells in the developing fetus, giving rise to the grandparent effects mentioned at the beginning of this chapter. It turns out that toxic environmental substances such as bisphenol A and other endocrine disruptors specifically affect germ cells in the developing fetus, giving rise to the grandmother effect in epigenetic toxicology (Ho, 2009d).

The first crack in the genetic paradigm appeared before genetic engineering research got underway. Howard Temin and David Baltimore (1972) discovered an enzyme in RNA tumor viruses that does the reverse of transcription, that is, making a copy of complementary sequence of DNA (cDNA) from an RNA sequence. The RNA viruses are retroviruses, implicated in acquired immune deficiency syndrome (AIDS) and many forms of cancer which have RNA as their genomes. The discovery resulted in a slight change in the Central Dogma: information goes from nucleic acids to proteins, and never in reverse. Little did they realize how that alone would allow torrents of counter-information flow from the environment and back to the genome, as RNA actually plays a central role in trafficking, subverting, and corrupting information from and back to the genome (see later).

Evidence that genes are neither stable nor immune from direct environmental influence has been accumulating almost as soon as genetic engineering began in the mid 1970s and applied to investigate the molecular basis of genetics. To their astonishment, molecular geneticists soon witnessed classical genetics being turned upside-down on their own lab benches. They found exceptions and violations to every tenet of classical genetics and the Central Dogma that had been accepted without question for decades. In direct contradiction to the concept of a relatively static genome with linear causal chains emanating from genes to the environment, they discovered constant cross talk between genome and environment. Feedback from the environment not only determines which genes are turned on where, when, by how much, and for how long, but marks, moves, and changes the genes themselves. So much so that by early 1980s, molecular geneticists have already coined the term, "the fluid genome" (Dover & Flavell, 1982) to capture what I have

later described as a molecular "dance of life" that is necessary for survival (see Ho, 2008b, for example).

The fluid genome spells the end of the genetic paradigm, and with it, mechanistic biology (Ho, 1998). I have described and updated these discoveries in successive reviews and commentaries (Ho, 1987, 1988a, 1988b, 1998, 2003, 2004a, 2004b, 2008b), each slipping out of date almost as soon as it was written. So, I shall do so again here, concentrating on the most profound discoveries especially since the human and other genomes have been sequenced, that blur the distinction between genetic and epigenetic, demonstrating that organisms are inextricably entangled with their experience of the environment.

The Vanishing Gene

Classically, the gene is a sequence of DNA in a defined location of the genome (a particular locus on a chromosome) that specifies the amino acid sequence of a polypeptide via a non-overlapping triplet code (three consecutive bases coding for each of 20 different amino acids). The coding sequence is flanked by regulatory signals for transcription to start at one end and stop at the other. Even this picture was not simple. Transcription in eukaryotes (organisms with a nucleus), especially requires not only the RNA polymerase enzyme (a protein encoded by another gene) that makes a complementary RNA copy of the gene sequence in the genome, but also transcription factors (proteins encoded by yet other genes) that help the enzyme access the DNA to initiate transcription. After transcription, a series of processing by further enzymes and cofactors (encoded by numerous other genes) chops and changes the transcript and adds a poly-A tail before the resultant messenger RNA (mRNA) is exported into the cytoplasm for translation.

Translation involves its own highly complex army of transfer RNAs, ribosomes, and regulatory machinery for initiation, elongation, and termination of the polypeptide chain. A lot of that was known or inferred before genetic engineering research really got underway.

Thus, the expression of each gene is inevitably entangled with numerous other genes, if not ultimately, with every other gene in the genome. It is impossible to tell where one gene function stops and the other begin. But that is far from the end of the story in the complexity of the gene.

It began with the discovery of overlapping genes in the bacteriophage (bacterial virus) ϕX174 in 1977, which meant that a single stretch of DNA specifies two different polypeptides by shifting the reading frame. Overlapping genes have since been identified in all organisms; the human genome has thousands of them (Veeramachaneni, Makalowski, Galdzicki, Sood, & Makalowka, 2004).

But it was the discovery of interrupted genes soon afterwards that ultimately threw the concept of a gene into disarray. In interrupted genes, the coding sequence is not continuous, instead, it is interrupted in many places by long intervening non-coding sequences (introns); the short coding sequences (exons) must be spliced together after transcription to make the mRNA that translates into a functional protein. It turns out that different exons could be spliced together to make distinct proteins. Furthermore, exons belonging to different genes could also be spliced together (trans-splicing) to create new proteins.

Alternative and trans-splicing of exons greatly expands the repertoire of proteins. In *Drosophila*, for example, alternative splicing of *Dscam* exons gives rise to 38,016 different isoforms of the cell surface protein, each with a different binding specificity, thereby contributing to the complex patterns of neuronal connections in the brain (Wojtowicz, Flanagan, Millard, Zipursky, & Clemens, 2004).

The sequences that code for proteins constitute a mere 1.5% of the human genome, but it is estimated that more than 60% of human genes are interrupted.

A consortium of 35 research groups took part in a project (ENCODE, 2007) to study 1% of the human genome in great detail to find out exactly how genes work, and came up with some "major surprises." As one journalist (Barry, 2007) commented: "genes are proving to be fragmented, intertwined with other genes, and scattered across the whole genome."

Indeed, not only are genes interrupted, exons contributing to a single protein can be in different parts of the genome. Coding sequences of different proteins frequently overlap. Regulatory signals are similarly scattered upstream, downstream, within the coding sequence, or in some other distant part of the genome. The potential repertoire of proteins that can be made by combining different exons is at least a thousand times the 20,000 genes identified in the human genome. Which exons are recruited to make specific proteins depends entirely on the microenvironmental contexts.

Even more dramatic headlines appeared in the business section of the *International Herald Tribune* (Carusco, 2007): "Change to Gene Theory Raises New Challenges for Biotech." The article went on to say: "The $73.5 billion global biotech business may soon have to grapple with a discovery that calls into question the scientific principles on which it was founded." And further on, commented that:

> the report is likely to have repercussions far beyond the laboratory. The presumption that genes operate independently has been institutionalized since 1976, when the first biotech company was founded. In fact, it is the economic and regulatory foundation on which the entire biotechnology industry is built.

She was right on all counts. I pointed that out 10 years previously in my book, *Genetic Engineering Dream or Nightmare, The Brave New World of Bad Science and Big*

Business (Ho, 1998), when findings in molecular genetics had already invalidated the genetic determinist paradigm underpinning the biotech industry. In fact, the paradigm had begun to unravel almost as the industry was starting up 20 years before, and that's why I said genetic modification of animals and plants was both dangerous and futile, which has proven so since (Ho, 2008b).

In the wake of the ENCODE project, fresh attempts are being made to redefine a gene in terms of a protein product (Zhang, Weissman, & Snyder, 2007), or a transcript (Gingeras, 2007), neither of which is satisfactory or would save the industry. All patents on genes based on the old concept are no longer valid; ultimately because the patent is awarded on a supposed function attached to a DNA sequence. But as genes exist in bits interweaving with other genes, so are functions. Multiple DNA sequences serve the same function, and conversely the same DNA sequence can have different functions. I had explained earlier why biotech patents are patently absurd (Ho, 2002), and should never have been awarded in the first place.

If genes are in disarray, that's nothing compared to how the genetic text in the genome is being subverted, corrupted, and rewritten by feedback from the environment.

Subverting and Rewriting the Genetic Text

Even as the human genome was announced, geneticists still believed that the most of the genome consisted of "junk" DNA that had no function at all; but that perception was soon to change.

According to the Central Dogma, DNA, the genetic text, is read out into RNA and RNA is translated into protein. RNA is rather like the scribe copying and translating the sacred text to direct the faithful, so the role of RNA is rather limited, and most of the action was supposed to be done by proteins.

But geneticists soon discovered a vast underworld of heresy to the Central Dogma where RNA plays a predominant role. RNA agents not only decide which bits of text to copy, which copies get destroyed, which bits to delete and splice together, which copies to be transformed into a totally different message and finally, which resulting message – that may bear little resemblance to the original text – gets translated into protein. RNAs even get to decide which parts of the sacred text to rewrite or corrupt (Ho, 2004a).

The whole RNA underworld also resembles an enormous espionage network in which genetic information is stolen, gets re-routed as it is transmitted, or transformed, corrupted, destroyed, and in some cases, returned to the source file in a totally different form.

And this underworld is big, really big. The protein-coding sequence is only about 1.5% of the human genome. Yet, around 97–98% of the transcriptional readout of

the human genome is non-protein-coding RNA, from parts of the genome not so long ago considered "junk" DNA (Semon & Duret, 2004).

The inescapable conclusion is that the job of mediating between DNA and protein is really the centre stage of molecular life. And who gives orders to the multitudes of RNA agents? In a sense it is everyone and no one, because the system works by perfect intercommunication. It is not the DNA, but rather, the particular environment in which the RNA agents find themselves that appears to decide what they do.

For the organism (organization) to survive, it needs to turnover the DNA text continuously, adapting to the realities of its environment. In the process, it keeps certain texts invariant (Ho, 2004b), while changing others rapidly in non-random ways (Ho, 2004c). It also needs to keep referring to texts that are relevant, modifying it, or updating the interpretation in keeping with the times (Ho, 2004d).

I describe some of the processes in greater detail especially in how they blur the distinction between genetic and epigenetic, organism and environment.

RNA Interference

RNA interference (RNAi) is present in all organisms that silence genes as well as viruses and transposons in the genome (Ho, 2004a); it is also involved in directing development. The agents are small RNAs ranging from 21 to 29 nt (nucleotides) in length, mainly short interfering RNAs (siRNAs) and microRNAs (miRNAs). Both are generated from double stranded RNA (dsRNA) precursors by members of the Dicer family of nucleases into small effector molecules. While miRNAs are encoded in the genome, siRNAs are externally derived. MiRNAs, especially those in animals, typically have incomplete base pairing to a target and inhibit the translation of many different mRNAs with similar sequences. In contrast, siRNAs typically base-pair perfectly and induce mRNA cleavage and destruction only in a single target (Pillai, Bhattacharyya, & Filipowicz, 2007).

MiRNAs are involved in regulating gene expression particularly during development. They also control a wide variety of neurologically important processes in both vertebrates and invertebrates including neuronal expression of chemoreceptor genes, neuron-specific splicing, circadian rhythms, morphogenesis of dendritic spine in neurons, learning, and memory.

RNA Editing

RNA editing is a process that systematically alters the genetic messages transcribed from the genome by changing its base sequence, thereby creating new coding and non-coding RNAs, and hence new proteins as well as RNAi (interfering RNA) species that regulate networks of genes. It involves insertion or deletion of specific

bases, as well as conversion of one base to another, such as cytosine (C) to uracil (U), or adenine (A) to inosine (I), which is read as a guanine (G).

RNA editing changes the genetic messages beyond recognition, and can act in concert with alternative splicing to further enhance transcript diversity (Jepson & Reenan, 2008). For example, in the *para* locus encoding a *Drosophila* voltage-gated Na^+ channel, 24 processing sites for alternative splicing and RNA editing can potentially combine to generating more than two million "isoforms" of the protein.

RNA editing occurs in all taxonomic groups of organisms, but increases dramatically in vertebrates, mammals, and primates, with humans exhibiting the highest levels of edited and multiply-edited transcripts. RNA editing occurs in most, if not all tissues, but is particularly active in the nervous system, where transcripts encoding proteins involved in fast neural transmission, such as ion channels and ligand-gated receptors are all subject to RNA editing (Jepson & Reenan, 2008; Mattick & Mehler, 2008). These species-specific alterations have profound importance for normal nervous system function.

A to I editing is much more abundant in humans than in mice, and over 90% of this increased editing occurs in *Alu* elements in mainly non-coding, regulatory regions. *Alu* elements are primate-specific retrotransposons that have undergone successive waves of amplification in the lineage leading to humans; Mattick and Mehler (2008) and others have suggested that the coincidental increase in *Alu* sequences and RNA editing play a crucial role in the evolution of the primate and human brain and complex behaviour (see Ho, 2009e).

A to I editing is catalyzed by members of the enzyme family adenosine deaminase that act on RNA (ADARs). ADARs have been shown to regulate neuronal gene expression through a variety of processes including modulation of RNAi, creation of alternative splice sites, and abolition of stop codons to generate new proteins. In addition, ADARs have a novel role in primates in the widespread editing of *Alu* elements to create new exons for proteins, among other things.

RNA editing appears to be vital for development, especially of cognitive functions. For example, the loss of A to I editing in mice lacking the editing enzyme ADAR1 leads to the mice dying at the embryonic stage from defects in the production of red blood cells and stress-induced programmed cell death, and degeneration of the liver. Mice lacking a second editing enzyme ADAR2 exhibit profound epileptic seizures and die shortly after birth.

In *Drosophila*, deletion of the single *adar* locus generates morphologically wild-type adult flies that display a range of behavioral abnormalities including severe non-coordination, temperature-sensitive paralysis, seizures, and a complete lack of courtship displays and mating. Deletion of the RNA editing enzymes ADR1 and ADR2 in *C. elegans* similarly results in chemosensory defects.

RNA editing alters transcripts from genes encoding proteins involved in neural cell identity, maturation, and function, as well as in DNA repair. In humans, three ADARs (1–3) exist, all preferentially expressed in the nervous system, with

ADAR3 being expressed exclusively in the brain. Within the brain, ADARs exhibit complex profiles of spatiotemporal regulation and dynamic changes in subcellular localization, and are themselves subject to alternative splicing. Moreover, the activities of ADARs are modulated by environmental cues to modify signal-transduction pathways containing edited targets. RNA editing is critical for cognitive behavior; the deregulation of ADAR activity and associated hyper- or hypo-editing of RNA transcripts is associated with an increased risk of neuro-degenerative disease and cancer, as well as neuro-developmental and psychiatric diseases in humans.

Intriguingly, transcripts from genes encoding a broad range of DNA surveillance and repair enzymes are also subject to RNA editing, suggesting that RNA editing may influence the fidelity of DNA replication, and hence the rate of mutation.

RNA-Directed Rewriting of Genomic DNA

Genomic DNA can be rewritten by reverse transcription (see Ho, 2004a). Around 45% of the human genome is derived from retrotranspositions that depend on reverse transcription.

Nobel laureate Howard Temin (1971) discovered the reverse transcription enzyme in a large class of RNA retroviruses that are related to the mobile genetic elements present in all genomes. Rothenfluh and Steele (1993) suggested that the immune system may use this mechanism to incorporate into the germline new antibody genes that have been generated by mutations in somatic cells during immune responses against foreign antigens.

Learning and memory in the brain is similar to the immune response in many ways. A key feature of the immune system is the alteration of a DNA sequence in the genome to generate receptor diversity, in part catalyzed by the APOBEC family of cytidine deaminases that catalyze cytosine to uracil (C to U) and cytosine to thymine (C to T) editing of RNA and DNA.

The possibility exists that DNA recoding – rewriting genome DNA – is a central feature of both the immune and nervous systems. DNA recoding may be involved at the level of establishing neuronal identity and neuronal connectivity during development, learning, and brain regeneration. And it appears that the brain, like the immune system, also changes according to experience.

Mattick and Mehler (2008) suggest that the potential recoding of DNA in nerve cells (and similarly in immune cells) might be primarily a mechanism whereby productive or learned changes induced by RNA editing are *rewritten* back to DNA via RNA-directed DNA repair. This effectively fixes the altered genetic message once a particular neural circuitry and epigenetic state has been established. (Steele (2008) has proposed a similar RNA-directed recoding of DNA for the immune system.)

The suggestion that memory formation involves RNA-directed DNA modifications similar to those in the immune system is supported by a range of circumstantial observations over many years. For example, two enzymes involved in generating diversity in the immune system (Rag1 and Rag2) are expressed in the central nervous system and in olfactory sensory neurons that are actively involved in experience-mediated neural plasticity. Furthermore, recombination catalyzed by Rag1 and Rag2 and programmed genomic rearrangements in other organisms are RNA directed, although it remains uncertain whether such recombination occurs in the brain and is relevant to brain function.

Members of the DNA polymerase Y family involved in somatic hypermutation of genes encoding immunoglobulins have reverse transcriptase activity (Franklin, Milburn, Blanden, & Steele, 2004). One of them, DNA polymerase-ι is expressed in areas of the brain associated with learning and memory, as is DNA polymerase M, which is involved in rearrangement of genes encoding immunoglobulins. The fact that transcripts from genes encoding enzymes putatively involved in DNA recoding are themselves edited suggests that the process is subject to contextual environmental control, which might explain why some memories are more vivid and enduring than others. Steele, Lindley, Wen, and Weiller (2006) suggested that RNA editing and reverse transcription are responsible for the somatic hypermutation for generating antibody diversity in the immune system.

It has been shown recently that RNA-directed DNA repair can indeed occur in eukaryotic cells. In addition, LINE1 (long interspersed nuclear element) that are active in the human genome encode several proteins, including a reverse transcriptase, and individual SINE (short interspersed nuclear element) including active Alu sequences can hijack and use the LINE1 reverse transcriptase.

The suggestion that there might be communication of RNA-encoded information back to the genome at the epigenetic and genetic levels would also potentially explain the surprising observation that diverse RNA species and associated regulatory signals are not only trafficked to the periphery of the nerve cell, but might also undergo retrotransport back to the nucleus. There is increasing evidence for retrograde transport of RNAs, including small RNAs, to the nucleus in a broad range of organisms, as well as RNA informational exchange between cells through "exosomes," specific RNA receptors and derivation of presynaptic RNA from surrounding glial cells.

There are clear evolutionary and functional parallels between members of the immunoglobulin (Ig) superfamily and the protocadherins, as well as many other subclasses of nervous system-selective Ig superfamily domain-containing proteins involved in neuronal cell identity, connectivity, synaptic plasticity, and developmental and adult brain homeostasis.

Mattick and Mehler (2008) suggest that environmentally induced changes in neural development and brain architecture, cell identity, and synaptic connectivity might become "hardwired in the genome, potentially defining the complex and emergent properties of long-term memories and other structural and functional adaptations on the developing brain."

They fall short of proposing that the RNA-templated recoding of the genome and the associated structural and functional adaptations could be transmitted to the next generation. This could be crucial for brain evolution in primates leading up to humans, so that the gains made by successive generations could be accumulated.

If the analogy with the immune system holds, then as suggested by Steele and colleagues, edited RNA messages or their reverse transcribed DNA counterparts could become inherited via the sperm (Steele, 1981; Ho, 2009f).

"Sperm-mediated gene transfer" has been well documented by Italian researcher Corrado Spadafora (2008) as a process whereby new genetic traits are transmitted to the next generation by the uptake of DNA or RNA by spermatozoa and delivered to the oocytes at fertilization. The interaction of exogenous nucleic acids with sperm cells is mediated by specific factors, among which, a reverse transcriptase that generates "retro-genes" through reverse transcription of exogenous RNA or through sequential transcription, splicing and reverse transcription of exogenous DNA. The result is to transmit low copy transcriptionally active extrachromosomal structures capable of determining new traits. Retro-genes can be further transmitted through sexual reproduction from founders to their F1 progeny as new genetic and phenotypic features, unlinked to chromosomes, and thus be generated and inherited in a non-Mendelian manner. Rare instances of retro-gene integration into the chromosome could also occur, providing further potential for evolution.

Adaptive Mutations, When to Mutate or Not to Mutate

The backbone of modern genetics and the neo-Darwinian theory of evolution by natural selection is that gene mutations occur *at random*, independently of the environment in which the organisms find themselves. Those mutations that happen to be "adaptive" to the environment are "selected," while those that are deleterious are weeded out.

The idea that genes do not mutate at random, but "adaptively," as though "directed" by the environment in which the organisms find themselves, is so heretical that most biologists simply dismiss it out of hand; or try to explain away the observations (Ho, 2004c).

Microbiologist Max Delbrück first used the term "adaptive mutations" in 1946 to refer to mutations formed in response to an environment in which the mutations are selected. The term was adopted more than 40 years later by a research team investigating gene amplification in rat cells. They distinguished between mutations that pre-exist at the time a cell is exposed to a selective environment from those "adaptive" mutations formed after exposure to the environment.

Other workers have followed the same definition. These "adaptive" mutations arise in non-growing or slowly growing cells *after* the cells were exposed to

conditions that favor the mutants, preferentially, though not exclusively, in those genes that could allow growth if mutated.

In one experiment, Cairns and Foster (1991) created an *E. coli* strain defective in the *lac* gene that leaves the cells unable to grow on lactose. They plated out the bacteria on a minimal medium with lactose, and looked for mutants that revert back to normal. As the cells used up the small amount of nutrient they stopped growing. But after some time, mutants began to appear that could grow on lactose. However, the mutations are not strictly directed to the gene in which mutations could be advantageous, as unselected mutations also accumulated. In fact, the mechanisms look like "inducible genetic chaos" (Rosenberg, 2001).

The defective *lac* gene in the *E. coli* strain was a frameshift mutant, in which a small deletion or addition of a nucleotide shifted the whole reading frame of the gene, so it became translated into a totally different enzyme that has little or no ability to break down lactose. The defective *lac* gene was on the F'-plasmid involved in bacterial conjugation. Two types of adaptive genetic change are now known to occur in the *lac* frameshift system: point mutations involving changes in base sequence of the DNA, and gene amplification involving the generation of multiple copies of the defective gene so that large amounts of defective enzyme can still function to metabolize enough lactose to allow the cells to grow.

The point mutation mechanisms are highly diverse, and include DNA breakage, recombination break repair, genome-wide hypermutation in a subpopulation of cells that give rise to some or all of the adaptive mutants, a special inducible mutation-generating DNA polymerase (polIV or DinB) that has homologues in all three domains of life. There are now many bacterial and yeast assay systems in which adaptive and stationary-phase mutations have been reported, and the mechanisms are equally diverse.

Some of the mechanisms that underlie adaptive genetic change bear similarities to genetic instability in yeast and in some cancer cells, and to somatic hypermutation in the immune system that generates antibody diversity. They might also be important in bacterial evolution to antibiotic resistance (see below), and the evolution of phase-variable pathogens, which evade the host immune system by frequent variation of their surface proteins.

At first, the phenomenon of adaptive mutations was observed only in laboratory strains. But similar stress-inducible mutagenesis has been found in stationary-phase bacterial colonies grown from strains culled from the wild (Bjedov et al., 2003). A total of 787 *E. coli* isolates were collected from habitats in air, water and sediments, and the guts of a variety of host organisms. Colonies formed during the exponential growth phase were subjected to starvation during a prolonged stationary phase, and the production of mutants was monitored in the starved aging colonies. The vast majority of colonies showed an increased number of mutants. In a sample of colonies, the authors were able to link the increased mutagensis to starvation and oxidative stress by showing that either additional sugar or anaerobic incubation could block the increased mutagenesis.

The bacteria were highly variable in their inducible mutator activity. The frequency of mutations conferring resistance to rifampicin (Rif^R) increased on average 7-fold to 4.03×10^{-8} between day 1 and day 7, while the median number of colony-forming units increased only 1.2-fold.

The mutagenesis in aging cells was genome wide in a large fraction of natural isolates. The mechanisms for generating mutations looked even more diverse than in the laboratory strains (Rosenberg & Hastings, 2003)

For many pathogenic bacteria, antibiotic resistance is also achieved by point mutation mechanisms and could be induced adaptively. Even antibiotics that cause lethality can be merely bacteriostatic at lower concentrations, such that stress-promoted mutation mechanisms might be significant in the development of resistance in clinical environments.

In multicellular eukarytoes, parallels between adaptive mutation and cancer have been noted, the key being that acquisition of mutations in growth-limited state (stress) allows cells to proliferate.

More and more geneticists now think that mutation is epigenetically regulated as suggested by Drake (1991), or at any rate, it is physiologically provoked, and highly non-random. In one study on 12 long-term *E coli* lines, 36 genes were chosen at random, and 500 bp regions sequenced in four clones from each line and their ancestors (Elena & Lenski, 2003). Several mutations were found in a few lines that evolved mutator phenotypes, but no mutations were found in any of the 8 lines that retained functional DNA repair throughout the 20,000 generations experiment. This confirms the low level of "spontaneous" or random mutation, even in bacteria.

Despite the correlation of genetic changes with physiological or cellular states, many still regard these genetic changes to be the result of "random" mutations which are then subject to internal or external selection. "Internal" selection is merely another name for physiological epigenetic interactions that ultimately give the required change, which is often highly predictable and non-random. Plants exposed to herbicides, insects to insecticides, and cultured cells to drugs, are all capable of changing their genomes repeatably by specific mutations or gene amplifications that render them resistant to the noxious agent (Pollard, 1988). Selection in any form was ruled out in at least one example: the predictable and repeatable changes in morphology associated with changes in genomic DNA which occurred simultaneously and uniformly in *all* the cells of the growing meristem in plants exposed to fertilizers that were then stably inherited in subsequent generations (Cullis, 1988).

Heredity and Evolution in the Light of the New Genetics and Epigenetics

How should we see heredity in the light of the new genetics and epigenetics? If the genome itself is so dynamic and fluid, where does heredity reside? It is clear that

heredity does not reside solely in the DNA of the genome. In the first instance, it resides in an epigenetic state, a dynamic equilibrium between interlinked genic and cellular processes. But even that is an abstraction and reification. It cannot be assumed that heredity is exhausted at the boundary of cells or organisms. For as organisms engage their environments in a web of mutual feedback interrelationships, they transform and maintain their environments which are also passed on to subsequent generations as home ranges and other cultural artifacts (Gray, 1988; Ho, 1984a, 1984b, 1986; Ho & Saunders, 1982). Embedded between organisms and their environment are social habits and traditions, an inseparable part of the entire dynamical complex that gives rise to the stability of the developmental process, and which we recognize as heredity (Ho, 1984a, 1984b, 1986, 1988b). Heredity is thus distributed over the whole system of organism-environment interrelationships, where changes and adjustments are constantly taking place, propagating through all space-time scales in the maintenance of the whole, and some of these changes may involve genomic DNA. Thus, the fluidity of the genome is a *necessary* part of the dynamic stability, for genes must also be able to change as appropriate to the system *as a whole*.

What implications are there for evolution? Just as interaction and selection cannot be separated, nor are variation (or mutation) and selection, for the "selective" regime may itself cause specific epigenetic variations or "adaptive" mutations. The organism experiences its environment in one continuous nested *process*, adjusting and changing, leaving imprints in its epigenetic system, its genome as well as on the environment, all of which are passed on to subsequent generations. Thus, *there is no separation between development and evolution*. In that way, the organism actively participates in shaping its own development as well as the evolution of its ecological community.

While the epigenetic approach fully reaffirms the fundamental holistic nature of life, it can give no justification to *simplistic* mechanistic ideas on arbitrary effects arising from use and disuse or the inheritance of acquired characters. And it does not lead to any kind of determinism, environmental or genetic. Organisms are above all, complex, nonlinear, dynamical systems (Saunders, 1992), and as such, they have regions of stability and instability that enable them to maintain homeostasis, or to adapt to change, or not, as the case may be. The appearance of novelties and of mass extinctions alike in evolutionary history are but two sides of the same coin, we cannot be complacent about the capacity of organisms to adapt to any and all environmental insults that are perpetrated. The dynamics of the developmental process ultimately holds the key to heredity and evolution, in determining the sorts of changes that can occur, in its resilience to certain perturbations and susceptibility to others.

Genetic and Epigenetic Paradigms in the Study of Behavior

A similar opposition between the genetic and epigenetic paradigms has dominated the study of animal behavior. Konrad Lorenz (1965) and Niko Tinbergen (1963)

conceptualized the development of behavior as a largely autonomous sequence of maturation of central neural mechanisms controlling the animal's behavioral repertoire. The environment, insofar as it enters in development, does so in the form of specific stimuli serving to release preformed patterns of behavior from central inhibition. A strict dichotomy is thereby maintained between the "innate" and "acquired" components of behavior, the "innate" being equated with species-typical or instinctive behavior. This fits easily within the genetic paradigm in terms of genes controlling behavior in a more or less straightforward and mechanical manner. Much of the theorizing in sociobiology and its derivative discipline of evolutionary psychology is based on just such an assumption, despite apologies to the contrary.

In opposition to the theory of Lorenz, comparative psychologists such as Lehrman (1956) and Schneirla (1965, 1966), showed that the "innate" and "acquired" are inextricably confounded. And that applies even to so-called instinctive behavior.

In a classic study on the chick, Kuo (1966), another prominent scientist who did not accept that instinctive behavior was preformed, showed how the embryonic heartbeat is instrumental in stimulating and entraining the raising and lowering of the head (resting on the heart), whose movements extend to the beak opening and closing, then to swallowing the amniotic fluid later on. The embryo not only develops an integrated sense of itself, but also a series of coordinated movements that are the tangible precursor of so-called instinctive behavior. Similarly, Gottlieb (1963) demonstrated how an isolated wood duckling learns to recognize the call of its conspecifics at hatching simply through hearing its own call while still in the egg. Thus, there is no preformed set of behavior encoded in the genes waiting to be released. Even an isolated animal is subject to self-stimulation arising from its own activities beginning early in embryogenesis, which in turn generates complex behavior. (This demonstrates the fallacy of isolation experiments that are carried out by ethologists and sociobiologists in an attempt to prove that particular behaviors are innate or instinctive.) Gottlieb had adopted the epigenetic approach since 1965; his probabilistic epigenetics model of development was in direct opposition to the Central Dogma approach, and explicitly affirmed the two-way traffic of information to and from the genome in the genesis of behavior (Gottlieb, 1992, 1998).

The comparative psychologists have been proven correct, as recent studies on the epigenetics of maternal behavior, in particular, has beautifully confirmed (see later).

The aim of comparative psychology, according to Schneirla, is to discover the similarities *and differences* between phylogenetic levels in how behavior is organized. This requires careful studies on the ontogeny of species-typical behavior which deal with the problem of organization. Maturational (biological/epigenetic) processes are inextricably linked with the experiential, each in turn defining and transforming the other. Through the interplay of maturational and experiential processes, the physiological and "meaningless" become psychological and meaningful by social reinforcement. There is thus a continuum linking the genetic/

epigenetic with the social and pyschological. A full understanding of how organisms evolve must ultimately take on board the whole spectrum of interrelationships (Tobach & Greenberg, 1984, 1988).

Comparative psychology is thoroughly epigenetic in its holistic attention to many levels of living organization, and its emphasis on how complex behavior is *generated* during development through the *formative* influence of experience. Gottlieb (1992) has extended comparative psychology to consider how new behavior defines new functions, and hence, new morphologies in evolution. This same step has been taken by developmental psychologist Jean Piaget (1896–1980) some years earlier.

Piaget rejected the idea that there is an innate cognitive structure that allows us to make sense of reality. Instead, much of his prodigious volume of work was devoted to showing how cognitive abilities are developed through the child's own activities in exploring and experiencing the world. One of his preoccupations in biology was to understand why form is so-well suited, or adapted to the "function" it serves. In his last works, Piaget (1979) returned to the study of biology in order to consider the evolutionary problem which he regarded insoluble within the neo-Darwinian framework: how is it that the form of an organ is invariably accompanied by the behavioral repertoire appropriate to its use? It stretches credulity to imagine, for example, that the woodpecker first got a long beak from some random mutations followed by other random mutations that made it go in search of grubs in the bark of trees. The only explanation for this coincidence of form and behavior in the execution of function is that the two must have evolved together through the organisms' own activity.

Experience, as we have seen, never involves the organism in a purely passive role. Organisms generally *act* (more than just *behave*) so as to give themselves the greatest chance of survival. This is brought about by various means ranging from avoidance reactions in unicellular organisms to the purposive or directed explorations of higher organisms. Thus, a change in habit may be the efficient cause of the change in form, which in turn accounts for the fit between form and function. If it is true that organisms generally act so as to maximize their prospects for survival, it follows that the resulting modification of form will most likely be "adaptive." The "adaptation" will involve feedback effects on its physiology, which include changes in gene expression, or in the genes themselves. (On the other hand, organisms may also act and develop "maladaptively," as human beings, in particular, seem capable of doing.)

I shall describe some recent work on maternal behavior to illustrate the continuity between biological and social, genetic and epigenetic.

Maternal "Instinct" Deconstructed

"Maternal Instinct is Wired into the Brain," says the headline of an article in the *New York Times* (Tonelli, 2008) reporting on a new study with magnetic resonance

imaging (MRI) on the brain of 13 mothers, each of whom had an infant about 16 months old. The finding had nothing to do with maternal instinct, whatsoever. All the study showed was that the mothers reacted differently and more strongly to videos of their own child crying or smiling than to those of other infants; which is not at all surprising, as after 16 months, the mothers would have developed a special bond with their own child. The reporting betrays the all too pervasive common misconception that certain behaviors are instinctive and hence "hardwired" in the brain or in the genes.

Anthropologist Sarah Blaffer Hrdy (2001) rejects both the "essentialist" Darwinian view of the preformed biological instinct, as well as the "social constructionist" view that maternal emotions are purely socially constructed, which has come to be framed as a dichotomy between nature and nurture "Completely overlooked was just how dynamic the multiple social and biological processes contributing to the emergence of maternal commitment – what humans mean by love – were likely to be."

Learning and prior experience caring for babies are particularly important in primates, compared with other mammals. That is why, Hrdy points out, mortality rates among firstborn infants are so high among primates.

There is abundant evidence that maternal behavior is not instinctive but learned and developed out of the reciprocal interactions between mother and infant before and after birth in which biological and social factors are interwoven (see also Lehrman, 1956).

All through pregnancy, the mother's body changes, lowering her threshold for responding positively to babies. The placenta produces progesterone that helps sustain the pregnancy and contributes to changes in estrogen and progesterone levels that prime the mother to respond maternally. During the birth process, further hormonal changes, particularly secretion of oxytocin produce the muscle contractions that push the baby out. Oxytocin also has an opiate-like, soothing effect, preparing the mother for her first encounter with the baby. Physical transformation in the mother's body continues after birth. Constant proximity to the infant and the act of caring for it produce yet other hormonal and neurological changes in the mother, writing new pathways in her brain. These paths lower the threshold of stimulation needed to elicit maternal responses in future. Memories interact with existential experience. Stimulation from the baby sucking on her nipples releases oxytocin, making her feel relaxed. At the same time (based on work with birds and lab rodents) higher circulating levels of prolactin may increase maternal protectiveness toward infants. Across many species even in birds and male mammals, higher prolactin levels are correlated with protective and caring responses. In some mammalian mothers, higher prolactin levels are correlated with fierce protectiveness of their offspring that animals behaviorists call "lactational aggression."

Even without all of the special hormonal changes associated with being pregnant and giving birth, females and even male primates and rodents can

develop maternal behavior. Particularly impressive parental care is exhibited in the male Djungarian hamsters, *Phodopus campbelli*, that actively participates in the birth and care of growing pups, even when prevented from any contact with pregnant females (Jones & Wynne-Edwards, 2002).

Hrdy (2001) pointed out that mothers in a broad range of insects, birds, and mammals rely on other group members to help rear their young, and proposed that humans must have evolved as cooperative breeders like tamarins (*Saguinus oedipus*), for how else could the human mother have managed, without help, to look after an offspring that remains helpless to varying degrees for 18 years?

Recent studies on the epigenetics of maternal behavior reveal the intricate molecular mechanisms accompanying the biological and social interactions, which gives no support to any kind of determinism, genetic or environmental, biological or social.

Epigenetics of Maternal Behavior

Champagne et al. (2006) at McGill University, Montreal, in Canada, showed that mother rats that cared adequately for their pups and others who don't, shape their offspring's response to stress accordingly for the rest of their lives. The responses are correlated with different states of expression in relevant genes.

The mother rat licks and grooms her pups in the nest and while nursing them also arches her back. Some (high-LG) do that more often than others (low-LG). The offspring of high-LG mothers grow up less fearful and able to cope with stress than those of low-LG mothers, and it works via the hypothalamus-pituitary-adrenal pathway. The magnitude of the hypothalamus-pituitary-adrenal (HPA) stress response is a function of the corticotrophin-releasing factor (CRF) secreted by the hypothalamus, which activates the pituitary-adrenal system. This is in turn modulated by glucocorticoid secreted in the hypothalamus, which feeds back to inhibit CRF synthesis and secretion, thus dampening the HPA response, and restoring homeostasis.

The adult offspring of high-LG mothers show increased glucocorticoid expression in the hippocampus, and enhanced sensitivity to glucocorticoid feedback. This enhanced sensitivity was due to increased expression of glucocorticoid receptor (GR) accompanied by the increased expression of a special transcription factor NGF-1-A, which binds to the promoter of the GR gene to increase its transcription. These differences in gene expression states are accompanied by significant differences in methylation of the GR promoter; with low methylation from offspring of high-LG mothers and high methylation from offspring of low-LG mothers. The researchers also found significantly higher acetylation of histone in chromatin protein around the GR gene (as consistent with active gene expression) in the offspring of high-LG than in the offspring of low-LG mothers.

Interestingly, cross-fostering the offspring of low-LG to high-LG mothers and *vice versa* at day 1 after birth induced changes in the offspring in line with the *foster* mother, with correlated changes in the gene expression states. (So, foster parents can influence their children biologically!)

The different gene expression states are acquired during the first week of life, and persist into adulthood. Pups of both high-LG and low-LG mothers start out practically the same. Just before birth, the entire region of the GR promoter was unmethylated in both groups. That is because most gene marks are erased in the germ cells. At day one after birth, methylation was also the same in both groups. But changes develop according to the behavior of the mother within the critical period of the first week of life, and remain stable thereafter.

Nevertheless, these changes in DNA methylation and histone acetylation could be reversed, even in adults, as demonstrated by the rather drastic method of infusing chemical activators or inhibitors into the brain, with concomitant changes in the adult's response to stress (Champagne, 2008). Thus, infusing the histone deacetylase inhibitor Tichostatin A (TSA) into the brains of offspring from low-LG mothers increased histone acetylation, and decreased methylation of the GR promoter, thus boosting GR expression to levels indistinguishable from the brains of offspring from high-performing mothers. And when tested for anxiety levels, they performed like offspring from high-LG mothers

On the other hand, injecting methionine, the precursor of S-adenosyl methionine (SAM) the co-factor of DNA methylase, into the brains of offspring from high-LG mothers increased methylation of the GR promoter to levels the same as those of offspring from low-performing mothers; thereby decreasing GR expression and caused them to switch their behavior accordingly to resemble that of offspring from low-LG mothers.

Thus, epigenetic states are stable yet dynamic and plastic, giving no support to any kind of determinism, genetic or environmental.

Maternal Care and Sex Hormones

What predispose mothers to be caring or otherwise? Apparently, the female offspring inherit the characteristics of their mothers when it comes to maternal care.

The hippocampus is the "emotion centre" of the brain. It is vulnerable to stress and richly supplied with receptors for the sex and reproductive hormones, and maternal care is regulated by those hormones.

In the rat, Champagne et al. (2006) also found oxytocin receptors linked to the expression of maternal behavior. Oxytocin (OT) is a hormone secreted by the posterior pituitary gland and stimulates the contraction of the uterus and ejection of milk. Variations in OT receptor levels in critical brain regions, such as the medial preoptic area (MPOA) of the hypothalamus, are associated with differences in

maternal care. OT receptor binding in the MPOA is increased in high-LG compared with low-LG mothers. Furthermore, differences in OT receptor binding in the MPOA between high-LG and low-LG females are estrogen-dependent; it is eliminated by ovariectomy, and reinstated with estrogen replacement. However, whereas ovariectomized high-LG females respond to estrogen with an increase in OT receptor binding, low-LG females show no such effect. Studies with mice suggest that estrogen regulation of OT receptor binding in the MPOA requires the α-subtype of the estrogen receptor (ERα). Significantly increased expression of ERα, but not ER β(another estrogen receptor subtype), was found in the MPOA of lactating high-LG and low-LG mothers as well as in their non-lactating, virgin female offspring.

ERα is a ligand-activated transcription factor that regulates gene transcription on binding estrogen. The cellular response to estrogen depends on the amount of ER present.

The researchers found that by day 6 after birth, ERα expression in the MPOA of female offspring from high-LG mothers is significantly increased compared with that of female offspring from low-LG mothers, and this state continues into adulthood, which is correlated with the female offspring of high-LG and low-LG mothers becoming high-LG and low-LG mothers accordingly, and this epigenetic state perpetuates itself via the female line until and unless disrupted by environmental intervention.

One effective environmental intervention is cross-fostering, in which the biological offspring of high- and low-LG mothers are reciprocally exchanged within 12 hours of birth, reared to adulthood, and then examined for ERα expression in the MPOA. Sure enough, the ERα expression in the MPOA of the adult females born to low-LG mothers but cross-fostered to high-LG mothers, became indistinguishable from that of the normal biological offspring of high-LG mothers; and conversely, ERα expression in the MPOA of adult females born to high-LG mothers but reared by low-LG mothers resembled that of normal biological offspring of low-LG mothers. Cross-fostering as such had no effect, so exchanging offspring between two low-LG mothers or two high-LG mothers did not alter the expression of ERα in the MPOA of the offspring.

Correlated with the high and low ERα expression in the MPOA were significant differences in the methylation of CpG sites across the entire ERα promoter, Overall, significantly elevated levels of methylation were found in the promoter of offspring with low ERα expression in the MPOA compared with high ERα expression in the MPOA.

Maternal Care Influences Brain Development and Many Gene Functions

Obviously, maternal care does not just influence a few genes. The McGill University team has previously found that in the rat, increased anxiety in response to stress in the offspring from low-LG mothers is associated with decreased neuronal development and density of synapses in the hippocampus. The offspring of high-LG mothers, on the other hand show increased survival of neurons and

synapses in the hippocampus, and improved cognitive performance under stressful conditions. These observations suggest a rather extensive influence of maternal care on brain development and gene expression.

In order to examine the effect on gene expression of high- and low-LG mothers and TSA or methionine infusion, the four different treatment groups were compared with their respective control groups using microarrays to monitor changes in 31,099 unique mRNA transcripts (Weaver, Meaney, & Szyf, 2006). A total of 303 transcripts (0.97%) were altered in the offspring of high-LG mothers compared to offspring of low-LG mothers: 253 transcripts (0.81%) up-regulated and 50 transcripts (0.15%) down-regulated. TSA treatment of offspring of low-LG mothers altered 543 transcripts (1.75%): 501 transcripts (1.61%) up-regulated and the rest, 42 transcripts (0.14%) down-regulated. Methionine treatment of offspring of high-LG mothers changed 337 transcripts (1.08%), with 120 (0.39%) up-regulated and 217 (0.7%) down-regulated.

The results suggest that maternal care during the first week of life determines the expression of hundreds of genes in the adult offspring, but they are nevertheless reversible even in the adult. Caring mothers tend to activate more genes in their offspring than mothers that do not provide adequate care. TSA treatment results predominantly in gene activation as expected, and methionine treatment results predominantly in silencing genes.

Implications for Mental Health

Although the epigenetic effects of maternal behavior have been worked out in most detail in rodents, there is potential for similar effects in other species including primates and humans, as pointed out by Champagne (2008).

Abusive behavior in rhesus and pigtail macaques has been demonstrated to be transmitted from mother to daughter with influences on multiple behavioral and neurobiological characteristics in the offspring. In humans, lack of parental care or childhood abuse can contribute to subsequent criminal behavior. Furthermore, lack of parental care and parental over-protection ("affectionless control") is a risk factor for depression, adult antisocial personality traits, anxiety disorders, drug use, obsessive-compulsive disorder and attention-deficit disorders. Conversely, people who reported high levels of maternal care were found to have high self-esteem, low trait anxiety and less salivary cortisol in response to stress.

Longitudinal studies demonstrated that mother-child attachment is crucial in shaping the cognitive, emotional, and social development of the child. Throughout childhood and adolescence, secure children are more self-reliant, self-confident, and have more self-esteem. Secure infants also have better emotional regulation, express more positive emotion and respond better to stress. Infant disorganized attachment has been associated with the highest risk of developing later psychopathology, including dissociative disorders, aggressive behavior, conduct disorder, and self-abuse.

Nutrition and Mental Health

The dramatic effects of TSA and methionine infusion in altering gene expression patterns in the rats also have obvious implications for drug intervention, or better yet, intervention/prevention through adequate nutrition, as stressed by the researchers (McGowan, Meaney, & Szyf, 2008).

In rats, dietary L-methionine has been shown to be crucial for normal brain development, and its deficiency implicated in brain aging, and neurodegenerative disorders. Synthesis of SAM (cofactor for DNA methyl transferase) is dependent on the availability of dietary folates, vit B12, methionine, betaine, and choline. Developmental choline deficiency alters SAM levels and global and gene-specific methylation. And prenatal choline availability has been shown to impact on neural cell proliferation and learning and memory in adulthood. Several studies have shown that additional dietary factors, including zinc and alcohol, can affect the availability of methyl groups for SAM formation and thereby influence CpG methylation. Maternal methyl supplements positively affect the health and longevity of the offspring.

Other studies have shown that certain dietary components may act as a histone deacetylase inhibitors (HDACis), including diallyl disulfide, sulforaphane and butyrate. For example, broccoli which contains high levels of sulforaphane, has been associated with H3 and H4 acetylation in peripheral blood mononuclear cells in mice 3–6 hours after consumption.

HDACis are an active area of research as anti-inflammatory and neuroprotective agents in autoimmune diseases such as lupus and multiple sclerosis. Sodium butyrate has been shown to have antidepressant effects in mice.

These experiments raise the possibility that diet can affect the phenotype through shaping the epigenotype. Thus, reversal of epigenetic damage may be triggered by stable variations in environmental conditions such as nutrition, and not just by pharmacologic agents.

All in all, these remarkable findings on the epigenetic effects of maternal care show how important it is for societies to look after the welfare of children and mothers to be, in order to ensure both the mental and physical health of the future generation.

The Epigenetic Approach and the Continuity between Development and Evolution

The epigenetic paradigm which encompasses both comparative psychology and biology may be broadly characterized as follows:

1. Development occurs by epigenesis, in which the experience of the organism's environment enters as necessary *formative* influences, there being no preformation or predetermination in the genes.

2. Evolutionary novelties arise from epigenetic changes during development.
3. These epigenetic changes are non-arbitrary, being defined by the dynamics of the epigenetic system itself.
4. Developmental changes may be assimilated into the new organism/environmental system as a whole, which sets the parameters for further evolution.
5. Epigenesis mediates between the biological and social levels serving to integrate the two into a structural and functional whole.
6. Development and evolution are continuous, with the organism participating in shaping its own development and future evolution.

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References

Baldwin, J. M. (1896). A new factor in evolution. *American Naturalist*, **30**, 441–451, 536–553.

Barry, P. (2007). Genome 2.0. Mountains of new data are challenging old views. *Science News*. Retrieved August 9, 2007, from http://www.sciencenews.org/articles/20070908/bob9.asp; http://mail.psychedelic-library.org/pipermail/theharderstuff/20070910/004262.html

Barthelemy-Madaule, M. (1982). *Lamarck, the mythical precursor* (M. H. Shank,Trans.). Cambridge MA: MIT Press.

Bird, A. (2007). Perceptions of epigenetics. *Nature*, **447**, 396–398.

Bjedov, I., Tenaillon, O., Gerard, B., Souza, V., Denamur, E., Radman, M., et al. (2003). Stress-induced mutagenesis in bacteria. *Science*, **300**, 1404–1407.

Blumberg, M. S. (2009). *Freaks of nature: What anomalies tell us about development and evolution*. Oxford: Oxford University Press.

Bonner, J. T. (Ed.) (1982). *Evolution and development*. Berlin: Springer-Verlag.

Brakefield, P. M. (2006). Evo-devo and constraints on selection. *Trends in Ecology and Evolution*, **21**, 362–367.

Burkhardt, R. (1977). *The spirit of systems*. Cambridge, MA: Harvard University Press.

Buss, L. (1987). *The evolution of individuality*. Princeton, NJ: Princeton University Press.

Cairns, J., & Foster, P. L. (1991). Adaptive reversion of a frameshift mutation in *Escherichia coli*. *Genetics*, **128**, 695–701.

Carroll, S. B. (2005). *The new science of evo devo, endless forms most beautiful*. New York: W. W. Norton.

Carusco, D. (2007). Change to gene theory raises new challenges for biotech. Retrieved July 3, 2007. from http://www.iht.com/articles/2007/07/03/business/biotech.php?page=1

Champagne, F. A. (2008). Epigenetic mechanisms and the transgenerational effects of maternal care. *Frontiers in Neuroendocrinology*, **29**(3), 386–397.

Champagne, F. A., Weaver, I. C. G., Diorio, J., Dyimov, S., Szyf, M., Meaney, M. J. (2006). Maternal care associated with methylation of the estrogen receptor-a-1b promoter and estrogen receptor-a expression in the midial preoptic area of female offspring. *Endocrinology*, **147**, 2909–2915.

Coyne, J. A. (2009). Evolution's challenge to genetics. *Nature*, **457**, 382–383.

Cullis, C. A. (1988). Control of variations in higher plants. In M. W. Ho & S. W. Fox (Eds.), *Evolutionary processes and metaphors*. London: Wiley.

Darwin, C. (1859). *On the origin of species by means of natural selection or the preservation of favoured races in the struggle for life*. London: Murray.

Dawkins, R. (1976). *The selfish gene*. Oxford: Oxford University Press.

Douady, S., & Couder, Y. (1992). Phyllotaxis as a physical self-organized growth process. *Physical Review Letters*, **68**, 2098–2101.

Dover, G. A., & Flavell, R. B. (Eds.). (1982). *Genome evolution*. London: Academic Press.

Drake, J. W. (1991). Spontaneous mutation. *Annual Review of Genetics*, **26**, 126–146.

Eldredge, N., & Gould, S. J. (1972). Punctuated equilibria: An alternative to phyletic gradualism. In T. J. M. Schopf (Ed.), *Models in paleobiology*. New York: Freeman.

Elena, S. F., & Lenski, R. E. (2003). Evolution experiments with microorganisms: The dynamics and genetic bases of adaptation. *Nature Reviews: Genetics*, **4**, 457–468.

ENCODE Project Consortium. (2007). Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. *Nature*, **447**, 799–816.

Fisher, R. A. (1930). *The genetical theory of natural selection*. Oxford: Clarendon Press.

Franklin, A., Milburn, P. J., Blanden, R. V., & Steele, E. J. (2004). Human DNA polymerase-h, an A-T mutator in somatic hypermutation of rearranged immunoglobulin genes, is a reverse transcriptase. *Immunology and Cell Biology*, **82**, 219–225.

Gilbert, S. F. (2003). The morphogenesis of evolutionary developmental biology. *International Journal of Developmental Biology*, **47**, 467–478.

Gingeras, T. R. (2007). Origin of phenotypes: Genes and transcripts. *Genome Research*, **17**, 682–690.

Goldschmidt, R. B. (1940). *The material basis of heredity*, New Haven, CT: Yale University Press.

Goodwin, B. C., Webster, G., & Sibatani, A. (Eds.). (1989). *Dynamic structures in biology*. Edinburgh: Edinburgh University Press.

Gottlieb, G. (1963). A naturalistic study of imprinting in wood ducklings (Aix sponsa). *Journal of Comparative and Physiological Psychology*. **56**, 86–91.

Gottlieb, G. (1992). *The genesis of novel behavior*. Oxford: Oxford University Press.

Gottlieb, G. (1998). Normally occurring environmental and behavioural influences on gene activity: From Central Dogma to probabilistic epigenetics. *Psychological Review*, **105**, 792–802.

Gould, S. J. (1977). *Ontogeny and phylogeny*. Cambridge, MA: Belknap Press.

Gray, R. (1988). Metaphors and methods in evolutionary theory. In M. W. Ho & S. W. Fox (Eds.). *Evolutionary processes and metaphors*. London: Wiley.

Green, R. E., Malaspinas, A. S., Kruase, J., Briggs, A. W., Johnson, P. L. F., Uhler, C., et al. (2008). A complete Neandertal mitochondrial genome sequence determined by high-throughput sequencing. *Cell*, **134**, 416–426.

Greenberg, G. (2007). Why psychology is not a biological science: Gilbert Gottlieb and probabilistic epigenesis. *European Journal of Developmental Science*, **1**(2), 111–121.

Haldane, J. B. S. (1932). *The causes of evolution*. London: Longmans.

Ho, M. W. (1983). Lamarck the mythical precursor – a book review. *Paleontological Association Circular*, **14**, 10–11.

Ho, M. W. (1984a). Where does biological form come from? *Rivista di Biologia*, **77**, 147–179.

Ho, M. W. (1984b). Environment and heredity in development and evolution. In M. W. Ho & P. T. Saunders (Eds.), *Beyond neo-Darwinism. An introduction to the new evolutionary paradigm* (pp. 267–290). London: Academic Press.

Ho, M. W. (1986). Heredity as process. *Rivista di Biologia*, **79**, 407–447.

Ho, M. W. (1987). Evolution by process, not by consequence: Implications of the new molelcular genetics on development and evolution. *International Journal of Comparative Psychology*, **1**, 3–27.

Ho, M. W. (1988a). How rational can rational morphology be? A post-Darwinian rational taxonomy based on the structuralism of process. *Rivista di Biologia*, **81**, 11–55.

Ho, M. W. (1988b). Genetic fitness and natural selection: myth or metaphor. In *Proceedings of the 3rd Schneirla Conference*. New Jersey: Erlbaum.

Ho, M. W. (1990). An exercise in rational taxonomy. *Journal of Theoretical Biology*, **147**, 43–57.

Ho, M. W. (1993). *The rainbow and the worm: The physics of organisms* (2nd ed. 1998, 3rd ed. 2008). Singapore: World Scientific.

Ho, M. W. (1998). *Genetic engineering: Dream or nightmare? The brave new world of bad science and big business*. New York: Continuum.

Ho, M. W. (2002). Why biotech patents are patently absurd. *Journal of Intellectual Property Rights*, **7**, 151–165. Retrieved March 10, 2010, from http://www.i-sis.org.uk/trips2.php.

Ho, M. W. (2003). *Living with the fluid genome*. London/Penang: ISIS/TWN.

Ho, M. W. (2004a). Subverting the genetic text. *Science in Society*, **24**, 6–10.

Ho, M. W. (2004b). Are ultra-conserved elements indispensable? *Science in Society*, **24**, 5.

Ho, M. W. (2004c). To mutate or not to mutate. *Science in Society*, **24**, 9–10.

Ho, M. W. (2004d). How to keep in concert. *Science in Society*, **24**, 12–13.

Ho, M. W. (2008a). In search of the sublime, significant form in science and art. *Science in Society*, **39**, 4–11.

Ho, M. W. (2008b). GM is dangerous and futile. *Science in Society*, **40**, 4–8.

Ho, M. W. (2009a). Epigenetic inheritance, "what genes remember." *Science in Society*. **41**, 4–5.

Ho, M. W. (2009b). Caring mothers strike fatal blow against genetic determinism. *Science in Society*, **41**, 6–9.

Ho, M. W. (2009c). From genomics to epigenomics. *Science in Society*, **41**, 10–12.

Ho, M. W. (2009d). Epigenetic toxicology. *Science in Society*, **41**, 12–15.

Ho, M. W. (2009e). Rewriting the genetic text in human brain development and evolution. *Science in Society*, **41**, 16–19.

Ho, M. W. (2009f). Epigenetic inheritance through sperm cells, the Lamarckian dimension in evolution. *Science in Society*, **42**, 40–42.

Ho, M. W., Matheson, A., Saunders, P. T., Goodwin, B. C., and Smallcombe, A. (1987). Ether-induced segmentation disturbances in *Drosophila melanogaster*. *Roux's Archives of Developmental Biology*, **196**, 511–524.

Ho, M. W., & Saunders, P. T. (1979). *Beyond neo-Darwinism*: An epigenetic approach to evolution. *Journal of Theoretical Biology*, **78**, 673–691.

Ho, M. W., & Saunders, P. T. (1982). Epigenetic approach to the evolution of organisms – with notes on its relevance to social and cultural evolution. In H. C. Plotkin (Ed.), *Learning, Development, and Culture: Essays in Evolutionary Epistemology* (pp. 343–361). Chichester: Wiley.

Ho, M. W., & Saunders, P. T. (Eds.). (1984). *Beyond neo-Darwinism. Introduction to the new evolutionary paradigm*. London: Academic Press.

Ho, M. W., & Saunders, P. T. (1994). Rational taxonomy and the natural system-segmentation and phyllotaxis. In R. W. Scotland, D. J. Siebert, & D. M. Williams (Eds.), Models in Phylogeny Reconstruction (pp. 113–124). *The Systematics Association Special* Vol. **52**.Oxford: Oxford Science.

Ho, M. W., Tucker, C., Keeley, D., & Saunders, P. T. (1983). Effects of sucessive generations of ether treatment on penetrance and expression of the bithorax phenocopy. *Journal of Experimental Zoology*. **225**, 1–12.

Hrdy, S. B. (2001). The past, present, and future of the human family. The Tanner lectures on human values, University of Utah, February, 27–28, 2001. Retrieved March 10, 2010, from http://www.citrona.com/TannerHrdy_02.pdf

Huang, S. (2008a). The genetic equidistance result of molecular evolution is independent of mutation rates. *Journal of Computer Science and Systems Biology*, **1**, 92–102. Retrieved March 10, 2010, from http://www.omicsonline.com/ArchiveJCSB/Ft01/JCSB1.092.html

Huang, S. (2008b). Ancient fossil specimens are genetically more distant to an outgroup than extant sister species are. *Rivista di Biologia*, **101**, 93–108.

Huang, S. (2009). Inverse relationship between genetic diversity and epigenetic complexity. *Nature Precedings*: doi: 101038/ripre2009/1751.2: Posted January 13, 2009.

Hunter, P. (2008). What genes remember. *Prospect Magazine*, May. Retrieved March 19, 2010, from http://www.prospect-magazine.co.uk/article_details.php?id=10140

Jepson, J. E. C., & Reenan, R. A. (2008). RNA editing in regulating gene expression in the brain. *Biochimica et Biophysica Acta*, **1779**(8), 459–470.

Jones, J. S., & Wynne-Edwards, K. E. (2002). Paternal behaviour in biparental hamsters, *Phodopus campbelli*, does not require contact with the pregnant female. *Animal Behaviour*, **62**(3) 453–464.

Kawai, M. (1962). On the newly-acquired behaviours of the natural troop of Japanese monkeys on Koshima Island. Abstracts of the papers read in the seventh annual

meeting of the Society for Primate Researches, November 22–24, 1962, Japan Monkey Centre, Inuyama. Retrieved March 10, 2010, from http://www.springerlink.com/content/w85j34568088u82q/

Kimura, M. (1968). Evolutionary rate at the molecular level. *Nature*, **217**, 624–626.

Kropotkin, P. (1914). *Mutual aid: A factor of evolution*. Boston, MA: Extending Horizon Books.

Kuo, Z. Y. (1966). *The dynamics of behavior development: An epigenetic view*. New York: Random House.

Lamarck, J. B. (1809). *Philosophie Zoologique*. Paris: Masson.

Lehrman, D. S. (1956). On the organization of maternal behavior and the problem of instinct. In *L'Instinct dans le Comportement des Animaux et de l'Homme* (pp. 475–514) Paris: Masson.

Lorenz, K. (1965). *Evolution and modification of behavior*. Chicago: University of Chicago Press.

Løvtrup, S. (1974). *Epigenetics*. London: Wiley.

Margoliash, E. (1963). Primary structure and evolution of cytochrome c. *Proceedings of the National Academy of Science*, **50**, 672–679.

Mattick, J. S., & Mehler, M. F. (2008). RNA editing, DNA recoding and the evolution of human cognition. *Trends in Neuroscience*, **31**, 227–233.

Maynard Smith, J., & Holliday, R. (1979). Preface. In J. Maynard-Smith & R. Holliday (Eds.), *Evolution of adaptation by natural selection*. London: The Royal Society.

Mayr, E. (1963). *Animal species and evolution*. Cambridge, MA: Belknap Press.

Mayr, E. (1984). The triumph of evolutionary synthesis. *Times Literary Supplement*, November 2, 1261–1262.

McGowan, P. O., Meaney, M. J., & Szyf, M. (2008). Diet and the epigenetic (re) programming of phenotypic differences in behaviour. *Brain Research*, **1237**, 12–24.

Miller, D. (2006). Miller D. Gilbert Gottlieb: development psychologist and theorist. Interview transcribed. Retrieved March 10, 2010, from http://web2.uconn.edu/millerd/GGTranscript.pdf

Palmer, A. R. (2004). Symmetry breaking and the evolution of development. *Science*, **306**, 828–833.

Pembrey, M. E., Bygren, L. O., Kaati, G., Edvinsson, S., Northstone, K., Sjöström, M., Golding, J. and ALSPAC Study Team. (2006). Sex-specific, male-line transgenerational responses in humans. *European Journal of Human Genetics*, **14**, 159–166.

Piaget, J. (1979). *Behaviour and evolution*. London: Routledge and Kegan Paul.

Pillai, R. S., Bhattacharyya, S. N., & Filipowicz, W. (2007). Repression of protein synthesis by miRNSa: How many mechanisms? *Trends in Cell Biology*, **17**, 118–126.

Pollard, J. W. (1988). The fluid genome and evolution. In M. W. Ho & S. W. Fox (Eds.), *Evolutionary processes and metaphors*. Wiley: London.

Rosenberg, S. M. (2001). Evolving responsively: Adaptive mutation. *Nature Reviews Genetics*, **2**, 504–515.

Rosenberg, S. M., & Hastings, P. J. (2003). Modulating mutations rates in the wild. *Science*, **300**, 1382–1383.

Rothenfluh, H. S., & Steele, E. J. (1993). Origin and maintenance of germ-line V genes. *Immunology and Cell Biology*, **71**, 227–232.

Saunders, P. T. (1984). Development and evolution. In M. W. Ho & P. T. Saunders (Eds.), *Beyond neo-Darwinism. An introdution to the new evolutionary paradigm* (pp. 243–263). London: Academic Press.

Saunders, P. T. (1989). Mathematics, structuralism and the formal cause in biology. In B. C. Goodwin, G. C. Webster, & A. Sibatani (Eds.), *Dynamic structures in biology* (pp. 107–120). Edinburgh: Edinburgh University Press. (Japanese translation: Yoshioka Shoten, Kyoto, 1991.)

Saunders, P. T. (1990). The epigenetic landscape and evolution. *Biological Journal of the Linnean Society*, **39**, 125–134.

Saunders, P. T. (1992). The organism as a dynamical system. In W. Stein & F. J. Varela (Eds.), *Thinking about Biology*. Reading, MA: Addison-Wesley.

Schneirla, T. C. (1965). Aspects of stimulation and organization in approach/withdrawal processes underlying vertebrate behavioral development. In D. S. Lehrman, R. Hinde, & E. Shaw (Eds.), *Advances in the study of behavior* (pp 1–71). New York: Academic Press.

Schneirla, T. C. (1966). Behavioral development and comparative psychology. *Quarterly Revue of Biology*, **41**, 283–302.

Semon, M., & Duret, L. (2004). Evidence that functional transcription units cover at least half of the human genome. *Trends in Genetics*, **20**, 220–232.

Spadafora, C. (2008). Sperm-mediated "reverse" gene transfer: a role of reverse transcriptase in the generation of new genetic information. *Human Reproduction*, **23**(4), 735–740.

Steele, E. J. (1981). *Somatic selection and adaptive evolution: on the inheritance of acquired characters* (2nd ed.revised with an author's postscript). Chicago: University of Chicago Press.

Steele, E. J. (2008). Reflections on the state of play in somatic hypermutation. *Molecular Immunology*, **45**, 2723–2726.

Steele, E. J., Lindley, R. A., Wen, J., & Weiller, G. F. (2006). Computational analyses show A-to-G mutations correlate with nascent mRNA hairpins at somatic hypermutation hotspots. *DNA Repair*, **5**, 1346–1363.

Temin, H. M. (1971). The protovirus hypothesis: speculations on the significance of RNA-directed DNA synthesis for normal development and for carcinogenesis. *Journal of the National Cancer Institute*, **46**, III–VII.

Temin, H. M. and Baltimore, D. (1972). RNA-directed DNA synthesis and RNA tumor viruses. *Advances in Virus Research*, **17**, 129–186.

Tinbergen, N. (1963). On aims and methods of ethology. *Zeitschrift für Tierpsychologie*, **20**, 410–433.

Tobach, E., & Greenberg, G. (Eds.). (1984). *Behavioral evolution and integrative levels*. New Jersey: Erlbaum.

Tobach, E., & Greenberg, G. (Eds.). (1988). *Behavior and evolution*. New Jersey: Erlbaum.

Tonelli, F. (2008). Maternal instinct is wired into the brain. *The New York Times*. Retrieved March 7, 2008, from http://well.blogs.nytimes.com/2008/03/07/maternal-instinct-is-wired-into-the-brain/

Valentine, J. W. (2004). *On the origin of phyla*. Chicago: University of Chicago Press.

Veeramachaneni, V., Makalowski, W., Galdzicki, M., Sood, R., & Makalowka, I. (2004). Mammalian overlapping genes: The comparative perspective. *Genome Research*, **14**, 280–286.

Waddington, C. H. (1957). *The strategy of the genes*. London: Allen and Unwin.

Weaver, C. G., Meaney, M. J., & Szyf, M. (2006). Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviours in the offspring that are reversible in adulthood. *Proceedings of The National Academy of Sciences of the USA*, **103**, 3480–3485.

Webster, G., & Goodwin, B. C. (1982). The origin of species: A structuralist approach. *Journal of Social and Biological Structures*, **5**, 15–47.

Whitelaw, E. (2006). Since of the fathers, and their fathers. *European Journal of Human Genetics*, **14**, 131–132.

Williams, G. C. (1966). *Adaptation and natural selection*, Princeton: Princeton University Press.

Wilson, E. O. (1975). *Sociobiology* Cambridge, MA: Belknap Press.

Wojtowicz, W. M., Flanagan, J. J., Millard, S. S., Zipursky, S. L., & Clemens, J. C. (2004). Alternative splicing of *Drosophila Dscam* generates axon guidance receptors that exhibit isoform-specific homophilic binding. *Cell*, **118**, 619–633.

Wright, S. (1964). Evolution, Organic. In *Encyclopedia Britannica*, **8**, 917–929.

Wright, S. (1969; 1978). *Evolution and the genetics of populations, Vols. II and IV*. Chicago: University of Chicago Press.

Zhang, Z. D., Weissman, S., & Snyder, M. (2007). What is a gene, post-ENCODE? History and updated definition. *Genome Research*, **17**, 669–681.

Zuckerkandl, E., & Pauling, L. B. (1962). Molecular disease, evolution and genetic heterogeneity. In M. Kasha & B. Pullman (Eds.), *Horizons in Biochemistry* (pp. 189–225). New York: Academic Press.

5

Probabilistic Epigenesis and Modern Behavioral and Neural Genetics

Douglas Wahlsten

In the years since our dear colleague Gilbert Gottlieb departed, the field of genetics, brain, and behavior has continued to progress. It is interesting to consider now the extent of Gottlieb's influence on this field, whether his principal ideas are supported in the light of current knowledge, and to what extent his ideological opponents in psychology have capitulated and embraced his thinking. These thoughts are presented here as brief commentary.

Gottlieb's Influence

It is very difficult to discern the influence of any one theorist on an entire field of knowledge because many of the influences are indirect and not evident in citation counts, such as contributions by former students and collaborators. It is too soon to evaluate Gottlieb's influence in any event, because it is the longer-term impact that matters most for one who addressed the broader issues in the way he did. In his final interview on June 10, 2006 with David Miller (http://icube.uconn.edu/GGvideo.mov; transcript available from D. Wahlsten), he expressed doubts about the extent of his influence: "I find that getting across the developmental point of view has been the largest failure of my career. I haven't succeeded, except with close colleagues that I've worked with and who have been sympathetic with that point of view." Most of his colleagues will disagree, and on this topic, their opinions matter more than those of a modest man about himself. Their efforts to

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promulgate his views through conferences (e.g., memorial symposia at the Society for Research on Child Development, March, 2007, and Midwestern Psychological Association, May, 2008) and memorial volumes such as the special issue of the *European Journal of Developmental Science* (Scheithauer, Ittel, Josephs, & Mack, 2007) alter the very phenomenon we might like to assess and put off an accounting of his influence for at least another year or two. The task is made doubly difficult because of the previous contributions of Kuo and Lehrman and continuing contributions by Lerner and other colleagues whose theoretical perspective shares much with that of Gottlieb. Who is responsible for precisely what aspect of the growth of a field is just about impossible to discern and really does not matter very much as long as the field continues to thrive. Post mortem analyses befit dead ideas, whereas Gottlieb's theory of probabilistic epigenesis is very much alive today.

Two small indicators suggest his influence extends well beyond the domain of developmental psychology. In the 2007 second edition of their book on neurobehavioral genetics, Jones and Mormede explicitly recognized Gottlieb as a foremost critic of one particular approach to the genetic analysis of behavior and invited him to contribute a chapter. In their preface, they noted his eminence as well as his decency: "We are particularly pleased to include a contribution by Dr. Gilbert Gottlieb, who has been an important and tough critic of behavioral genetics. Fortunately, he has always been kind to behavior geneticists!" Gottlieb in his interview with Miller cited that chapter (Gottlieb, 2007a) as one of his two most important contributions, the other being his article in *Developmental Science* (Gottlieb, 2007b). At the 2008 Gordon Research Conference on behavior genetics in Il Ciocco, Italy, two of the keynote speakers, both prominent neuroscientists who work outside the area traditionally defined as developmental psychology, independently showed a slide of Gottlieb's famous diagram of development as four interacting levels across time. For them, his formulation helped to integrate a vast amount of information from diverse fields. Several of his concepts were well expressed recently in a prominent review in a special section of *Science* on "Genetics and behavior" (Robinson, Fernald, & Clayton, 2008).

Developmental System Theory in the Light of Modern Genetics

Some of the more enthusiastic reductionists in biology and psychology foresaw that completion of the entire DNA sequence of humans and mice would directly and almost immediately answer many vexatious questions about genes and development. This did not happen, and the reasons for the apparent setback are instructive. Gottlieb already had a sense of what was happening (Gottlieb, 1998) and took delight in it but did not live to see it in full blossom.

Development System Complexity

The complexity of the developmental system is proving to be far greater than almost anyone anticipated. Once the full DNA sequence of the human and mouse genomes became available, it was no longer so clear where a gene begins and ends (Pennisi, 2003), or whether regulatory sequences should be considered part of the gene (Gerstein et al., 2007). Because of this uncertainty, the estimate of the number of human genes fluctuated from an initial 21,541 to 21,714 in 2008 and 22,258 in May 2009 (www.ensembl.org/Homo sapiens). The old view of a contiguous series of codons flanked by start and stop codes that is transcribed neatly into mRNA and then translated into protein obtains for a very few genes but not most of the others. Only about 2% of the total genome (the exons) is translated into amino acids in protein, whereas the bulky 98% of introns has a rather obscure role. It has been termed "junk" DNA by some authors, but a growing body of evidence suggests that many sites in the introns are critically important for the developmental regulation of gene action (Pennisi, 2004). It is now fair to speculate that the portion of the genome involved in control of transcription and translation is far larger than the portion that is actually rendered into protein. Thus, the importance of genetics for developmental psychology is not simply that for some genes there are different alleles that code for slightly different forms of a protein that in turn lead to different phenotypes. For the genome as well as psychology, development is central.

As if life were not complex enough already, it is now well documented that in mammals most genes consist of several exons, sometimes more than a dozen exons separated by introns, and after transcription from the DNA, these can be spliced together into a large number of different combinations, each forming a unique mRNA molecule that codes for a distinct protein (Alberts, Johnson, Lewis, Roberts, & Walter, 2008). For example, the α-tropomyosin gene has 18 exons and codes for at least10 different forms of the protein in skeletal and smooth muscles as well as in different kinds of fibroblasts (Lees-Miller, Goodwin, & Helfman, 1990). The phenomenon of alternative splicing is not some esoteric oddity of interest only to cell biologists. The neural cell adhesion molecule (NCAM) exists in the brain in three alternative forms derived from the same gene (Kolkova, 2008). These forms appear at different ages in human prefrontal cortex and may play a role in neurodevelopmental disorders (Cox et al., 2009). Psychological and physiological processes such as stress can themselves alter which forms of protein are synthesized from the DNA template of the same gene (Shaked, Zimmerman, & Soreq, 2008; Singh, Tapia-Santos, Bebee, & Chandler, 2009; Xie and McCobb, 1998). Modern genetics calls for a major rewriting of psychology textbooks that portray a simplistic one gene: one protein view (Hickman & Cairns, 2003).

Even the simplest form of neural transmission involves substantial numbers of genes that synthesize, store, release, transport, re-uptake, and break down neurotransmitters as well as those that specify the structure of receptor complexes and the intricate second messenger cascades that convey signals to the cell nucleus

where gene activities are then altered. At one time it was believed that this process of neural transmission might be understood on the basis of just a few dozen genes, but that number turns out to reflect the limit of how many labels can be affixed to a diagram. In reality, post-synaptic receptor complexes contain more than 1,000 different kinds of proteins (Coba et al., 2009), and the number of viable combinations of different proteins in one macro-molecular cluster is astronomical.

Thus, the new molecular biology has opened a window into a world of complexity that boggles the mind and appeals to the poets among us to create better metaphors (Gough & Foley, 2009). It most certainly has not divulged easy answers to the questions with which Gottlieb grappled during a long and illustrious career. Instead, the complexity of the biology now seems to correspond better to the complexity of behaviors that psychology already knew so well (Barnett, Buckley, & Bullock, 2009; Bascompte, 2009).

Bi-Directional Features of Nervous System Functioning

Bi-directional features of nervous system functioning are now well documented through the medium of gene expression arrays that can detect which among thousands of genes is expressed most abundantly in specific kinds of cells (Eviskov et al., 2006) and under the influence of specific kinds of environmental factors. There is now no room for doubt that environment regulates gene activity. The interesting question at this stage of the investigation is which specific features of environment have the greatest impact on genes involved in which neural processes. The idea of genes specifying behaviors in any kind of direct way has rather rapidly become extinct. Gene expression arrays also speak to the issue of complexity, because even simple environmental influences such as the entrained day-night cycle or enriched environment (Rampon et al., 2000) are found to alter the expression levels of hundreds of genes. It is not the case that bi-directional influences are typical of just a few esoteric examples. Instead, they seem to extend into every domain of neural and behavioral functioning. The entire genome appears to be dynamic and show cycles of activity (Klevecz, Li, Marcus, & Frankel, 2008). For those who have devoted a career to the study of development, this all makes good sense. The antiquated notion of gene coding for a specific phenotype independently of the organismic environment never made any sense to the developmentalist, and now we can see that it makes no sense to the molecular biologist either. Only in the realm of quantitative human behavior genetics do the old notions continue to claim adherents.

Context and Phenotypic Expression

The importance of context for phenotypic expression is now well established for a wide range of genetic phenomena. Consider the transgenic methodology that

creates targeted mutations (knockouts) of any known gene in inbred mice. If we symbolize the normal form of the gene at a locus as the + allele and the disabled form or knockout allele as −, a study might be done to compare littermates having +/+, +/− and −/− genotypes. The knockout methodology works especially well with embryonic stem cells in tissue culture that are derived from the 129S1 inbred strain. The 129S1 strain is itself a rather odd creature with its own problems such as absence of the corpus callosum (Wahlsten, Crabbe, & Dudek, 2001). Consequently, it is common practice to backcross the knockout onto another inbred strain background such as C57BL/6 (Crusio, Goldowitz, Holmes, & Wolfer, 2009). In many instances, the developmental consequences of the knockout depend strongly on whether the background strain is 129S1 or C57BL/6 (Crusio, 2004).

The genetic background problem is just one form of the more general phenomenon of gene-gene interaction or epistasis, which occurs when the developmental or physiological effects of a genetic difference at one locus depend on the genotype at another locus, perhaps one on a different chromosome. Epistasis is a valuable clue in deciphering pathways of gene action because two genes that interact statistically must somehow be part of a common physiological or developmental process. So many instances of epistatic interaction have been detected in various species that efforts are now underway to map the *interactome*, the full array of gene-gene interactions that are characteristic of a particular genome (Marco & Marin, 2009). Given that most organisms have at least 10,000 genes, just the number of pairwise combinations to test is huge, and progress to date in this ambitious enterprise is most advanced in single-cell organisms. Nevertheless, the same thing is being attempted with mice where the first stage of the project is to knock out every gene and later combine the knockouts into double mutants.

The environmental context is equally important for gene expression, and one can contemplate a massive study of how single gene mutations interact with various features of a mouse's environment. This is not currently being done. The features of environment have not yet been systematized adequately. It seems likely that environment is even more complex than the genome and it clearly can increase in complexity without limit as humans create more environmental novelties through their inventions and accidents. Consider just the chemical environment: in 2008 the Chemical Abstract Service (www.cas.org) registered the 40 *millionth* distinct chemical form. In Canada, over 23,000 chemicals are subject to regulation by the Environmental Protection Act of 1999 (www.ec.gc.ca/CEPARegistry).

Specific Gene-Environment Interactions Pertinent to Human Mental Disorders

Specific gene-environment interactions pertinent to human mental disorders are now being published regularly in leading scientific journals such as *Science* and

Nature. These include evidence that the level of adverse events in childhood interacts with the form of the serotonin transporter (5HTTP) allele to influence depression (Caspi et al., 2003) and that childhood maltreatment interacts with the form of the MAOA (monoamine oxidase type A) gene to influence adult antisocial behavior (Alia-Klein et al., 2008; Caspi et al., 2002). Whether breast feeding of an infant augments childhood IQ appears to depend in part on genotype at the FADS2 locus (fatty acid desaturase type 2) (Caspi et al., 2007). The upsurge in reports of this nature appears to reflect more than just an increasing interest in interactions. Investigators who have detected these interactions have focused attention on aspects of the environment as well as specific genes that are known from separate lines of investigation to be pertinent to processes involved in depression, antisocial behavior, or brain development (Alia-Klein et al., 2008; Caspi & Moffitt, 2006). Furthermore, they have employed larger samples of subjects that are needed to document interactions.

Epigenetic Processes Involving Behavior

Epigenetic processes involving behavior have been known to developmental psychologists for decades, but recently they have seized the attention of neuroscientists because a molecular mechanism has now been demonstrated in certain instances. Social grooming of rat pups by their mother can alter the methylation of specific sites along the genome and thereby affect the reponsiveness of specific genes to later environmental influences (Szyf, McGowen, & Meaney, 2007; Weaver et al., 2004). When methyl groups are bound to DNA at the promoter region of a gene, the gene is functionally silenced (Rottach, Leonhardt, & Spada, 2009). This has special significance for the understanding of cancer because tumors that grow rapidly show numerous sites that are de-methylated (Piekarz & Bates, 2009). Now it is clear that methylation can mediate longer-term changes in behavioral states, even spanning generations and thereby appearing as durable changes in heredity that do not require a change in DNA sequence. Epigenetic changes may be involved in longer-term, durable changes in learning and memory (Lattal, Barrett, & Wood, 2007; Vecsey et al., 2007) as well as psychiatric disease (Mill et al., 2008). Methods now exist to document all methylated sites across the genome, which opens the way to more extensive studies of the functions of gene silencing (Ballestar & Esteller, 2008; Butcher & Beck, 2008).

It is interesting to contemplate the theorizing of Lamarck and McDougall on the inheritance of acquired characters in the light of recent evidence of epigenetic inheritance. In 1807 Lamarck (Lamarck, 1984) argued that exercise of a mental faculty could gradually, over many generations, improve the hereditary basis for that ability in the organ of thought: "Now every change that is wrought in an organ through a habit of frequently using it, is subsequently preserved by reproduction, if it is common to the individuals who unite together in fertilization for the

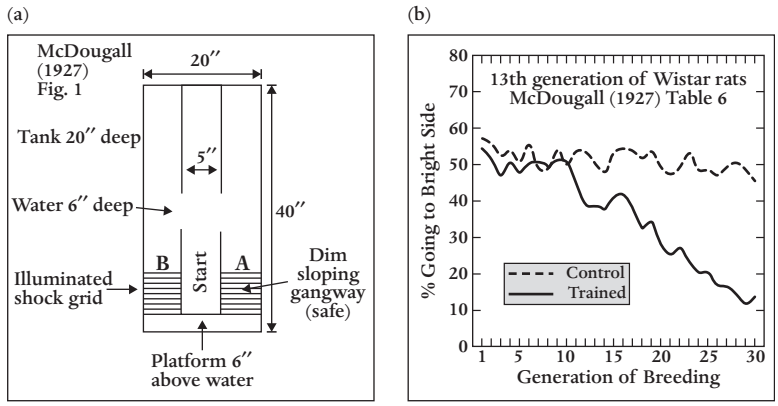

Figure 5.1. (a) McDougall's (1927) water maze; (b) Rate of learning for the 13th generation of Wistar rats.

propagation of their species. Such a change is thus handed on to all succeeding individuals in the same environment, without their having to acquire it in the same way that it was actually created." McDougall (1927) sought to demonstrate this by training rats in a maze and breeding the trained offspring to show that rate of learning gradually improved over generations in the absence of genetic selection. Figure 5.1a illustrates the water maze used by McDougall to train rats. The illuminated grid ramp provided an electric shock that animals learned to avoid, while the dimly lit ramp offered a means to escape from the water onto a platform. Several minor variations were introduced into the apparatus and training procedure as the study progressed. Figure 5.1b graphs the rate of learning to avoid the brightly lit side that involved electric shock. In the 13th generation of breeding, the rats from the line that had experienced training on previous generations showed superior learning. By our current standards of evidence, those Lamarckian experiments on behavior that were so prominent from 1920 to 1950 in psychology lacked essential control groups to prove their point, things such as replicate lines and lines trained to approach the brightly lit ramp as well as the dim ramp. Nevertheless, recent findings on epigenetic effects suggest that we might want to take a fresh look at the old arguments.

The Future of Additivity Theory in Psychology

One of Gottlieb's prime targets for his critique of the use of heredity in psychology was the notion that genotype and environment act separately in development and therefore additively in algebra. Algebraic additivity allows estimates of the percentage of variance in a phenotype that arises from genetic sources. Variance partitioning is central to the application of quantitative genetics in psychology, as embodied in heritability analysis based on comparisons of monozygotic and

dizygotic twins. Many shortcomings in this kind of analysis have been identified (Devlin, Daniels, & Roeder, 1997; Kempthorne, 1978, 1990; Wahlsten, 1979, 1994, 2007). We can now ask whether these critiques have had any impact on those who espouse heritability analysis and whether advances in molecular biology have aided or impeded their mission.

It appears that there has been little change within academic psychology. If anything, psychology is becoming more remote from contemporary biology as biological psychology is deleted from the curriculum from an increasing number of departments and animal labs are disappearing from the undergraduate experience. Many of the leading exponents of heritability analysis are preaching the doctrine of additivity unchanged by recent findings. One noteworthy exception is McClearn (McClearn, 2006).

Looking at the larger world of neuroscience and biomedical science, searches of PubMed reveal that heritability and twins studies, the stock in trade of quantitative human genetics applied in psychology, are a small fraction of the total corpus of work in these fields (Table 5.1, Figure 5.2). Table 5.1 reveals that less than 1% of published studies dealing with genetics utilize the term heritability at all, and the fraction is even smaller when comparison is made with the collection of studies that focus on single gene inheritance (terms such as gene, mutation, knockout, linkage). Even for twin studies, the vast majority do not utilize heritability. Figure 5.2 shows how work with mice and mouse models has grown substantially during the past decade, while mouse studies utilizing heritability have remained mired at a pathetic 20 to 24 studies per year. The use of heritability in human genetic research, which in any year has been a minuscule fraction of the total corpus of work, appears to be on the decline. At the recent meeting of the International Behavioural and Neural Genetics Society in Dresden, Germany, there seemed to be no interest whatsoever in the debates that animated our discussions two decades ago. Colleagues young and old have now taken the plunge into the sea of new genetic knowledge and are struggling to stay on top of the latest findings and utilize new techniques to help understand behavior.

Table 5.1. Search of PubMed for keywords by decades

Search term	*1970–1979*	*1980–1989*	*1990–1999*	*2000–2009*
gene	31,848	117,495	425,730	776,760
genetic	46,819	97,470	244,606	449,618
mutation	38,757	59,242	156,859	260,224
knockout	0	0	8,266	58,534
linkage	5,662	12,462	27,070	40,635
inbred (mouse OR mice)	27,637	70,542	98,167	135,801
twin OR twins	5,037	6,685	9,993	15,516
heritability	328	729	1,429	4,024

Source: http://www.ncbi.nlm.nih.gov/pubmed.

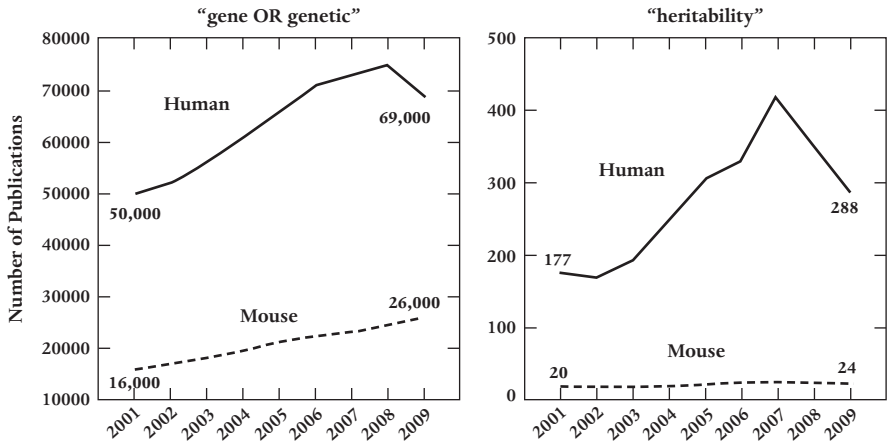

Figure 5.2. Number of publications utilizing "gene or genetic" or "heritability" from 2001 to 2009.

Note: Based on a search of the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed), plotted separately for those involving humans or mice. The counts for mice used the search phrase "mouse OR mice." Human and mouse counts are not entirely mutually exclusive, because a few publications may have addressed both species. For the year 2009, the number of articles for the first 6 months of the year was doubled to obtain an estimate.

To their credit, several colleagues in psychology have made serious efforts to utilize one of the tools provided by the human genome sequence, one that is valuable for detecting genetic variants that may influence behavior both abnormal and in the normal range of variation. Variants that increase or decrease a phenotype by a small amount are known as quantitative trait loci (QTLs). We now know there are hundreds of thousands of apparently neutral genetic markers scattered widely across the genome. A single nucleotide polymorphism (SNP) exists when people in a population have different nucleotides at one specific location in the genome. If one of these SNPs resides on a chromosome close to an unknown QTL that alters intelligence, for example, then there should be an association between the marker genotype and IQ test score in a large sample (Altshuler, Daly, & Lander, 2008). The methodology for conducting a whole genome scan for such QTLs is well developed, and researchers are cognizant of the need to study large samples in this quest (McKay, Stone, & Ayroles, 2009).

Five large studies were reported recently of whole genome scans in search of QTLs influencing human intelligence in the normal range. A summary of that evidence (Posthuma & de Geus, 2006) indicated that there were very few regions of a chromosome where any study found evidence of a statistically significant association of IQ with a SNP and, more importantly, there was only one region (p arm of chromosome 6) where there was any noteworthy agreement among the studies, with four of the five reporting at least suggestive evidence for that region.

Even then, there was no indication of what gene, if any, might be giving rise to the cluster of associations, and hundreds of genes were present in the interval. More startling and sobering was the observation, supported by at least four of the five studies, that 12 previous reports in the literature of a specific gene allegedly altering IQ was *not* associated with any nearby SNP. Thus, not only did the studies fail to locate any new gene influencing IQ, but they also demonstrated that the entire published literature on single gene effects on IQ in the normal range of variation consists of false positives.

The SNP methodology is not capable of proving that there are no genes influencing IQ. All available evidence from twins, adoptees, and correlations of relatives suggests that there must be such genes scattered widely across the genome. What the method can and now has shown, however, is an upper limit for the effect size of such genes. With the large sample sizes used in the recent studies, there was considerable statistical power to detect any gene associated with an IQ difference of 0.1 standard deviation or more (Dreary, Spinath, & Bates, 2006). As Plomin and colleagues remarked, IQ must be influenced "by many more QTLs of much smaller effect size than previously imagined." (Plomin, Kennedy, & Craig, 2006) By any standard, one-tenth of a standard deviation is a very small effect, one in which the genetic polymorphism could account for a trivial 1% of variation in IQ scores in the population.

It appears that the new molecular biology has given tools to the psychologist that demonstrate dramatically the minuscule size of any specific gene effect on IQ. When effects are so small, there is no way that psychologists will ever be able to utilize other molecular genetic tools to either anticipate or enhance human intelligence. This particular line of investigation appears to have reached a dead end. Few in the field of human behavior genetics may be ready to admit this painful reality. Of course, Gilbert Gottlieb would not have experienced pain when reading these recent findings. Neither would they have inspired joy in his heart. I do believe he would lament the waste of resources that have been and are still being devoted to the quest for genes for IQ.

Despite the many advances in genetics and powerful new tools for detecting and understanding the involvement of specific genes in behavior (Wahlsten, 1999), an influential group of authorities in psychology continues to cling to a belief in additivity of genetic and environmental effects and emphasizes partitioning of variance. According to Gilbert Gottlieb in his last interview: "These points of view persist because they offer simplistic answers, and that is also why they are doomed to fail."

References

Alberts, B., Johnson, A., Lewis, J., Roberts, K., & Walter, P. (2008). *Molecular biology of the cell* (5th ed.). New York: Garland Science.

Alia-Klein, N., Golstein, R. Z., Kriplani, A., Logan, J., Craig, I. W., Henn, F., et al. (2008). Brain monoamine oxidase-A activity predicts trait aggression. *Journal of Neuroscience*, **28**, 5099–5104.

Altshuler, D., Daly, M. J., & Lander, E. S. (2008). Genetic mapping in human disease. *Science*, **322**, 881–888.

Ballestar, E., & Esteller, M. (2008). SpanShot: The human DNA methylome in health and disease. *Cell*, **135**, 1144.

Barnett, L., Buckley, C. L., & Bullock, S. (2009). Neural complexity and structural connectivity. *Physical Review E Statistical Nonlinear Soft Matter Physics*, **79**, 51914.

Bascompte, J. (2009). Disentangling the web of life. *Science*, **325**, 416–419.

Butcher, L. M., & Beck, S. (2008). Future impact of integrated high-throughput methylome analyses on human health and disease. *Journal of Genetics and Genomics*, **35**, 391–401.

Caspi, A., McClay, J., Moffitt, T. E., Mill, J., Martin, J., Craig, I. W., et al. (2002). Role of genoytpe in the cycle of violence in maltreated children. *Science*, **297**, 851–854.

Caspi, A., & Moffitt, T. E. (2006). Gene-environment interactions in psychiatry: Joining forces with neuroscience. *Nature Reviews Neuroscience*, **7**, 583–590.

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., et al. (2003). Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science*, **301**, 386–389.

Caspi, A., Williams, B., Kim-Cohen, J., Craig, I. W., Milne, B. J., Poulton, R., et al. (2007). Moderation of breastfeeding effects on the IQ by genetic variation in fatty acid metabolism. *Proceedings of the National Academy of Sciences, USA*, **104**, 18860–18865.

Coba, M. P., Pocklington, A. J., Collins, M. O., Kopanitsa, M. V., Uren, R. T., Swamy, S., et al. (2009). Neurotransmitters drive combinatorial multistate postsynaptic density networks. *Science Signalling*, **2**, ra19.

Cox, E. T., Brennaman, L. H., Gable, K. L., Hame, R. M., Glantz, L. A., LaMantia, A. S., et al. (2009). Developmental regulation of neural cell adhesion molecule in human prefrontal cortex. *Neuroscience*, **162**, 96–105.

Crusio, W. E. (2004). Flanking gene and genetic background problems in genetically manipulated mice. *Biological Psychiatry*, **56**, 381–385.

Crusio, W. E., Goldowitz, D., Holmes, A., & Wolfer, D. (2009). Standards for the publication of mouse mutant studies. *Genes Brain and Behavior*, **8**, 1–4.

Devlin, B., Daniels, M., & Roeder, K. (1997). The heritability of IQ. *Nature*, **388**, 468–471.

Dreary, I. J., Spinath, F. M., & Bates, T. C. (2006). Genetics of intelligence. *European Journal of Human Genetics*, **14**, 690–700.

Eviskov, A. V., Graber, J. H., Brockman, J. M., Hampl, A., Holbrook, A. E., Singh, P., et al. (2006). Cracking the egg: Molecular dynamics and evolutionary aspects of the transition from the fully grown oocyte to embryo. *Genes & Development*, **20**, 2713–2727.

Gerstein, M. B., Bruce, C., Rozowsky, J. S., Zheng, D., Du, J., Korbel, J. O., et al. (2007). What is a gene, post-ENCODE? History and updated definition. *Genome Research*, **17**, 669–681.

Gottlieb, G. (1998). Normally occurring environmental and behavioral influences on gene activity: From central dogma to probabilistic epigenesis. *Psychological Review*, **105**, 792–892.

Gottlieb, G. (2007a). Developmental neurobehavioral genetics: Development as explanation. In B. C. Jones, & P. Mormede (Eds.), *Neurobehavioral genetics* (2nd ed., pp. 17–27). Boca Raton, FL: CRC Taylor & Francis.

Gottlieb, G. (2007b). Probabilistic epigenesis. *Developmental Science*, **10**, 1–11.

Gough, N. R., & Foley, J. F. (2009). Focus issue: Unraveling signaling complexity. *Science Signaling*, **2**, eg10.

Hickman, M., & Cairns, J. (2003). The centenary of the one-gene one-enzyme hypothesis. *Genetics*, **163**, 839–841.

Jones, B. C., & Mormede, P. (2007). *Neurobehavioral genetics: Methods and applications* (2nd ed.). Boca Raton FL: CRC Taylor and Francis.

Kempthorne, O. (1978). Logical, epistemological and statistical aspects of nature-nurture data interpretation. *Biometrics*, **34**, 1–23.

Kempthorne, O. (1990). How does one apply statistical analysis to our understanding of the development of human relationships? *Behavioral and Brain Sciences*, **13**, 138–139.

Klevecz, R. R., Li, C. M., Marcus, I., & Frankel, P. H. (2008). Collective behavior in gene regulation: The cell is an oscillator, the cell cycle a developmental process. *FEBS Journal*, **275**, 2372–2384.

Kolkova, K. (2008). Biosynthesis of NCAM. *Neurochemical Research*, *DOI 10.1007/s11064-008-9773-y*.

Lamarck, J. B. (1984). *Zoological philosophy*. Chicago: University of Chicago Press.

Lattal, K. M., Barrett, R. M., & Wood, M. A. (2007). Systemic or intrahippocampal delivery of histone deacetylase inhibitors facilitates fear extinction. *Behavioral Neuroscience*, **121**, 1125–1131.

Lees-Miller, J. P., Goodwin, L. O., & Helfman, D. M. (1990). Three novel brain tropomyosin isoforms are expressed from the rat alpha-tropomyosin gene through the use of alternative promoters and alternative RNA processing. *Molecular and Cellular Biology*, **10**, 1729–1742.

Marco, A., & Marin, I. (2009). Interactome and gene ontology provide congruent yet subtly different views of a eucaryotic cell. *BMC Systems Biology*, **3**, 69.

McClearn, G. E. (2006). Contextual genetics. *Trends in Genetics*, **22**, 314–319.

McDougall, W. (1927). An experiment for the testing of the hypothesis of Lamarck. *Journal of Psychology*, **17**, 267–304.

McKay, T. F. C., Stone, E. A., & Ayroles, J. F. (2009). The genetics of quantitative traits: challenges and prospects. *Nature Reviews Genetics*, **10**, 565–577.

Mill, J., Tang, T., Kaminsky, Z., Khare, T., Yazdanpanah, S., Bouchard, L., et al. (2008). Epigenomic profiling reveals DNA-methylation changes associated with major psychosis. *American Journal of Human Genetics*, **82**, 696–711.

Pennisi, E. (2003). Gene counters struggle to get the right answer. *Science*, **301**, 1040–1041.

Pennisi, E. (2004). Searching for the genome's second code. *Science*, **306**, 632–635.

Piekarz, R. L., & Bates, S. E. (2009). Epigenetic modifiers: Basic understanding and clinical development. *Clincal Cancer Research*, **15**, 3918–3926.

Plomin, R., Kennedy, J. K. J., & Craig, I. W. (2006). The quest for quantitative trait loci associated with intelligence. *Intelligence*, **34**, 513–526.

Posthuma, D., & de Geus, E. J. C. (2006). Progress in the molecular-genetic study of intelligence. *Current Directions in Psychological Science*, **15**, 151–155.

Rampon, C., Jiang, C. H., Dong, H., Tang, Y. -P., Lockhart, D. J., Schultz, P. G., et al. (2000). Effects of environmental enrichment on gene expression in the brain. *Proceedings of the National Academy of Sciences, USA*, **97**, 12880–12884.

Robinson, G., Fernald, R., & Clayton, D. (2008). Genes and social behavior. *Science*, **322**, 896–900.

Rottach, A., Leonhardt, H., & Spada, F. (2009). DNA methylation-mediated epigenetic control. *Journal of Cellular Biochemistry*, 10.1002/jcb.22253.

Scheithauer, H., Ittel, A., Josephs, I. E., & Mack, W. (2007). Integrating nature and nurture through developmental systems: The contributions of Gilbert Gottlieb. *European Journal of Developmental Science*, **1**, 97–207.

Shaked, I., Zimmerman, G., & Soreq, H. (2008). Stress-induced alternative splicing modulations in brain and periphery: Acetylcholinesterase as a case study. *Annals of the New York Academy of Sciences*, **1148**, 269–281.

Singh, R. K., Tapia-Santos, A., Bebee, T. W., & Chandler, D. S. (2009). Conserved sequences in the final intron of MDM2 are essential for the regulation of alternative splicing of MDM2 in response to stress. *Experimental Cell Research*, 10.1016/j.yexcr.2009.07.017.

Szyf, M., McGowen, P., & Meaney, M. J. (2007). The social environment and the epigenome. *Environmental and Molecular Mutagenesis*, **49**, 46–60.

Vecsey, C. G., Hawk, J. D., Lattal, K. M., Stein, J. M., Fabian, S. A., Attner, M. A., et al. (2007). Histone-deacetylase inhibitors enhance memory and synaptic plasticity via CREB: CBP-dependent transcriptional activation. *Journal of Neuroscience*, **27**, 6128–6140.

Wahlsten, D. (1979). A critique of the concepts of heritability and heredity in behavioral genetics. In J. R. Royce, & L. Mos (Eds.), *Theoretical advances in behavioral genetics* (pp. 425–481). Alphen aan den Rijn, the Netherlands: Sijthoff and Noordhoff.

Wahlsten, D. (1994). The intelligence of heritability. *Canadian Psychology*, **35**, 244–258.

Wahlsten, D. (1999). Single-gene influences on brain and behavior. *Annual Review of Psychology*, **50**, 599–624.

Wahlsten, D. (2007). Progress in the developmental genetic analysis of behavior. *European Journal of Developmental Science*, **1**, 131–137.

Wahlsten, D., Crabbe, J. C., & Dudek, B. C. (2001). Behavioral testing of standard inbred and 5HT1B knockout mice: Implications of absent corpus callosum. *Behavioural Brain Research*, **125**, 23–32.

Weaver, I. C. G., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., et al. (2004). Epigenetic programming by maternal behavior. *Nature Neuroscience*, **7**, 847–854.

Xie, J., & McCobb, D. P. (1998). Control of alternative splicing of postassium channels by stress hormones. *Science*, **280**, 443–446.

6

The Roles of Environment, Experience, and Learning in Behavioral Development

George F. Michel

In the science of behavioral development, we want to understand how the complete behavioral repertoire of an individual develops along trajectories (starting from conception and proceeding through differences in forms and processes across the lifespan) that yield both individual differences and species-typical similarities. For some decades, the conceptual frame for examining such questions was that behavior developed from an organism-environment interaction. The task for the developmental scientist was to demonstrate that both organism variables (e.g., genes, hormones, brain processes) and environmental variables (e.g., rearing conditions, social conditions, training) contributed to the developmental expression of some behavioral trait (e.g., memory, anxiety, parental care). Demonstrating that individual differences in the level of some hormone, presence of some genetic factor, or the activation of some brain area were responsible for differences in some behavioral trait (e.g., parental care) was always qualified by the recognition that environmental factors also played a role. Similarly, although rearing conditions, social influences, or teaching conditions were demonstrated to produce differences in the behavioral trait (e.g., parental care), these demonstrations were qualified by recognition that organism factors also played a role.

Essentially, there were two methods for examining such interaction: 1) hold some aspects of a group of individuals constant (e.g., their genotype, hormonal condition) while varying some aspect of their environment; 2) hold some aspects of the environment constant (e.g., general rearing conditions) while varying some aspect of the organism (e.g., genotype). More complex designs would permit statistical analysis that would partition the phenotypic variability among individuals in the groups into those parts that were related more strongly to the

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individual (presumably organism factors), those that related more strongly to the individual's environment, those that related to the interaction between the individual and environmental factors (and this interaction could be partitioned further into those shared and unshared environments of individuals), and error.

These studies always revealed that the organism-environment interaction was related to the variability of the behavioral traits among individuals. With more sophisticated characterizations of both organism and environment, such research methodologies revealed rather striking relations of the differences in the behavioral trait to the interaction. For example, individual variability and similarity in the expression of conduct disorders in human children was highly related to the genotype-environment interaction (Jaffee et al., 2005). Individuals who were maltreated as children were more likely to be diagnosed as adults with antisocial personality or conduct disorder if they had a polymorphism for low expression of the monoamine oxidase A (MAOA) genotype compared to those with a genotype resulting in high levels of expression of MAOA (Caspi et al., 2002).

Unfortunately, these research methodologies reveal nothing about the actual developmental trajectories that led to the expressed phenotypes of individuals. There is no account of the relationship of the individual's earlier appearing phenotypes to the phenotypes of interest or of the mechanisms governing the transitions (or lack thereof) in succession of expression among these developing traits. Nor, in this common gene-environment interaction approach, have we explanations for why some individuals with both the "predisposing" polymorphism and the "predisposing" child abuse did not become adults with antisocial personalities (we must presume that somehow they are functioning with the disorder). Also, left unexplained is how some individuals without either predisposing condition, nevertheless did develop an antisocial personality as adults. The developmental trajectory simply is assumed to reflect some interaction among these factors during development which results in the phenotype of interest. What appears to be a developmental study with a solid organism-environment focus does not reveal anything about the influences that directly affect the creation of the developmental trajectories underlying the differences between individuals.

As a small step toward developing a solid science of behavioral development, this chapter attempts to clarify the meaning of the constructs environment, experience, and learning as they play a role in creating the trajectories of behavioral development. Therefore, I will provide definitions of these three constructs. Also, I will emphasize the distinction between the roles that the environment, experience, and learning play in affecting the developmental trajectories of the organism and the roles they play in affecting adjustments in the functioning of the organism. This chapter represents an extension of the conceptual frame of probabilistic epigenesis, originally proposed by Gilbert Gottlieb (1976, 1999) and represented in his illustration shown in Figure 6.1. In Gottlieb's scheme, developmental trajectories of behavior emerge from the reciprocal influences among several levels of organization (i.e., environment, behavior, neural activity, and genetic activity in

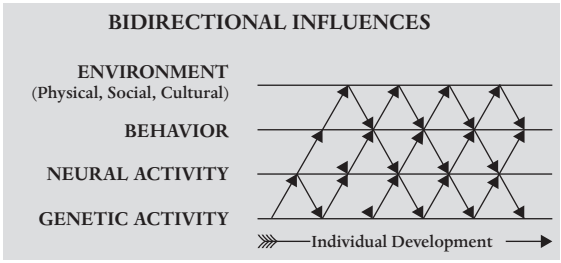

Figure 6.1. Gilbert Gottlieb's general scheme of probabilistic epigenesis.
Source: Gottlieb (1992, p. 186).

the current illustration). The illustration nicely captures Gottlieb's notion that environmental influences can be identified at the level of genetic activity and the influences of genetic activity can be identified at the level of the environment.

Organism-Environment Transaction in Behavioral Development

Although the organism-environment interaction may be an appropriate initial classification for the analysis of such trajectories, neither aspect of the interaction is stable during development. Of course, independently of the organism, the environment can change. However, during development, both the organism and its environment change as a consequence of the organism-environment interaction. What the individual is like at point X + Y in time will depend on what the individual was like at point X, what the environment was like at point X, and the kind of interaction that the individual had with its environment during the transition from X to X + Y. It is these transformations of the individual's phenotype across the lifespan that mark the developmental trajectory. Given this description, we can identify relationships among individuals according to the pattern of their trajectories. Comparison of trajectory analyses permit identification of: 1) those with different pathways that lead to some commonality at a particular later phase of development (equifinality); 2) those with parallel pathways of different phenotypes at all phases of development; 3) those with diverging pathways that begin with a set of early common phenotypes but separate from thereon; 4) those with fluctuating pathways that frequently converge and diverge during development. To understand both individual and species-typical developmental trajectories, we have to determine exactly how the environment interpenetrates with the individual's characteristics throughout the lifespan. This process of organism-environment interpenetration produces the characteristics expressed as the successive phases of the developmental trajectory.

The organism-environment interaction should not be confused with the heredity-environment or genes-environment interaction. Heredity is concerned with why offspring resemble parents. Heredity consists of those things transmitted from parent to offspring that contribute to the development of that resemblance. In 1909, Johannsen (1909, 1911) introduced the word "gene" as an abstraction to be used to describe whatever it was that parents passed to their offspring so that they exhibited parent-like traits. Johannsen also asserted that an organism's appearance, physiology, and behavior (its phenotype) derive from an interaction between its genes and its environment. With the "gene" representing inheritance, the heredity-environment interaction became the genes-environment interaction. During the six decades following Johannsen's introduction of the gene construct, scientific research transformed "gene" from an abstraction to a concrete reality. By 1970, a gene became a specific stretch of DNA containing the instructions to make a protein molecule.

Every time a cell divides, it replicates its DNA (the location of its genes), and parents passed along some of their genes to their offspring through their gametes. If you inherited any trait or a disposition for a trait from your mother or father, genes on the DNA were considered the mechanism for such inheritance. It was presumed that the genes ensured the occurrence of the trait, or the disposition to express the trait, by controlling development. However, if we acknowledge that the developmental process is a consequence of the transaction between an organism and its environment, then, although it is easy to consider the activity of DNA as playing a role in such transactions, it is unlikely that DNA acts as a "blueprint," "director," or "controller" of development.

The gene construct has undergone extensive transformation during the past two decades (Gerstein et al., 2007). In the 1970s and 1980s a gene was defined as those specific arrangements of DNA bases (A, G, C, T) that serve as instructions for piecing together the body's proteins. There was a "one gene yields one protein" assumption, with "messenger" RNA (long strands of RNA or mRNA) representing the information on the DNA that is needed to construct a particular protein. However, a recent report from a project created to determine the function of every piece of DNA in the human genome (called ENCODE – Encyclopedia of DNA Elements), revealed that only about 1% of the three billion paired bases of human DNA (or likely that of most species) contribute to protein codes. Moreover, via a process of alternative *splicing*, a sequence of bases associated with the production of a particular protein (previously considered to be a single gene) can contribute to the making of more than one protein. Thus, molecular biological research in the 21st century has completely challenged the definition of a gene as a specific arrangement of DNA bases specifying a particular protein.

To understand *splicing*, we need to know that those DNA sequences that contribute to coding a particular protein (exons) are interspersed with sequences of bases that do not contribute to that coding (introns). The introns are removed (spliced) to make the actual mRNA transcripts for specifying the protein. Somehow,

a cell can select different combinations of bases in a sequence to make different mRNA transcripts which leads to the production of different proteins. Some cells combine exons from other coding regions (even from different chromosomes) to make certain mRNA transcripts. Experimental evidence indicates that all of the ''genes'' are products of splicing and not simply sequences on the DNA. The ENCODE estimates are that the average protein coding region on DNA produces nearly 6 different mRNA transcripts and different types of cells produce different transcripts from the same coding region. DNA operates more like a raw material than a blueprint.

In addition to being sensitive to alternative splicing, some base sequences encode short clips of RNA molecules which act like DNA and transmit genetic information to the next generation. Also, some RNA sometimes act like a protein, catalyzing chemical reactions that affect the relative proportions of other molecules in the cell. Thus, although DNA seems to function as an important ingredient in the biochemical activities of a cell (and hence in the functioning of an organism), it is not the blueprint, or master controller of either development or protein formation. Indeed, whether or not some piece of DNA was available for transcription, splicing, and eventual translation into a protein depends on whether certain proteins and other molecules had bound onto bits of DNA. DNA is studded with millions of proteins and other molecules, which seem to determine those coding sequences that can produce transcripts and those that cannot. New cells inherit those molecules along with DNA. In other words, heredity operates through a second, ''epigenetic,'' mechanism.

Epigenetic inheritance involves changes in ''gene'' expression patterns without any changes in the DNA sequence. Such effects include DNA methylation and histone modifications, among others. DNA is packaged around spool-like bundles of proteins called histones which can ''wind up'' a stretch of DNA so that the cell cannot make transcripts from it. Gene expression can only occur when DNA can be unwrapped from the histone proteins to expose sequences of its nucleic acids. Histones have tails that stick out from the core. In most cases, methylation of those regions of DNA that promote the exposure of other DNA regions for transcription inhibits that transcription. In contrast, acetylation of the histone tail enhances transcription (Dolinoy, Weidman, & Jirtle, 2007; Gore, 2008; Ho and Tang, 2007). All of the molecules that affect DNA transcription are called epigenetic ''marks'' and they are essential for the form and function of cells in the organism. As an embryo continues to develop, epigenetic marks in different cells are altered, and as a result the cells develop into different tissues. Once the final pattern of epigenetic marks is created, it remains as the cell divides; the marks on gamete cells ensure that descendants carry the same set of marks.

Epigenetic marks are intriguing not just for their effects on DNA transcription, but also for how they are created. To place methyl groups on a sequence of DNA, a cluster of proteins must be guided to the right place by an RNA molecule specific to DNA sequence. The so-called RNA interference (RNAi) system can direct this

activity, via small RNA strands. In addition to controlling DNA methylation and modifying histones, these RNAi molecules target mRNA and break it down into small segments. In this way, the RNAi molecules ensure that a certain sequence of DNA cannot be translated into its protein. The ENCODE project estimates that 93% of the human genome produces various RNAi transcripts. Thus, any environmental events that affect RNAi can control the activity of coding sequences of DNA.

If, as currently defined, a gene is the smallest unit underlying inherited traits, it has to include not just a collection of exons, but the epigenetic marks on them and those marks that affect the ability of those exons to be transcribed, as well. It, also, must include the various RNAi factors that affect transcription. All of those additions to the DNA can reflect the consequences of environmental factors. Thus, the modern concept of ''gene'' includes the DNA's history of environmental exposures. This removes the gene construct further away from its instantiation as simply a sequence of DNA and replaces it with a more abstract definition, similar to that provided originally by Johannsen. Moreover, since epigenetic marks can represent the influence of the environment, more research is examining the influence of environment on the inheritance of phenotypic traits. Such research has led to the growth of the discipline of ecological developmental biology (Gilbert & Epel, 2009).

Epigenetics and Ecological Influences in Developmental Biology

Ecology can be defined broadly as the study of the relations of organisms to one another and to their physical surroundings. The function of ecological developmental biology (eco-devo) is to unravel ecology's role in development. Because, traditional developmental biology focused on a few species (model organisms), studied in uniform lab environments, it seemed that environmentally contingent development was rare or unimportant. Whenever environmental influences were observed, they typically disrupted normal development. Consequently, the scientific field of teratology examined the influence of drugs (from drugs of abuse to those provided medicinally), disease and parasitic organisms, chemical additives to food and household products, etc. on the normal development of the individual. However, demonstrating that some environmental factor can disturb development is theoretically quite different from demonstrating that some environmental factor contributes to normal development. In the former, the environment can be merely a permissive factor in development whereas in the latter, it can be instructive.

Eco-devo expanded the analysis of development to a diverse set of organisms which has revealed that information in the genome is intertwined with ecological influences from the environment in different ways at different periods throughout

the lifespan (c.f., Gerhart & Kirschner, 1997; Gilbert, 2006; Kirschner & Gerhart, 2006; Raff, 1996; Schlichting & Pigliucci, 1998). The DNA is simply a part of a complex system (network) of causes that operates throughout the lifespan to produce phenotypic variability. Cells are chemical manufacturing plants controlled by an intricate and dynamic set of chemical messengers that travel within and between cells to permit the transcription (or not) of specific parts of the DNA, and the timing of these actions is important. Such ''gene switching'' sets the steps in motion that lead to protein synthesis or other key changes in cell function which is the basis of development.

The layer of biochemical reactions that do or do not permit the transcription of DNA is called the ''epigenome.'' There are many ways by which environmental information can affect the epigenomic process and new ways continue to be discovered. The epigenome plays a major part in heredity, as well as in development and health. These epigenomic processes begin before conception during the formation of germ cells (eggs and sperm) and continue throughout the lifespan. Because the epigenome can change according to an individual's environment and can be passed reliably from generation to generation, there has been a revolutionary change in biological thinking about heredity and evolution. For many scientists, evolution is no longer changes in gene frequencies.

DNA is part of a network of developmental causes that lead to the manifestation of traits that have general properties in common across individuals while retaining individual differences. Evolution is change in the frequencies of alternative developmental causes that yield variations in developmental trajectories (a phrase that is more cumbersome than ''changes in gene frequencies'' but nevertheless more accurate). Consequently, modern evolutionary biology has turned to developmental biology as a source for information about how trait variability (the substrate upon which natural selection and other evolutionary mechanisms operate) can emerge during development. This approach is called ''evo-devo'' (e.g., Fox Keller, 2002; Gilbert, 2006; Jablonka & Lamb, 2005; Raff, 1996; West-Eberhard, 2003). For evolutionary developmental biology, understanding how organisms are built (development) must proceed understanding how the process of building organisms can be changed (evolution). Evolution by natural selection cannot occur without the variability created by development. Natural selection operates at the phenotypic level and the openness of developmental processes to environmental influences creates the individually unique trajectories that result in phenotypic variability.

Thus, research in eco-devo, teratology, and evo-devo reveal that certain environmental modifications of development can be passed stably across generations. In the case of disease, these environmental influences can profoundly influence the health of descendents living in a perfectly benign environment and with no apparent genetic disposition for the disease. Similar effects apply to an individual's behavioral (including social/emotional) functioning. Understanding the influences on development of both present and past environments provides

important insights to the causes of cross-generational stability in behavior and physiology. In contrast to teratology, however, knowledge of ecology's role in development also is demonstrating the essential influence of environmental factors on how organisms develop normally and evolve.

Thus, the concept of heredity has been expanded in the past 15–20 years to include the epigenetic consequences of environmental events. Moreover, research has shown that there are other types of environmental conditions that parents transmit to their offspring including viruses, bacteria, fungi, various parasites, habitats and shelters (constructed nests), relatives, food (prey types and edible vegetation – c.f., Mennella, Ziegler, Briefel, & Novak, 2006; Walker et al., 2007). As West, King, and Arberg (1988) note, parents provide an ecological niche for the offspring, which ensures a delimited range of environmental events and a delimited range of potential experiences for the offspring. If offspring inherit a niche from the parents, then whatever constitutes the environment for an organism cannot be presumed a priori but must be specified for that organism. Therefore, the measurement of any environmental variable must be defined, in part, by reference to the organism of interest. This makes the notion that the heredity-environment interaction involves an interaction of two separate factors, problematic, at best.

Considering the environment from the organism's perspective is not new. von Uexkuell (1957) called this personal quality of environments the *Umwelt*. He defined Umwelt by the perceptual processes and biomechanical/physiological actions possible for an individual of that species at that point in its lifespan. For example, a particular level of viscosity of the medium within which the organism dwells depends on the morphological features of the organism as well as the medium. Thus, water is a different viscosity for sea anemone than for the clown fish hiding among its tentacles and the soil's viscosity is different for earthworms and the moles that eat them. This effective environment involves many other features that potentially could impact the organism. Some features can be quite consistent across a wide range of organisms (e.g., gravity, atmospheric pressure, oxygen content of the medium, ultraviolet radiation for most land and water dwelling organisms, heat from sun or earth's core); whereas others are quite delimited to a particular niche (consider the atmospheric pressure in deep seas versus mountain tops).

Thus, determination of the effective environment for the development of a particular kind of organism requires extensive knowledge about the range of environments that are typically experienced by that type of organism. Environmental factors outside of that range may impinge on the organism and produce individual differences in development, including abnormalities. However, it is unclear as to what such impingement means for its normal individual or species-typical development. This is an issue that the eco-devo approach to development raised against the use, by traditional developmental biology, of about six model organisms, all of which had been selected for small body size, large litter size, rapid embryonic development, early sexual maturation, the nearly immediate separation of the germline from somatic lines, and the ability to develop within the laboratory

(Gilbert, 2001). Moreover, the highly controlled lab environment both eliminates potentially important environmental influences on development and creates unintended, artificial, environmental influences, which remain stable within the lab environment. Similar critiques of the role of model organisms in behavioral research have been made (Michel, in press). Thus, in addition to DNA, the individual inherits an epigenetic pattern, an ecological and social niche, and perceptual and behavioral biases from the parents.

The Concept of Environment

Defining environment as conditions and events that are outside of the organism, but nonetheless impinge on it, can be confusing in biology because it depends on the level of organism examined and the permeability of the boundary of the organism (e.g., membrane or skin). In biology, cell theory maintains that all living things (with the exception of viruses) consist of cells and all cells have a membrane. In single-celled organisms, the membrane and everything inside of it constitutes the organism and everything outside the membrane constitutes the environment. Single-celled organisms have membrane boundaries that can be differentially permeable to various substances in the environment. Protein constructed gaps in the membrane and transport proteins in the membrane permit certain substances to enter and leave the cell depending on their size, shape, and biochemical properties. Moreover, certain proteins in the membrane are ''activated'' (i.e., change form/biochemical properties) or ''deactivated'' by substances in the environment of the cell.

These changes in the membrane proteins subsequently induce changes in the biochemical activity of the cell leading to changes in permeability of the membrane and/or alterations in the cell's protein production and biochemical activities. Moreover, because the cell membrane is a double layer of lipids, many lipid compounds can fuse with the membrane and pass into the cell directly. Certain physical properties of matter (e.g., temperature, electromagnetic energy) are not hindered by the cell's boundary but do affect the cell's biochemical operations and characteristics. All of these transactions with the environment need to be considered when examining an environmental influence on a single-celled organism.

The questions raised by an environmental influence on a single-celled organism are: is the environmental factor: 1) affecting the single-celled organism by entering the cell; 2) altering the membrane characteristics of the cell so that other environmental factors can enter which, in turn, affect the activities of the organism; 3) changing the biochemical activities of the organism by affecting the proteins in the membrane of the single-celled organism; 4) affecting the temperature of the cell, etc.? These mechanisms of influence may enhance or hinder protein production, the enzymatic activity of proteins, the structure of proteins (which affects their

properties), the organism's energy production, etc. Indeed, at this level it is quite possible for an environmental factor to directly interact with the organism's DNA, even to the point of inducing a mutation in the DNA (e.g., radiation). I propose that consideration of some or all of these environmental influences as experiential would be inappropriate, even if the organism seems to exhibit learning-like changes in behavior as a result of changes in the environmental factor, as was once demonstrated for Paramecia (Day & Bentley, 1911).

The cell-environment transactional mechanisms that operate in single-celled organisms are repeated in all cells of multicellular organisms and permit the ''communication'' among cells needed to regulate the establishment of differentiated tissue and organs that occurs during the developmental transition from a single cell to a multicellular organism. This process is called secondary induction in embryology (Gilbert, 2001). Thus, cell division creates the conditions of environmental differentiation because some cells are completely surrounded by other cells; whereas other cells are not. The substances leaked or secreted through the membranes of the daughter cells contribute to the environment of each other. Variability in the spatial landscape of the cells, as they divide, also contributes to their variability in the secretions they produce as environments for neighboring cells and in their responsiveness to the secretions of their neighbors (c.f., Michel & Tyler, 2007a).

As cellular differentiation and morphogenesis complete the creation of tissues and organs, the cell-environment transactional sensitivity continues to permit the communication among the developing tissue and organs that is needed to regulate complex functions (e.g., digestion, metabolism, movement) in multicellular organisms. Indeed, several systems develop (cardio-vascular, lymphatic, ventricular, etc.) which facilitate the communication and interaction among tissues and organs. Other systems (nervous, endocrine, immune, etc.) accentuate and enhance such communication.

The cell-environment transactional mechanisms during development also permit the organization of tissues and organs receptive to particular environmental physical manifestations (e.g., sensory systems), others that are capable of generating movement toward or away from such environmental manifestations (e.g., skeletal-muscular systems), and still others that are capable of rapidly mediating the relation between sensory systems and skeletal-muscular systems (e.g., nervous system, endocrine system). Such cell-environment transactional mechanisms are responsible for the functioning of the cells, tissue, organs, organ systems, and the organism as well as their developmental trajectories. However, not all environmental factors that affect cell-environment transactional mechanisms affect developmental trajectories. Identifying which do requires systematic empirical investigation.

Multicellular organisms have specific tissues that define the boundary of the organism. For example, in humans these tissues include the skin and the linings of the digestive and respiratory tracts. Everything inside that boundary is the

organism whereas everything outside of that boundary is the environment. This is the usual definition of environment for most psychologists. However, there can be confusion. For most animals, food often needs to be digested (catabolized to a simpler biochemical form such as glucose and amino acids) before it can be transported into the organism. The digestive tract is a boundary that keeps food stuffs from entering the organism until it is digested into a form capable of being absorbed into the organism's circulatory system. Environment, as used in common parlance, often fails to recognize that what is ingested remains part of the environment until it is transported from the boundary cells of the digestive tract to the circulatory system. Similarly, volatile substances remain part of the environment until they are transported across the lining of the respiratory tract.

Thus, most of the waste eliminated from the digestive tract, or expelled during respiration, always was, and continues to remain, a part of the organism's environment. Although transformed as a consequence of the digestive process, the eliminated waste is still part of the organism's environment. This aspect of the organism's environment can be used in the construction of shelters (e.g., in certain communal insect societies), to mark territories (e.g., giraffes), to lure (e.g., horses) and arouse mates (e.g., hippopotamuses), etc. as well as to influence the activities of other species. When employed in this manner, waste products have the potential to act as an experiential factor.

Of course, certain ingested chemical substances are absorbed directly into the organism without the need for digestion. Also, certain volatile substances (besides O_2 and CO and CO_2) are absorbed through the olfactory mucosa and the lungs and are transported throughout the body by the circulatory system. Indeed, some substances can be absorbed through the skin. All such directly absorbed factors, along with those that are converted by digestion, are capable of affecting the organism's cell-environment transactional mechanisms. As such, these would constitute an environmental influence on the organism. This process is called tertiary induction in embryology (Gilbert, 2001). Many medicines and drugs of abuse are environmental influences on humans because of their absorption across our boundaries.

Many environmental factors associated with diet can have developmental consequences on the individual and these consequences can include affecting the development of the individual's offspring without any change to the sequencing of DNA (Patisaul & Adewale, 2009). Environmental "pollutants" such as PCBs (polychlorinated biphenyls that make up many common plastics) alter endocrine physiology in ways that mimic or block the action of the organism's internally produced hormones. Known as endocrine disrupting chemicals (EDCs), such toxins trigger processes that act very early in development and in very small amounts. Because hormone processes regulate DNA expression, these disruptions can affect heredity. The effects of these EDCs may not be immediately apparent; rather, they are often manifest much later in life as decreases in fertility, susceptibility to diseases, such as cancer, diabetes, and obesity, and altered brain structure.

Anway, Cupp, Uzumcu and Skinner (2005) demonstrated, using male mice, that fungicides and pesticides used in food and wine may alter heredity for as many as four future generations. Decreases in fertility, sperm defects, and increased incidence of several diseases, including cancer, prostate disease, and immune defects, were found in 90% of the descendents of exposed adults. These toxin-induced changes involve mechanisms that modify sperm production in the exposed generation at the time of gamete formation. Consequently, they are passed on to future generations, because altered sperm or eggs give rise to gametes that retain the original hereditary change for many generations, even in the absence of toxic exposure (Anway & Skinner, 2006; Jirtle & Skinner, 2007). The effect appears to permanently alter the familial line. Epidemiological research on humans suggests that similar exposure may be linked to disease patterns in humans (e.g., Ma et al., 2002). Epigenetic factors also can affect behavior. Abel, Moore, Waselewsky, Zajac, and Russell (1989) found that male mice that had inhaled cocaine passed memory problems onto their pups. Although their sperm showed no apparent DNA damage, there were changes in the seminiferous tubules, where sperm are produced, in the levels of two enzymes involved in methylating DNA (Anway et al., 2005).

It is important to distinguish between environmental factors that pass the individual's boundaries to affect development via tertiary induction and those that do not pass the boundaries but rather affect the individual via specific receptor cells. Some of the cellular mechanisms that permit the transaction of single-celled organisms with their environment are accentuated in multicellular organisms in the development of receptor cells. That is, the cell-environment transactional mechanisms during development permit the organization of receptor cells, tissues, and organs (especially, but not only, in the boundary structures) that detect certain changes in environmental substances and events. These receptors transduce the detected changes in the environment into products that can affect cell-environment transactional mechanisms. That is, the receptor cells change their properties in response to certain environmental events (e.g., changes in environmental energies, concentrations, and/or other kinds of impact on the organism's boundary cells). This change in the receptor cell enables it to affect the properties of neighboring cells. Thus, receptor cells use secondary induction to enable tertiary induction effects for those environmental influences that do not pass the organism's boundaries.

Receptor cells are often called ''sensory'' (sensing the environmental conditions). The transduction of environmental stimuli into neurophysiological and neuroendocrinological processes permits the environment to become a part of the epigenome that, in turn, affects the transcription of DNA. Such environmental transduction defines an ''experience'' for an individual. Thus, experiential influences are a sub-class of environmental influences because they require transduction, via a specialized cell, of an environmental stimulus into a product that can operate with the organism's cell-environment transactional mechanisms.

Experiential influences on development operate as a kind of tertiary induction initiated via a secondary induction mechanism (perhaps, experiential induction might best be called quartenary induction).

For example, the chemical substances in food activate specific receptor cells in the boundary tissues of the organism that eventually produce changes in hormonal secretions and/or changes in the nervous system that affect both the digestive process and the behavior of the organism. This activation of receptor cells marks an essential aspect of the definition of experience for the organism. Thus, the sight, taste, smell, sound, and feel of food are experiential factors affecting the digestive and ingestive processes of the organism.

In humans, the presence of sugars on the tongue affects receptors that initiate neural activities that increase salvation, activate stomach muscles and secretions, stimulate pancreatic secretion, etc. but also receptors for sugar eventually stimulate dopaminergic tracts in the tegmental area of the brain that increase the probability of ingesting similar sources of such sugars. In addition, there are similar neural and endocrine reactions to certain lipids, salts, alkalis, acids, etc.; all of which aid digestion and absorption. Stretch receptors in the stomach help stop ingestion by affecting the activity of the nervous system directly; they also, prompt the secretion of hormones that influence the neural mechanisms controlling ingestion. All of these activations of receptor cells mark potential experiential influences on the trajectory of development. However, many of the experiential factors that regulate ingestion do not contribute toward the developmental trajectory of the individual. Some evidence from the Dutch famine of 1945 suggests that the developmental trajectory for human preferences for certain sensory components of food (in this case, fats) may be altered by early experiences (Lussana et al., 2008).

There are many events and substances that pass across the organism's boundaries and many environmental conditions that are transduced into experiential events that affect the state of the organism without affecting any developmental trajectories. Although some parasites, viruses, stimuli, toxic chemicals, etc. affect developmental trajectories, most do not. Similarly, some experiences affect developmental trajectories but most do not. As with disease, toxic chemicals, etc., it is important to identify which environmental factors do and do not have consequences on developmental trajectories, so, too, it is equally important to identify which experiences do and do not have developmental consequences. It is likely that most experiential influences adjust the functioning of the individual's systems rather than affect the developmental trajectories of the individual's phenotypic traits.

I propose that the construct of experience be reserved for multicelluar organisms possessing a set of specialized cells (e.g., skin) that create a boundary that separates the organism from its environment. Experience would require specific ''receptor'' cells which transduce an environmental event (stimulus) into an electrochemical property involving changes in the cell's biophysics and biochemistry. Such

transduction should result in alteration of the cell's secretions and/or membrane potential. These alterations would have consequences on the receptor cell's neighboring cells with potential consequences on the entire organism. Whether these consequences affect any developmental trajectories must be a matter of empirical investigation.

The importance of distinguishing an environmental from an experiential influence on a developmental trajectory may be illustrated by the example of the differences in the development of the sex phenotypes in certain species of reptile and fish. Male and female turtles of most species have no genetic differences, but adult males and females exhibit substantial morphological, physiological, and behavioral differences. Sex is determined in these reptiles by the temperature at which the eggs are maintained during embryonic development (Bull 1983). In some species, males develop when the eggs are laid in warm areas (unshaded sand) and females develop when the eggs are laid in the shade of vegetation. This environmental difference in temperature during incubation affects the action of certain enzymatic processes in the cells that initiate different trajectories in anatomical (males are a fraction of the size of females), reproductive physiological, and behavioral development. There are no specific receptors for temperature in the developing turtle embryo that must be activated to create this environmental influence on development. Instead, temperature operates directly on the biochemical processes of differentiation of cells and morphogenesis.

Now compare the turtle situation with certain social species of coral reef fish. These fish are capable of substantial morphological, physiological, and behavioral changes throughout their lifespans when they alter their sex. They use the social behavior of their companions as cues for switching from the more common female form to the relatively rarer male form (Demski, 1987; Shapiro, 1983). The activation of receptors for light patterns, pressure waves, and such are transduced into products that affect the changes in the fish's endocrine and nervous system responsible for the transformation into males. These differences in how the environment affects sexual differentiation illustrate the importance of distinguishing between an environmental and an experiential influence on development. Moreover, the sexual differentiation of the coral reef fish illustrates the difference between an experiential influence on development and an influence of learning. Experience is essential but the fish does not ''learn'' to be male.

Thus, environmental factors can affect development in multicellular organisms via the digestive tract, skin absorption, and ''breathing.'' Moreover, there are other environmental events (e.g., electomagnetic energies, ultrasonic vibrations) that are unaffected by the boundary systems of the organism and can affect the organism's cells directly. Although these environmental factors can have profound influences on development, we would not classify them as experiential events because they lack receptor mechanism for transducing these factors into products that affect cell-environment transactional mechanisms. When investigating the organism-environment relationship, it is important to recognize that developmental

trajectories can be affected by environmental factors that directly affect the organism's cell-environment transactional mechanisms whereas others only affect it via specific receptor transduction.

If development is identified as the trajectory of phenotypic change in an organism during its lifespan as a result of its transaction with its environment, and if aspects of that relationship require receptors to transduce environmental events into processes that can affect the organism, then that environment must be defined, in part, based on capabilities of the organism's receptors and how it processes and uses the tranduced information about the environment. The same physical energies may have an effect on one organism but not another or on the organism at one point in development but not at another, all depending on the character of the receptors and how the information that they provide is processed. As noted earlier, von Uexkuell's (1957) concept of Umwelt was meant to direct research attention to this personal quality of an individual's environment. The morphology, physiology, and behavior of the individual combine to determine which aspects of ''physical reality'' may become part of the individual's Umwelt. Appreciation of the Umwelt is essential for understanding how the same environmental conditions can have differing effects on the developmental trajectories of different individuals.

The Umwelt in Behavioral Development

The concept of Umwelt forces us to recognize that individuals construct, alter, transduce, and modify their environment throughout their lifespan (Lewontin, 1982; Michel & Moore, 1995). For example, certain organisms construct nests for shelter and raising offspring. Consequently, the influence of the nest on the nest constructor's further development is self-generated. Moreover, the nest is part of the niche provided by parents that may affect the development of the offspring (West et al., 1988). As development continues through this individual-experience relation, those factors that define environmental reality for the individual will change, in part, as a consequence of the constructions previously assembled.

Individuals will alter their environments in both subtle and obvious ways. The physical alteration can arise by consuming food, depositing waste, and constructing artifacts (burrows, dams, etc.). Although individuals create many of the conditions that are necessary for their life, paradoxically, their activities can have destructive consequences for themselves or their offspring. In ecology, this alteration of the environment by living organisms is a major source for ecological succession. During behavioral development, especially for social species, creation of artifacts or social routines and ''expectancies'' with companions during early phases of development has consequences for social and physical experiences available in later phases. Therefore, the concept of Umwelt must connote a process and not stasis,

if it is to be used to understand its role in individual and species-typical developmental trajectories.

Moreover, because receptors and the consequences of their activity exhibit both absolute and relative thresholds, continuous changes in the physical environment are not necessarily identified as continuous by the individual. That is, whereas there is both an upper and lower bound to the range of environmental stimuli that the receptor will detect, within that range, continuous variations in environmental change are likely not to be matched by continuous variation in receptor activity. Rather the receptor will mark (change activity) only after certain levels of environmental change have occurred. Even if the receptor cells themselves mark continuous change in the environmental stimulus, the rest of the system will operate non-linearly. In perception research, these changes are described as just noticeable differences (JNDs) and they enable us to detect quickly the rate of change in intensity of physical stimuli rather than the level of change. Consequently, although we would avoid entering a bath of water with 130-degree temperature because of the rate of change in skin temperature that would produce, we are likely not to notice a slow incremental change in bath water temperature, even to 130-degrees.

Exposure of the receptors to the environmental stimuli to which they are sensitive also can profoundly influence the developmental trajectory of the receptor and the sensory system itself (cf., Hirsch, Tieman, Barth, & Ghiradella, 2001). For example, the normal development of the olfactory bulb in mammals requires exposure to olfactory stimuli. Blocking one of the nares of an infant rat stunts the development of the olfactory on that side (Brunjes & Frazier, 1986). Exposure to specific odors during early postnatal development of rat pups results in the enhanced development of the parts of the olfactory bulb that are active during the processing of those odors in adults (Coopersmith & Leon, 1988). Axons from olfactory receptors terminate in the layer of the olfactory bulb that is organized as clusters of dendrites (glomeruli) of second-order neurons, relay neurons, and interneurons. Specific glomeruli are responsive to specific olfactory stimuli and the glomeruli of adult rats who were exposed to a particular odor (e.g., peppermint) as infants are larger and more active in processing that stimulus. Similar developmental consequences of receptor activity upon exposure to environmental stimuli occur in the visual, auditory, gustatory, etc. sensory systems (see Hill, 2001). Thus, during development, the receptors (and the sensory system) that transduce physical stimuli themselves undergo transformation, in part, as a consequence of earlier transductions of previous environment stimuli.

The physical environment also consists of a flux of rhythmic variations in frequency and amplitude of energies. Some of these fluctuations reflect diurnal, lunar, seasonal, and climatological regularities. Thus, the pattern of rhythmic flux can reliably signal future states of the environment. Since environmental variations are transduced by the individual, individuals can dampen or amplify the fluctuations. Therefore, the impact of cycles and rhythms of environmental stimuuli can

be ignored or transduced, combined, and modulated in various ways to increase individual variability in developmental trajectories. Such environmental fluctuations do not have a linear effect on the individual.

These forms of individual-experience relationhips demonstrate that the Umwelt is both a product and a process of development. Developmental changes occur as a consequence of a reciprocal relation between an active organism and a changing environment. Just as the environmental context provides the opportunities for experiences that change the individual, the individual changes the context of environmental stimuli. By being both a product and producer of their contexts, individuals affect their own development. As Schneirla (1966) noted, the organism and its environment may be separated for analytical purposes, but for the organism, there is no separation. Some of the organism's own activities contribute to the construction of its own environment, which in turn become the milieu for subsequent activity and further developmental change. Thus, it is just as reasonable to consider the consequences of self-stimulation on developmental trajectories as it is to consider the effects of stimulation arising from the individual's environment.

Although it is easy to accept that the individual may have an impact on how the social and physical environment interacts with him/her (a self-generated experience), it is more difficult to identify how the individual's self-produced stimuli can affect his/her development. One of the difficulties with the concept of ''self-stimulated experience'' is how to study it. However, a good example of self-stimulative experience on development is provided by Gottlieb's research on the behavior of mallard ducklings, who approach and follow their mother primarily when she utters a special ''assembly call.'' The behavior of the ducklings is not dependent on prior exposure to this maternal call since incubator hatched ducklings also will approach the source of this call. Indeed, even duck embryos exhibit signs of being responsive to the call.

Gottlieb (1971) demonstrated that the duckling's responsiveness depends on prior exposure to either the pre-hatching peeps of its nest mates or its own peeping. These ducklings begin to vocalize 3–4 days before hatching. When deprived of the sound of their own peeping and that of their siblings during these 3–4 days, they show no selective approach to the maternal assembly call after hatching. Indeed, they are susceptible to developing a preference for the calls of another species (chicken) even in the presence of their own species call. Although the complete maternal call does not sound at all like the peeping the embryo makes before hatching, Gottlieb found that the auditory characteristics of the maternal call to which the duckling is initially responsive is similar to the prehatching peep. Thus, the duckling's self-produced stimulation (hearing itself peep before hatching) affects the development of social behaviors vital for its survival (following its mother).

Shortly after Gottlieb's publications, Impekoven and Gold (1973) reported an effect of self-generated experience on the development of a species-typical behavior pattern in laughing gulls that illustrates the difference from Gottlieb's demonstration of a self-stimulative experience. Newly hatched laughing gull chicks

will approach and peck the parent's bill in response to the sound of the parent's ''croon call.'' Such pecking stimulates the parent to regurgitate food to the chick. Because of the vital importance of the response to the croon call it might be argued that the chicks are designed with the ability to make the response. Indeed, even embryos can discriminate the croon call from other adult calls two days before hatching. However, the ability to respond appropriately to the croon call is developmentally dependent on the chicks having heard the call before they hatch. Impekoven and Gold discovered how the embryos generated that experience for themselves.

Parent gulls frequently make croon-like calls while occasionally resettling on their eggs during incubation. Resettling involves shifting the position and orientation of the eggs, thereby providing the embryo with vestibular stimulation and neural activation to accompany the auditory stimulation. Several days before hatching, the parent's croon-like calls tend to increase activity of the embryo and increase peeping along with the activity. The peeping inside the egg, in turn, stimulates the parent to stand up and investigate the eggs and, subsequently, to make more resettling movements and hence, more croon calls. Chicks hatched in incubators crouch and hide in response to a croon call. Thus, the embryo's peeping increases the embryo's exposure to the croon-like calls of the parent and that, in turn, appears to facilitate development of the chick's post-hatching ability to ''recognize'' and approach the croon call of its parent. Approaching the croon call of a neighbor increases the risk of the chick being eaten by the neighbor because as a consequence of this reciprocal pre-hatching communication, the parents are able to ''recognize'' the calls of their own chicks.

Note that the laughing gull embryo is generating experiences (exposure to croon-like calls) by its own behavior (peeping) but unlike Gottlieb's duckling embryos, it is not the laughing gull embryo's exposure to its own peeping that enables it to approach, post-hatching, the call of the parent. The duckling embryo's experience is self-stimulated whereas the gull embryo's experience is self-generated.

The developmental organization of the ring dove (*Streptopelia risoria*) reproductive cycle also provides a good illustrations of the difference between self-stimulation and self-generated stimulation (Michel, 1986; Michel & Tyler, 2007b). In ring doves, as in many other species, the expression of specific species-typical behaviors during the progression of a breeding cycle is associated with the secretion of specific hormones. Although the hormones do not simply cause the expression of behavior, they do augment or potentiate the expression of the associated behaviors. In doing so, a given hormone operates in conjunction with a host of other internal factors, some of which have been created by concurrent social and physical environmental stimuli, some by the trace effects of previous social and physical environmental stimuli, and some by the previous and concurrent presence of certain other hormones (Lehrman, 1965).

Successful reproduction requires the tight coordination of the physiological progression of the female's reproductive system (from the formation of gametes

through feeding and weaning the offspring) with the hormonal changes that occur for both parents (in this biparental species) and with the necessary behavioral actions that characterize the phases of the cycle (courtship, nest-building, incubation, brooding). Since the same hormones that regulate the physiological progression of reproduction also augment the expression of specific behaviors, hormones can coordinate the parents' behavior so that they are in synchrony with one another and with the appropriate phases of each of their physiological cycles. Parents must synchronize their behavioral expression not only with the physiological events essential for reproduction, but also with one another so that the behavior of each either complements or supplements that of the other. Antagonistic behavior or phase differences in expression disrupt the progression of the cycle and reduce breeding efficiency.

Because hormones that are secreted by parents help to regulate the temporal organization of both the physiological and behavioral aspects of a breeding cycle, behavioral synchrony between mates can be assured by having the secretion of some hormones sensitive to the stimuli provided by the behavior of the mate. Indeed, reception of specific stimuli provided by the mate can directly affect a dove's hormonal secretion (Michel, 1986). Another way by which stimuli provided by the mate can affect the dove's hormonal status is, indirectly, through feedback from the dove's own behavior which has been generated in response to the mate-produced stimuli (self-stimulated experience).

For example, during courtship, male ring doves "bow-coo" to their mates and these behaviors are responsible for initiating the physiological process of producing eggs (along with associated behaviors of selecting a nest site via "nest-cooing" and building a nest). Cheng (2003), in a series of systematic studies was able to demonstrate that the male's bow-coos and nest-coos stimulated the female's nest-coos and that it was hearing herself nest-coo (and not the coo-stimuli provided by her mate) that elicited the hypothalamic control of the hormones needed to stimulate the female's production of eggs. Females who could not nest-coo or could not hear themselves nest-coo, could not lay eggs and could not proceed with the reproductive cycle no matter how actively their mates' performed for them. Cheng is systematically tracing the neural pathways in the transduction of these self-stimulative experiences to identify how they alter the organization and functioning of neural circuits, the sensitivity to hormonal conditions, and the transformation of hormones into more effective influences on behavior.

The Role of Experience in Development

The range of events that might be considered as possible experiences contributing to the development of an organism's behavioral repertoire can be very broad. Indeed, it is not possible to describe all of the categories of experience a priori. What

is and what is not an experiential factor contributing to the development of some behavioral characteristic is frequently more a matter of an empirical investigation than reasonable deduction. Of course, knowledge of the organism's receptors, their thresholds and state of developmental functioning, the developmental status of the organism's biomechanical properties and the organism's niche can help narrow the focus of investigation. However, failure to find an effect of a particular experiential factor that is expected to influence the development of some behavioral trait, unfortunately, cannot preclude the influence of other experiential factors. Experience must be systematically investigated for each organism and for the development of each behavioral trait of that organism.

Empirical investigations have both eliminated the influence of certain experiential factors and demonstrated the influence of other, unexpected or unanticipated, experiential factors (e.g., Blumberg, 2005, 2008). Also, such investigation has shown that the role of experience in development is not synonymous with the role of learning when learning is defined as training, conditioning, practice, or observation. Learning, as customarily defined, can be considered a part of an organism's experience. However, whether or not learning is an important contributor to the developmental trajectory of some behavioral trait is an interesting and important question, but not the only interesting and important question about the role of experience in the development of that trait.

Gottlieb (1976, 1992) noted that trace effects of experience are woven into all aspects of the developing organism and that there are three ways by which experience contributes to both the anatomical development of an organism and the physiological functioning involved in the organization of its behavior. *Maintenance* experiences can sustain achieved states of particular anatomical, physiological, and behavioral trait. A maintenance experience may not be necessary to achieve the developed state of that feature, but it is necessary for that feature to remain in that state. A *facilitative* experience affects when, during development, a particular anatomical, physiological, and behavioral feature appears by promoting or inhibiting the rate of its development. However, even without the facilitative experience, the feature would emerge at some time during development. In contrast, an *inductive* experience is necessary for the development of a particular anatomical, physiological, and behavioral feature. That is, the feature will not develop without the experiential event; the trait is experience dependent.

Although a useful first step, one of the consequences of Gottlieb's characterization of experiential influences is that it has been easy for some theorists to consider only the inductive experiences as contributing to the development of behavior. A facilitating experience can be considered as simply fine-tuning the developmental expression of a genetically controlled or programmed developmental process. Of course, the appearance of the trait without exposure to the facilitating experience does not preclude the role of other experiences in the development of that trait. Maintenance experience also has been considered to be irrelevant to

the development of behavior because it merely maintains a behavior pattern that has already developed.

However, Michel and Moore (1995) argued that both facilitating and maintaining experiences can have profound consequences on development that can be equal to inductive experiences. If a feature (hormone level, receptor for a hormone or neuromodulator, numbers of neurons or synaptic relationships, muscle activity or paralysis, etc.) is maintained by experience, it can participate with other features in the developmental emergence of a new feature (e.g., probability of expressed anxiety, level of social attractiveness, rate of skill acquisition and/or level of skill achieved). As such, a maintaining experience can be a necessary condition for the development of many other features. The failure of maintenance also can affect development.

Experience and Behavioral Sex Differences in Rats

Moore and Morelli (1979) reported that the frequency of every rat dam's maternal anal-genital licking (AGL) expressed toward her pups during the two-three week nursing period, is sharply greater toward each of her male versus female pups. This high frequency AGL helps to maintain certain neurons in the lumbar spinal cord of the male (Moore, Dou, & Juraska, 1992). Moore (1992) also observed that the frequency of maternal AGL that a rat pup received preweaning positively affected the frequency of juvenile AGL self licking. Thus, the individual maintains the AGL experienced during the juvenile period at a level related to that provided by the mother during the nursing period. This high-level self-AGL facilitates the development of puberty in the male.

As adults, the experience-maintained spinal motor neurons control the movements of the penis during copulation that remove vaginal plugs and help ensure fertilization of the female. Other experience-maintained neurons in this area enable the feedback during copulation needed for the maintenance of intromission and eventual ejaculation. Moore (1982; Moore & Chadwick-Dias, 1986) discovered that testosterone results in the dam engaging in more AGL of testosterone stimulated pups than those without testosterone. It is both testosterone's metabolites and the testosterone dependent secretions of the preputial gland in the pup's urine that attracts the dam's AGL. The preputial gland is present at birth in both male and female pups but the normal perinatal secretion of testosterone from the male's gonads keeps the preputial gland active postnatally; whereas it diminishes postnatally in female pups. The reason for the dam's preference for licking (ingesting) testosterone metabolites and the secretions of the preputial gland are unknown but it is known that dams will lick pieces of filter paper with a drop of male pup urine (Moore, 1985).

When female pups were injected with testosterone, they were licked as much as the control males and, as adults, these females exhibited many of the male

stereotypical adult behaviors involved in sexual reproduction (Moore, 1982, 1984). Before Moore's research, the early injections of testosterone in female pups were thought to organize directly the structures and functioning of her brain so that she behaved as a male when adult (Gorski, Gordon, Shryne, & Southam, 1978; Phoenix, Goy, Gerall, & Young, 1959). Male pups, that were gonadectomized at birth, were thought to develop a ''female'' nervous system because they were deprived of the brain organizing influence of early testosterone secretions. They behaved as females when adult. However, Moore (1984) found that when males are gonadectomized at birth, they were licked only as much as control female; then, as adults, they had lower mounting and intromission (attempts at copulation) episodes (as would be typical of normal females) despite having normal perinatal exposure to their own testosterone secretion.

Moore et al. (1992) further found that when the dam is made anosmic (unable to smell) she treats both sexes the same and they both receive the AGL that a female under ''normal'' conditions would receive. Subsequently, the male offspring (whose brains had experienced normal levels of early testosterone) of these anosmic dams behaved, as adults, equivalently to males who had had their testes removed at birth. In another study, Moore (1985) found that female pups, who have little endogenous testosterone but who received extra stimulation of their AGL region, behave more like males when adult. Thus, an experience that is responsible for maintaining the presence of certain neurons and their connections is responsible for striking differences in the adult neuroanatomy and behavior between the sexes.

Clearly, the experience associated with maternal behavior is an important aspect of the development of sex-typical reproductive behaviors in rats. The experience of maternal AGL both maintains and facilitates the development of striking variations in the male offspring's adult reproductive success. Indeed, Moore (Moore & White, 1996; Moore, Wong, Daum, & Leclair, 1997) found that mothers of different strains of rats exhibited differences in the frequency of AGL of their male offspring. When males from these different strains are in competition for mating, those males from strains that engage in less AGL leave significantly fewer offspring than those males from strains that engage in more AGL. Clearly, there is a disadvantage to low frequency AGL of male offspring. The work of Moore and colleagues clearly demonstrate that differences in maternal behavior can provide experiences for the pups that affect their developmental trajectories (eco-devo) sufficient enough to produce the variability on which natural selection potentially can act to create species (evo-devo).

Since heterochrony in development can be important for evolution (de Beer, 1958; Gould, 1977), maintaining a feature or trait means that it can participate with others in the developmental emergence of some new features or traits. If experience maintains a trait beyond the developmental phase in which it may be most appropriate, the trait could become an important exaptation for the emergence of a new species. For example, juvenile males in many species of rodent exhibit alloparental care of pups (Michel & Tyler, 2007a). Such parental

behavior disappears after puberty (adult males of many species kill pups and hence most rodents are uniparental). There are several experiential conditions (specific circadian light and/or temperature conditions) that seem to maintain the male's alloparental care into adulthood. These experiential conditions (which are associated with particular habitats) may have been the foundation for the evolution of biparental care in certain rodent species (Michel & Tyler, 2007a). Maintaining experiences can be a necessary condition for the development of many other features. Also, maintenance of a feature or trait may prevent reorganization of the system as a result of further experience.

Similarly, by altering the rate at which certain features develop, facilitating experiences can become a fundamental source of the production of new patterns of organization. Differential change in the rate of development among phenotypic features (heterochrony) is a fundamental source of the new developmental traits that serve as the variability on which natural selection can operate to produce new species (Jablonka & Lamb, 2005; Michel, 2007; Michel & Moore, 1995; West-Eberhard, 2003). Distinctly different individuals can emerge from alterations in the relative rate of development of specific features. It would not be surprising if delaying or accelerating a human child's language abilities created differences in thinking, problem-solving skills, intelligence, social skills, emotional range, and other aspects of personality. Since living organisms are organized entities, altering the development progression of any feature by affecting the feature's maintenance or the timing of its emergence can ramify to affect the development of other features. Such ramifications can mimic the effects of an inductive experience.

Thus, facilitative and maintenance experiences can have as profound an effect on developmental trajectories as inductive experiences. Indeed, whether an experience is identified as maintaining, facilitating, or inductive may depend on the type of measure that is used. For example, the observed differences in the size and shape of certain spinal and brain-stem neural structures between the two sexes, which depend on the differences in the pup's experience with maternal-AGL, might be classified as inductive. However, measures of the rate of cell division or cell loss in the nucleus might reveal that the experience was facilitative or that the experience maintained some set of cells that would otherwise have died via apoptosis. As we noted, whether or not some event plays an experiential role in development depends on empirical investigation. If the event is demonstrated to play a role in development, whether that event is classified as an inductive, facilitative, or maintenance experience depends on how the empirical investigation was conducted.

As I have proposed, experience is a construct that applies only to multicellular organisms because it requires the transduction of certain physical manifestations of the environment (e.g., photic, chemical, thermic, mechanical stimuli) into substances that can operate at the level of the cell-environment transactional mechanisms. This transduction uses the differentiation of cells into organized tissues that are sensitive to particular physical environmental factors (e.g., cells

receptive to photons within a 400 to 700 nm range of vibration; cells receptive to cooling or heating of skin and blood; cells receptive to deformation of the skin, parts of the digestive tract, vibrations in air or water, inertia and gravity; cells receptive to chemicals volatile in air or water). The differentiation of tissues in development also yields receptors sensitive to stretch of muscle, position of joints, changes in pH, CO_2, osmotic pressure, and even substances like histamine released by cells as they die. All of these receptors are sources of potential components of experience for the organism. All contribute to the functioning of the organism's systems and some may contribute to the trajectory of development. Early in their development, exposure of these receptors to the environmental stimuli that they detect also contributes to the development of their sensitivity, and that of their neighboring cells, to these environmental events.

Transduction of environmental events (stimuli) by receptors can eventuate in neural activity in the ''sensory'' subsystem of the nervous system and this activity, in turn, affects other neural subsystems and may eventuate in effects on the ''motor'' neural subsystems. The motor subsystems create the biochemical conditions that stimulate the contraction of muscles that transform the relation between articulated rigid tissue (e.g., exoskeleton or endoskeleton) involved in movement of the whole organism. Or, motor system activity can affect the movements of the digestive tract, respiratory tract, cardiovascular system, etc. Other neural subsystems affect the secretion of specific secretory cells whose products (hormones) are carried to various parts of the organism to affect a wider variety of cells using the cell-environment transactional mechanisms. The ramifications of any experience can be quite extensive.

Experiential Consequences of Rat Maternal Behavior

Consider the lengthy period of prenatal and postnatal mother-infant interaction in mammals. The pattern of care received by an infant early in life can produce changes in the development of way that the neural system regulates responses to novelty and social behavior (Meaney, 2001). During the first week postpartum in rats, lactating dams engage in a high frequency of pup licking and grooming (LAG) in addition to nursing. This behavior serves to stimulate pups. The warm paws of the dam heat parts of the pup's body while other parts are exposed to cooling air. The pup's muscles and joints are stretched and flexed by the dam's manipulations. The dam's warm tongue creates cooling post lick as the dam's saliva evaporates. The dam's manipulations stimulate the pup's vestibular system. Finally, the dam's manipulations provide olfactory (from the saliva) and auditory stimulation to the pup (both from the pup's own squealing and the dam's sounds). The LAG also modifies the pup's overall body and brain temperature (Sullivan, Shokrai, & Leon, 1988; Sullivan, Wilson, & Leon, 1988). The dam's licking of the pup's anal-genital region not only stimulates the pup's urination and

defecation but also permits her to reclaim nutrients, salt, and water lost as a result of the physiological demands of lactation (Gubernick and Alberts, 1983, Gubernick & Alberts, 1985).

It has been known for some time that early handling (EH), a 15 minute daily separation of the pups from the mother for 2–3 weeks postnatally (Lehmann & Feldon, 2000; Levine, 1957), affects the development of the offspring's hypothalamic-pituitary-adrenal cortex (HPA) functioning. Such separation results in better coping responses to novel stimuli as adults when compared to undisturbed control animals (Levine, 1957; Meaney, Viau, Aitken, & Bhatnagar, 1989; Parfitt et al., 2004). Thus, when EH rat pups are restrained as adults, their secretion of corticosterone (CORT) from their adrenal cortex and the secretion of adrenocorticotropin releasing hormone (ACTH) from the anterior pituitary is lower compared to non-handled control animals (Núñez et al., 1995; Plotsky & Meaney, 1993). These results suggest that EH leads to adults that are better able to cope with, or are more resilient to, environmental stressors.

In contrast, adult offspring who were separated daily from their mothers for 3 or more hours for 2–3 weeks of nursing (MS) were less able to cope with stressors (Fernández-Teruel, Escorihuela, Driscoll, Tobeña, & Battig, 1991; Huot, Thrivikraman, Meaney, & Plotsky, 2001; Meaney et al., 1994; Plotsky et al., 2005). Offspring of MS mothers spend less time in the open arms of the elevated plus maze compared to non-handled controls (Huot et al., 2001) and display higher levels of CORT and ACTH when exposed to an airpuff startle. In addition, there was increased receptor binding to corticotropin releasing factor receptor subtype 1 (CRF1 R) in the paraventricular nucleus of the hypothalamus in MS than in the EH animals. The CRF1 receptors seem to be the mediator of corticotrophin releasing hormone (CRH) and HPA activation.

It has been argued that it is the reunion behaviors of the dam that are the important experiential factor mediating the developmental differences in EH and MS offspring (Lee & Williams, 1974; Levine, 2002). By providing a set of foster pups to the rat dam during separation from her own pups, Huot, Gonzalez, Ladd, Thrivikraman, and Plotsky (2004) were able to eliminate some of the effects that MS can have on the development of her natural born pups. Adult offspring that experienced maternal separation exhibited a higher ACTH response to an air puff startle only if the dam was not given foster litters during the separation. Thus, the separation somehow was different for the dam when she is given a foster litter during separation and consequently she did not interact with her biological pups in the manner that facilitates their development of the hyper-responsiveness to stress that is traditionally associated with MS experience. Thus, this study does provide some support for the notion that there are important factors in the dam-litter interaction upon reunion after separation that are responsible for the EH and MS offspring differences in emotional coping ability. These differences can be ameliorated, if not eliminated, by providing MS dams with foster pups during their separation from their own pups.

Liu et al. (1997) reported that EH results in pups receiving twice the frequency of LAG compared to a non-handled control group. They also reported that arched back nursing (ABN) is highly correlated with LAG; thus, EH rat pups are experiencing more tactile stimulation from LAG and possibly more milk or more thermoregulation from the mother's ABN than non-handled pups. Boccia and Pedersen (2001) exposed rat pups to EH or MS on PND 2–14 and observed that pups experiencing EH received higher frequencies of LAG from their mothers upon reunion than MS pups across all days. Again this demonstrates that EH pups receive more stimulation from the dam compared to another experimental group.

Avishai-Eliner, Eghbal-Ahmadi, Tabachnik, Brunson, & Baram (2001) identified the ways in which EH affects HPA responsivity during early pup development. EH resulted in the down-regulation (decreased genetic expression) of hypothalamic CRF-mRNA by PND 9. Up-regulation (increased genetic expression) of hippocampal glucocorticoid receptor mRNA did not occur until after PND 23. Although there were no differences between EH and non-handeled (NH) animals in the hormonal (ACTH & CORT) responses to the stressor at PND 9, the change in secretion of these hormones in response to the stressor emerged after PND 9 and before PND 23. Thus, the effects of early handling likely initially affect hypothalamic (paraventricular nucleus) regulation of the HPA and subsequently affect the development of hippocampal glucocorticoid receptors. Subsequent work (Fenoglio, Brunson, Avishai-Eliner, Chen, & Baram, 2004) revealed that EH (PND 2–9) had increased CRFmRNA in amygdaloid central nucleus by PND 6. The authors speculate that increases in maternal licking and grooming of the EH pups is conveyed by the spinal cord to brain stem nuclei and from there to the paraventricular nucleus of the thalamus which projects heavily to the amygdaloid central nucleus.

Since pup CORT levels were observed to rise about 15 minutes after being returned to the dam, stimulation from the mother's behavior likely increases CORT secretion. The presence of CORT in the blood results in an up-regulation of CRF-mRNA in the amygdaloid central nucleus and a down-regulation of CRF-mRNA in the paraventricular nucleus of the hypothalamus in immature rats (Hatalski, Guirguis, & Baram, 1998). Thus, CORT stimulated by maternal care during reunion after EH, may up-regulate CRF-mRNA in the amygdaloid central nucleus such that subsequent stimulation (at other reunions) facilitate CORT release to down-regulate CRF-mRNA expression in the paraventricular nucleus of the hypothalamus. This results in a reduction of CORT over age and hence, a reduced response to a stressor. That is, the stressor initiates a release in CORT but activation of the glucocorticoid receptors (GR) for CORT in the hippocampus and amygdala inhibit further secretion of corticotrophin releasing hormone (CRH) from the paraventricular nucleus. Hence, ACTH secretion subsides and, in turn, CORT secretion subsides.

Meaney and colleagues (2001, 2005) have argued that maternal behaviors such as ABN and LAG can have a profound impact on development of the molecular

processes involved in HPA activation. The frequency with which dams engage in LAG varies considerably between individuals yet shows a high level of stability within individuals (Champagne, Francis, Mar, & Meaney, 2003). In general, females can be characterized as engaging in High, Mid, or Low levels of maternal LAG. Adult offspring, who were reared by Low-LAG dams, exhibit elevated HPA activity as a consequence of restraint stress. That is, they have a decreased capacity to down regulate the release of corticotrophin releasing hormone (CRH) from the hypothalamus, adrenal cortical trophic hormone (ACTH) from the pituitary, and glucocorticoids (e.g., corticosterone, cortisol) from the adrenal cortex (Caldji et al., 1998; Liu et al., 1997).

The release of glucocorticoids following activation of the HPA axis has negative-feedback effects on the stress response through interaction with hippocampal glucocorticoid receptors (GR) (Sapolsky, Meaney, & McEwen, 1985). Decreased levels of GR mRNA in the hippocampus result in a decreased capacity to achieve baseline levels of corticosterone following the cessation of a stressor. Thus, hippocampal neurons with many receptors for glucocorticoids are able to more quickly provide negative feedback on the secretion of CRH. More precise regulation of the production of glucocorticoids results in an individual better able to respond effectively to novel and unusual events and situations. Thus, differences in the pattern of maternal care results in differences in the maintenance of the expression of GR in the hippocampus. Behaviorally, these neuroendocrine changes result in decreased exploratory behavior and increased inhibition on tests such as the open-field and elevated plus maze (Caldji et al., 1998).

Since these differences in maternal behavior affect hippocampal physiology, it is perhaps not surprising that offspring of Low-LAG dams also exhibit impaired performance on tests of spatial learning and memory. This impairment may be a consequence of decreased neuronal survival and increased apoptosis in the hippocampus (Bredy, Grant, Champagne, & Meaney, 2003; Weaver, Grant, & Meaney, 2002). In addition to these HPA effects, GABA subunit expression is altered by maternal LAG with implications for benzodiazepine binding (Caldji, Diorio, & Meaney, 2003) and offspring of Low-LAG dams have a decreased density of benzodiazepine receptors in the amygdala (Caldji, Francis, Sharma, Plotsky, & Meaney, 2000; Francis, Caldji, et al, 1999; Liu, Caldji, Sharma, Plotsky, & Meaney, 2000; Liu et al., 1997). In males, dopaminergic release associated with stress responsivity is also altered as a function of LAG (Champagne et al., 2004).

Meaney & Szyf (2005) proposed a model for a molecular cascade in pups that experience high ABN/LAG during maternal care that underlies their difference in HPA functioning when compared to those who receive low ABN/LAG. Weaver, Diorio, Seckl, Szyf, & Meaney (2004) observed demethylation (DNA can be expressed) of the exon 17 promoter region of the glucocorticoid receptors in the hippocampus of adults and pups (at PND 6, 21, and 90) born to dams that exhibit

high ABN/LAG. Offspring born to dams that exhibit ABN/LAG were found to have this exon 17 promoter region methylated. For pups born to both high and low ABN/LAG, the exon 17 promoter region was methylated in both the pre-natal period and just after birth.

The tactile stimulation of licking increases serotonin (5-HT) which in turn stimulates serotonin receptor production. High ABN/LAG also leads to increased levels of cyclic AMP (cAMP) production and protein kinase A (PKA). Both 5-HT and cAMP/PKA production increase expression of a transcription factor called nerve-growth-factor inducible factor A. Increased binding of nerve-growth-factor inducible factor A to the exon 17 promoter region may lead to increased expression of glucocorticoid receptors (GR). Higher levels of GR in the hippocampus mean that the HPA axis is more sensitive to CORT in the bloodstream. Increased binding of CORT in the hippocampus will decrease CRH expression which then turns off the activation of the HPA. Low LAG/ABN pups will not experience these increases in 5HT and cAMP and thus the exon 17 promoter region of their GR will remain methylated. Thus, the process of demethylation occurs post-natally, as a consequence of the maternal care (Champagne, 2008).

Experiential Influences on Transgenerational Patterns of Maternal Care

The pattern of maternal care has consequences not only on the offspring's ability to cope with socio-emotional situations but it also affects the parental care that the offspring provide for their own offspring (a grandmother experiential effect). Offspring of High-, Mid-, and Low-LAG dams exhibit levels of LAG that are highly correlated to the behavior exhibited by their mothers (Champagne et al., 2003; Fleming et al., 2002; Francis, Diorio, Liu, & Meaney, 1999). Moreover, cross-fostering female offspring between High and Low-LAG dams confirms that postnatal care mediates this transmission. Females born to Low-LAG dams and then fostered to High-LAG dams will exhibit high levels of LAG toward their own pups whereas females born to High-LAG dams and then fostered to Low-LAG dams will exhibit low levels of LAG (Champagne et al., 2003; Francis, Diorio, et al., 1999).

In an elegant set of experiments, Fleming et al. (2002) demonstrated that rat dams maintain patterns of maternal care similar to those of their mothers and grandmothers. By removing a dam's olfactory bulb (or interfering with its functioning) before they gave birth to their pups, they profoundly reduced the frequency of maternal licking of the pups. Subsequently, one female pup from each of the litters of these anosmic mothers was selected to be bred and to be observed with her pups. Those dams that were raised with a mother that was bulbectomized, exhibited less licking of her pups than a control group of offspring from SHAM

operation dams. Furthermore, this same pattern of reduced pup-licking was observed when females from the second generation became adults and had their own litters.

Although each generation exhibited a similar pattern in parental care (reduced pup-licking), the mechanism for the transmission across the generations obviously was different. In the first generation, the decreased licking was due to the olfactory disruption (bulbectomy) and the set of events (unknown still) dependent upon that disruption. By the second generation, the decreased licking was due to the developmental consequences of not having experienced certain patterns of licking very early in development. Thus, variations in early postnatal experience created by maternal behavior can affect not only the developing offspring, but future generations through its impact on the offspring's maternal behavior.

Although the precise mechanism by which this transgenerational influence is created is unknown, it may involve expression of estrogen receptor alpha (ERa) sites available in the medial preoptic area (MPOA) of the hypothalamus (Champagne et al., 2006). Low frequencies of maternal LAG are associated with high levels of DNA methylation and low levels of ER alpha expression in the MPOA of the offspring which results eventually in their low frequency of LAG when they become mothers.

Thus, individual variation in maternally provided care to the pups create experiences for the developing pups that alter the rate of production of many hormones, neuromodulators, the activity of many neural systems, etc. which will result in variability in the trajectories of their normal psychobiological development. Maternally created experiences that facilitate the production of receptors for hormones and neuromodulators in particular parts of the offspring's nervous system result in individuals who are more or less vulnerable to stressful situations. Maternally created experiences for the pups also create trajectories in the offspring which leads them to provide their own offspring with experiences that insure the transgenerational transmission of these behavioral phenotypes.

Developmental psychobiology

Much of the research that has revealed the effects of experience during development emerged from a discipline called Developmental Psychobiology (DPB). Whereas eco-devo and teratology have focused on the influence of environmental factors in the embryological (morphological/physiological) development of species and evo-devo has focused on the constraints developmental processes place upon evolution, DPB has been a major source of much of the empirical evidence demonstrating the roles of environment and experience in the development of both the species-typical and individually specific behavior patterns (Michel &

Moore, 1995). The research has been conducted with a wide variety of animals (including species of mammals, birds, reptiles, fish, insects, mollusks, crustaceans, etc.) and the organism is expected to have an adaptive relation to its environment throughout its lifespan (ontogenetic adaptations).

DPB investigations tend to integrate neurophysiology, endocrinology, genetics, immunology, psychology, and ecology. Moreover, developmental psychobiologists treat developmental phenomena as existing simultaneously at many levels of description (cellular, tissue, organ systems, the individual, family units, groups, etc.). In DPB a complete explanation of any developmental phenomenon would require: 1) descriptions at all these levels without giving primacy to any one level; 2) a set of rules that would permit translation of these descriptions across levels so that meaning is not confused; 3) identification of the patterns of reciprocal causation among these levels; and 4) the integration of evidence from neurophysiology, endocrinology, genetics, immunology, psychology, and ecology for the construction of explanatory theories. Of course, no single research program in DPB has provided such a complete explanation; nevertheless, it is the explicit goal of the discipline.

DPB research shows that experiential influences on developmental trajectories are not synonymous with the influences of learning in development. DPB research also has shown that many aspects of behavioral and neural development are explained by experiential factors that are not intuitively obvious and may be functionally discontinuous with the behavioral system under study.

DPB devised four strategies for identifying potentially important but not intuitively obvious experiential influences on development:

1. *Describe development within the ''natural'' ecological context within which the behavior develops*. This will reveal interesting aspects of the individual's ecology from which hypotheses about potential environmental or experiential influences can be generated.
2. *Describe the same phenomenon in more than one way*. Trying to provide alternative descriptions of the behavioral components of a developmental trajectory can provide insight into the ecological context from which potential environmental and experiential influences contributing to the development of that trait arise.
3. *Study various species*. A comparative perspective promotes greater understanding of the web of causality associated with the different developmental trajectories of among similar behaviors in different species.
4. *Examine the developmental trajectories of several systems (neural, endocrine, etc.) and several behaviors (reproductive, ingestive, cognitive, etc.) of the individual*. This will illuminate a broader range of potential causal influences. These strategies complicate the research enterprise but improve the science of behavioral development.

Learning and Behavioral Development

There is no doubt that learning often plays a powerful role in the adjustment of an organism's behavior to the non-random fluctuating conditions of its environment (its Umwelt). However, the role that learning plays in the organization of the developmental trajectories of species-typical or even indivudal behavioral phenotypes has been controversial. It commonly acknowledged that frequently learning is constrained by when, during a developmental trajectory, the learning opportunity occurs (Hinde & Stevenson-Hinde, 1973). Learning early in the lifespan may have long-lasting effects on preferences and response biases of the individual (Wilson, Fletcher, & Sullivan, 2004) or it may have no long lasting influences unless there are periods of reinstatement (Rovee-Collier, 2001). Acquisition of learned responses may occur more quickly for juvenile and adolescent animals but exhibit more mistakes than in sexually mature animals (Vince, 1974). The most typically investigated developmental constraint on learning is represented by a phenomenon known as a ''critical'' or ''sensitive'' period (Michel & Tyler, 2005; Tyler, 2006). That is, the learning will be most effective only if it occurs when the organism is in a particular phase of its developmental trajectory. Exposure to the learning conditions either before or after the occurrence of that phase in the developmental trajectory results in less than optimal learning, if any learning occurs, at all.

To account for this developmental constraint on learning, the Nobel Lauriate, Konrad Lorenz, proposed that a phenomenon of ''instinct-training interlocking'' characterized the development of many species-typical behavior patterns.

> Instinctively innate and individually acquired links often succeed each other directly in the functionally uniform action chains of higher animals. . . Where we are dealing with the effects of experience, we must first discuss the interlocking of ''instinct and training''. . . [the] essence [of this interlocking] lies in the fact that a conditional action is inserted in an innate chain of acts at a certain also innately determined point. . . [Therefore,] the functional units of behavior are made up of two fundamentally different components. (Lorenz, 1937, in Schiller, 1957, p. 137)

Any modifiability of species-typical behavior can occur only ''in those preformed places where built-in learning mechanisms are phylogenetically programmed to perform just that function'' (Lorenz, 1965, p 45).

Consequently, for Lorenz, learning plays an important role in the developmental trajectory of species-typical behaviors but only if the developmental program for that behavior in that species has an innately specified opportunity for learning. Lorenz considered these opportunities to be critical periods during development when the trajectory awaits some input from the environment. The phenonmenon of imprinting is a common example of this process.

As Lorenz observed for certain species of bird, the young chick reaches a stage of development when exposure to a particular visual or auditory event creates a developmental pattern whereby that visual and/or auditory stimulus will elicit affiliative behavior and, when the chick becomes sexually mature, sexual behavior. Thus, for Lorenz, the critical period for imprinting seemed to be an excellent example of instinct-training interlocking. Unfortunately, research during the past 40 years has drastically revised the concept of imprinting (Michel & Moore, 1995) and the concept of critical period has changed similarly (Michel & Tyler, 2005; Tyler, 2006).

To understand the role of learning in the establishment of developmental trajectories in behavior requires a good definition of learning. Generally, the concept of learning refers to the process by which an organism is able to use experience to modify its behavior in order to achieve some goal. For any behavioral modification to reflect learning, it must not occur as a result of general growth processes in the nervous system, muscle fatigue, or sensory adaptation (the failure of sensory receptors to register the presence of the stimulus). Clearly, the processes of habituation, conditioning, practice, training, observation, and imitation (the customary paradigms for identifying learning) are aspects of experience that permit better adjustment of the individual to its environment.

The procedures typically used to measure learning, examine 1) the increases or decreases the frequency of acts in an individual's repertoire, 2) the creation of new chains of acts into actions, and 3) the substitution of new stimuli for those typically eliciting or terminating an act. All of these measures reflect the use or functioning of certain levels of sensory and motor system development. Only then, can rehearsal, practice, correction, and familiarization contribute to further development.

Obviously, the use or functioning of a system often improves the efficiency or competence with which the system can be used, extends or restricts the range of its use, converts a vaguely specified system into one that is more precise or detailed, or alters a preference. However, for use to contribute to further development, it is necessary to first have a nascent system that has developed to a usable state. That begs the question: How did this initial nascent state develop? Pondering this question likely led Lorenz to the notion that learning has a restricted role in the development of species-typical behavioral traits.

Whether or not learning (as customarily defined) is an important contributor to the developmental trajectory of any specific behavioral trait is an interesting and important question that requires empirical support. However, the contribution of learning is not the only interesting and important experiential question that may be asked about development. Experience may play an essential role in the development of those nascent systems that permit learning to contribute to further development. In the development of any particular behavior pattern, learning may play a relatively minor role whereas experience may play a major role. Conversely, for another behavior, learning may play an essential role in influencing the developmental trajectory of that behavior.

Consider the development of bird song; learning seems to be a fundamental component of a species-typical trajectory. There are more than 4,500 species of song birds. Discovering the contributions of environment, experience, and learning to the development of each species song would be a Herculean task. Therefore, most research is conducted using a very few species. Indeed, nearly 67% of the research has been conducted with two species – zebra finches and canaries (Williams, 2004). The zebra finch is proposed to have an innate crude "template" (established in a neural circuit) that guides the memorization of the conspecific song heard during an early sensitive period. Exposure to the conspecific song converts the template into a more complex acquired template (early sensory learning). Young birds convert this acquired template into a sensorimotor pattern by comparing the auditory feedback from their initial poorly constructed song to the memorized model (subsong and plastic song). Eventually a crystallized adult song is achieved, consisting of a repertoire of elements, notes, syllables, phrases, motifs, or songs.

Research on the model species provides a very sophisticated account of the neural mechanisms involved in song (Zeigler & Marler, 2004). There appear to be neural mechanisms involved in acquisition that do not operate during eventual production and it is sometimes presented as though these mechanisms operate as relatively isolated neural circuits with restricted functions. For example, Hessler and Doupe (1999) report that the pattern of singing-related neural activity in several high level neural areas (L-MAN, Area X) specialized for song learning (but not production) depends on whether the zebra finch sings alone or to another zebra finch. Also, multielectrode single-unit recording in juvenile Zebra finches identified single HVC (High Vocal Center of the forebrain) neurons that appear to integrate sensory and motor information during the sensorimotor phase of vocal learning (Crandall, Aoki, & Nick, 2007). This was interpreted to mean that the premotor HVC is part of the circuit involved in the sensorimotor shaping through activity-dependent mechanisms characteristic of song learning. In contrast, recent evidence (Day, Kinnischtzke, Adam, & Nick, 2008) suggests that the HVC affects song variability rather than song features.

Indeed, studies show that many song characteristics of zebra finches (e.g., syllable order) can vary depending on how syllables are defined by an experimenter. Kojima and Doupe (2008) intentionally used different syllable definitions in the same song to reveal different mean syllable durations and that the syllable order selectivity of each bird's neurons varied depending on those durations. As Meitzen, Thompson, Choi, Perkel, & Brenowitz (2009) demonstrated, measuring either song quantity or quality alone does not provide a complete picture of how song behavior changes during seasonal transitions in breeding physiology. Song rate, song phonology, and song syntax changed on different time scales in response to changes in breeding condition. Thus, although seemingly simple in character, the particular definitions that a researcher chooses for defining the zebra finch song alters how the song is related to neural and endocrine mechanisms.

Since the definitions used to characterize the phonology, syntax, and rate of expression of the zebra finch's song can create much variability in the relation of the song to neural and endocrine mechanisms, it would not be surprising that consideration of the diversity of song acquisition patterns among other species would dramatically alter the value of popular models of song learning. Such diversity reveals that: song learning can occur early (as in the typical model systems) or remain lifelong (European starling and pied flycatcher); a species may sing only one simple song type (the white-crowned sparrow) or, in contrast, have extensive repertoires of several hundred song types like the mockingbird, the Australian lyrebird, and the European nightingale, or even more (the brown thrasher sings more than 1,000 song types); a species may closely mimic a conspecific song (as in the zebra finch) or, in contrast, improvise by modifying song elements to create novel songs (sedge wrens); a species may require early exposure to a model (as in the zebra finch) or in contrast, a species may develop the song in isolation (grey catbirds and sedge warblers); a species may copy tutor material only if it fits tightly constrained species-typical parameters (chaffinches) or a species may copy nearly anything heard (northern mockingbird and Marsh warblers).

Although for many species, young birds acquire the song of their parents and neighbors, Wheelwright et al. (2008) showed that most male Savannah sparrows acquired their songs from neither their biological nor their foster-reared ''social'' father but rather from neighbors. Moreover, Beecher, Burt, O'Lloghlen, Tempelton, and Campbell (2007) reported that young song sparrows learned from two kinds of adult ''song tutors'': one with whom the subject interacted vocally, and one whom the subject only overheard singing with another young bird. Unlike Zebra finches, song sparrows learned more than twice as many songs from the ''overheard'' tutor. Thus, in some species, birds may learn more by eavesdropping than by direct interaction. In contrast, some model species (e.g., zebra finch) require an interactive relationship either with an adult bird or with just a key that needs to be pecked to produce the playback from a speaker; whereas, others (e.g., canary) will acquire song just from hearing it via a speaker.

Within species, there is much adult individual variability in the song which reflects, among other influences, differences in the sources from which the song was acquired, how the song was assembled during acquisition, and the responses of adult listeners. Moreover, song is a consequence of certain acoustical and structural properties of the bird, the specific properties of the salient tutor, as well as improvised aspects. Thus, there may be no ''typical'' songbird developmental process except, perhaps, the comparison of auditory feedback from self-generated song to some internal model (Brenowitz & Beecher, 2005).

The examination of bird song development demonstrates that learning can contribute to the developmental trajectory of a species-typical behavior. It also

demonstrates that the learning depends upon the foundation of a certain level of development of the bird's sensory and motor systems. Unfortunately, we know too little about the role of experience in the development of that foundation. We can be certain, however, that there are environmental and experiential influences (that are not learning) that contribute to the developmental trajectory of those sensory and motor systems.

Environment, Experience, Learning and the Questions about Behavioral Development

There are, at least, four developmental questions about behavior that require consideration of the various contributions of the individual's environment, experience and learning. These are:

1. What developmental trajectories underlie the expression of species-typical behavioral traits? How does the environment, experience, or learning contribute to the pattern of those trajectories? This is a question that prompts eco-devo and DPB investigations.
2. What factors facilitate and constrain the individual's progression along such trajectories? How does the environment, experience, or learning contribute to such facilitation or constraint? This is a question that has received too little investigation.
3. What alterations of these factors produce new traits? This is the question that prompts evo-devo research because these new traits are the variations on which natural selection and other evolutionary processes can operate to create new species.
4. What alterations of these factors can reinstate a more typical path? This is the question that prompts research on the rehabilitation techniques for correcting distorted trajectories and research on what factors are responsible for resilience in behavioral development. This question reflects the public health and ecological conservation application of the sciences of eco-devo, evo-devo, and DPB.

A science of behavioral development can be created only by empirically investigating these questions for a wide variety of animals using the proper statistical tools for identifying and specifying the character of the phenotypic trajectory (Michel, 2001; Singer & Willett, 2003). Such investigation will benefit from a clear account of what distinguishes an environmental, from an experiential, from a learning influence on the trajectory of a behavioral phenotype.

References

Abel, E. L., Moore, C., Waselewsky, D., Zajac, C., & Russell, L. D. (1989). Effects of cocaine hydrochloride on reproductive function and sexual behavior of male rats and on the behavior of their offspring. *Journal of Andrology*, **10**(1), 17–27.

Anway, M. D., Cupp, A. S., Uzumcu, M., & Skinner, M. K. (2005). Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science*, **308**, 1466–1469.

Anway, M. D., & Skinner, M. K. (2006). Epigenetic transgenerational actions of endocrine disruptors. *Endocrinology*, **147**(6), 43–49.

Avishai-Eliner, S., Eghbal-Ahmadi, M., Tabachnik, E., Brunson, K. L., & Baram, T. Z. (2001). Down-regulation of hypothalamic corticotropin-releasing hormone messenger ribonucleic acid (mRNA) precedes early-life experience induced changes in hippocampal glucocorticoid receptor mRNA. *Endocrinology*, **142**, 89–97.

Beecher, M. D., Burt, J. M., O'Lloghlen, A. L., Tempelton, C. N., & Campbell, S. E. (2007). Bird song learning in an eavesdropping context. *Animal Behavior*, **73**(6), 929–935.

Blumberg, M. S. (2005). *Basic instinct: The genesis of behavior*. New York: Thunder's Mouth Press.

Blumberg, M. S. (2008). *Freaks of nature: What anomalies tell us about development and evolution*. New York: Oxford University Press.

Boccia, M. L., & Pedersen, C. A. (2001). Brief vs. long maternal separations in infancy: Contrasting relationships with adult maternal behavior and lactation levels of aggression and anxiety. *Psychoneuroendocrinology*, **26**, 657–672.

Bredy, T. W., Grant, R. J., Champagne, D. L., & Meaney, M. J. (2003). Maternal care influences neuronal survival in the hippocampus of the rat. *European Journal of Neuroscience*, **18**, 2903–2909.

Brenowitz, E. A., & Beecher, M. D. (2005). Song learning in birds: Diversity and plasticity, opportunities and challenges. *Trends in Neuroscience*, **28**(3), 127–132.

Brunjes, P. C., & Frazier, L. L. (1986). Maturation and plasticity in the olfactory system. *Brain Research Reviews*, **11**, 1–45.

Bull, J. J. (1983). *Evolution of sex determining mechanisms*. Menlo Park, CA: Benjamin/Cummings.

Caldji, C., Diorio, J., & Meaney, M. J. (2003). Variations in maternal care alter GABA(A) receptor subunit expression in brain regions associated with fear. *Neuropsychopharmacology*, **28**, 1950–1959.

Caldji, C., Francis, D., Sharma, S., Plotsky, P. M., & Meaney, M. J. (2000). The effects of early rearing environment on the development of GABAA and central benzodiazepine receptor levels and novelty-induced fearfulness in the rat. *Neuropsychopharmacology*, **22**, 219–229.

Caldji, C., Tannenbaum, B., Sharma, S., Francis, D., Plotsky, P. M., & Meaney, M. J. (1998). Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proceedings of the National Academy of Sciences, USA*, **95**, 5335–5340.

Caspi, A., McClay, J., Moffitt, T. E., Mill, J., Martin, J., Craig, I. W., et al. (2002). Role of genotype in the cycle of violence in maltreated children. *Science*, **297**(5582), 851–854.

Champagne, F. A. (2008). Epigenetic mechanisms and the transgenerational effects of maternal care. *Frontiers in Neuroendocrinology*, **29**, 386–397.

Champagne, F. A., Chretien, P., Stevenson, C. W., Zhang, T. Y., Gratton, A., & Meaney, M. J. (2004). Variations in nucleus accumbens dopamine associated with individual differences in maternal behavior in the rat. *Journal of Neuroscience*, **24**, 4113–4123.

Champagne, F. A., Francis, D. D., Mar, A., & Meaney, M. J. (2003). Variations in maternal care in the rat as a mediating influence for the effects of environment on development. *Physiology and Behavior*, **79**, 359–371.

Champagne, F. A., Weaver, I. C., Diorio, J., Dymov, S., Szyf, M., & Meaney, M. J. (2006). Maternal care associated with methylation of the estrogen recCeptor-alpha1b promoter and estrogen receptor-alpha expression in the medial preoptic area of female offspring. *Endocrinology*, **147**, 2909–2915.

Cheng, M.-F. (2003). Vocal self-stimulation: From the ring dove story to emotion-based vocal communication. In P. J. B. Slater, J.S. Rosenblatt, C. T. Snowdon, & T.J. Roper (Eds.), *Advances in the study of behavior, Vol. 33*, (pp. 309–353). New York: Academic Press.

Coopersmith, R., & Leon, M. (1988). The neurobiology of early olfactory learning. In E. M. Blass (Ed.), *Handbook of behavioral neurobiology. Vol. 9. Developmental psychobiology*. New York: Plenum.

Crandall, S. R., Aoki, N., & Nick, T. A. (2007). Developmental modulation of the temporal relationship between brain and behavior. *Journal of Neurophysiology*, **97**, 806–816.

Day, L., & Bentley, M. (1911). A note on learning in Paramecium. *Journal of Animal Behavior*, **1**, 67–73.

Day, N. F., Kinnischtzke, A. K., Adam, M., & Nick, T. A. (2008). Top-down regulation of plasticity in the birdsong system: "Premotor" activity in the nucleus HVC predicts song variability better than it predicts song features. *Journal of Neurophysiology*, **100**, 2956–2965.

de Beer, G. (1930). *Embryology and evolution*. Oxford: Clarendon Press.

de Beer, G. R. (1958). *Embryos and ancestors* (3rd ed.). London: Oxford University Press.

Demski, L. S. (1987). Diversity in reproductive patterns and behavior in teleost fishes. In D. Crews (Ed.), *Psychobiology of reproductive behavior: An evolutionary perspective* (pp. 1–27). Englewood Cliffs, NJ: Prentice-Hall.

Dolinoy, D. C., Weidman, J. R., & Jirtle, R. L. (2007). Epigenetic gene regulation: Linking early developmental environment to adult disease. *Reproductive Toxicology*, **23**(3), 297–307.

Fenoglio, K. A., Brunson, K. L., Avishai-Eliner, S., Chen, Y., & Baram, T. Z. (2004). Region-specific onset of handling induced changes in corticotropin-releasing factor and glucocorticoid receptor expression. *Endocrinology*, **145**, 2702–2706.

Fernández-Teruel, A., Escorihuela, R. M., Driscoll, P., Tobeña, A., & Battig, K. (1991). Infantile (handling) stimulation and behavior in young Roman high- and low-avoidance rats. *Physiology & Behavior*, **50**, 563–565.

Fleming, A. S., Kraemer, G. W., Gonzalez, A., Lovic, V., Rees, S., & Melo, A. (2002). Mothering begets mothering: The transmission of behavior and its neurobiology across generations. *Pharmacology, Biochemistry, and Behavior*, **73**, 61–75.

Fox Keller, E. (2002). *Making sense of life: Explaining biological development with models, metaphors and machines*. Cambridge, MA: Harvard University Press.

Francis, D. D., Caldji, C., Champagne, F., Plotsky, P. M., & Meaney, M. J. (1999). The role of corticotropin-releasing factor – norepinephrine systems in mediating the effects of early experience on the development of behavioral and endocrine responses to stress. *Biological Psychiatry*, **46**, 1153–1166.

Francis, D. D., Diorio, J., Liu, D., & Meaney, M. J. (1999). Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science*, **286**, 1155–1158.

Gerhart, J., & Kirschner, M. (1997). *Cells, embryos, and evolution: Toward a cellular and developmental understanding of phenotypic variation and evolutionary adaptability*. Malden, MA: Blackwell Science.

Gerstein, M. B., Can, B., Rozowsky, J. S., Zheng, D., Jiang, D., Korbel, J. O., et al. (2007). What is a gene, post-ENCODE? History and updated definition. *Genome Research*, **17**, 669–681.

Gilbert, S. F. (2001). Ecological developmental biology: Developmental biology meets the real world. *Developmental Biology*, **233**, 1–12.

Gilbert, S. F. (2006). The generation of novelty: The province of developmental biology. *Biological Theory*, **1**(2), 209–212.

Gilbert, S. F., & Epel, D. (2009). *Ecological developmental biology*. Sunderland, MA: Sinauer.

Gore, A. C. (2008). Developmental programming and endocrine disruptor effects on reproductive neuroendocrine systems. *Frontiers in Neuroendocrinology*, **29**(3), 358–374.

Gorski, R. A., Gordon, J. H., Shryne, J. E., & Southam, A. M. (1978). Evidence for a morphological sex difference within the medial preoptic area of the brain. *Brain Research*, **148**, 333–346.

Gottlieb, G. (1971). *Development of species identification in birds*. Chicago: University of Chicago Press.

Gottlieb, G. (1976). The roles of experience in the development of behavior and the nervous system. In G. Gottlieb (Ed.), *Studies in the development of behavior and the nervous system. Vol. 3. Neural and behavioral specificity* (pp. 25–54). New York: Academic Press.

Gottlieb, G. (1992). *Individual development and evolution: The genesis of novel behavior*. New York: Oxford University Press.

Gottlieb, G. (1999). *Probabilistic epigenesis and evolution*. Worcester, MA: Clark University Press.

Gould, S. J. (1977). *Ontogeny and phylogeny*. Cambridge, MA: Harvard University Press.

Gubernick, D. J., & Alberts, J. R. (1983). Maternal licking of young: Resource exchange and proximate controls. *Physiology and Behavior*, **31**, 593–601.

Gubernick, D. J., & Alberts, J. R. (1985). Maternal licking by virgin and lactating rats: Water transfer from pups. *Physiology and Behavior*, **34**, 501–506.

Hatalski, C. G., Guirguis, C., & Baram, T. Z. (1998). Corticotropin releasing factor mRNA expression in the hypothalamic paraventricular nucleus and the central nucleus of the amygdala is modulated by repeated acute stress in the immature rat. *Journal of Neuroendocrinology*, **10**, 663–669.

Hessler, N. A., & Doupe, A. J. (1999). Social context modulates singing-related neural activity in the songbird forebrain. *Nature Neuroscience*, **2**(3), 209–211.

Hill, D. L. (2001). Taste development. In E. M. Blass (Ed.), *Handbook of behavioral neurobiology. Vol. 13*: *Developmental psychobiology* (pp. 517–550). New York: Kluwer/Plenum.

Hinde, R. A., Hinde, J. (Eds.) (1973). *Constraints on learning*. New York: Academic Press.

Hirsch, H. V. B., Tieman, S. B., Barth, M., & Ghiradella, H. (2001). Tunable seers: Activity dependent development of vision in fly and cat. In Blass E. M. (Ed.), *Handbook of behavioral neurobiology*, Vol. *13*: *Developmental Psychobiology* (pp. 81–141). New York: Kluwer Academic/Plenum.

Ho, S. M., & Tang, W. Y. (2007). Techniques used in studies of epigenome dysregulation due to aberrant DNA methylation: An emphasis on fetal-based adult diseases. *Reproductive Toxicology*, **23**(3), 267–282.

Huot, R. L., Gonzalez, M. E., Ladd, C. O., Thrivikraman, K. V., & Plotsky, P. M. (2004). Foster litters prevent hypothalamic-pituitary-adrenal axis sensitization mediated by neonatal maternal separation. *Psychoneuroendocrinology*, **29**, 279–289.

Huot, R. L., Thrivikraman, K. V., Meaney, M. J., & Plotsky, P. M. (2001). Development of adult ethanol preference and anxiety as a consequence of neonatal maternal separation in Long Evans rats and reversal with antidepressant treatment. *Psychopharmacology (Berl)*, **158**, 366–373.

Impekoven, M., & Gold, P. S. (1973). Prenatal origins of parent-young interactions in birds: A naturalistic approach. In G. Gottlieb (Ed.), *Behavioral embryology*, *Vol. 1*: *Studies on the development of behavior and the nervous system* (pp. 325–356). New York: Academic Press.

Jablonka, E., & Lamb, M. J. (2005). *Evolution in four dimensions*. Cambridge, MA: MIT Press.

Jaffee, S. R., Caspi, A., Moffitt, T. E., Dodge, K. A., Rutter, M., Taylor, A., et al. (2005). Nature × Nurture: Genetic vulnerabilities interact with child maltreatment to promote conduct problems. *Development and Psychopathology*, **17**, 67–84.

Jirtle, R. L., & Skinner, M. K. (2007). Environmental epigenomics and disease susceptibility. *Nature Reviews Genetics*, **8**(4), 253–262.

Johannsen, W. (1909). *Elemente der exacten Erblichkeitslehre*. Jena: Gustav Fischer.

Johannsen, W. (1911). The genotype conception of heredity. *American Naturalist*, **45**, 129–159.

Kirschner, M. W., & Gerhart, J. C. (2006). *The plausibility of life: Resolving Darwin's dilemma*. New Haven, CT: Yale University Press.

Kojima, S., & Doupe, A. J. (2008). Neural encoding of auditory temporal context in a songbird basal ganglia nucleus, and its independence of birds' song experience. *European Journal of Neuroscience*, **27**, 1231–1244.

Lee, M. H. S., & Williams, D. I. (1974). Changes in licking behavior of rat mothers following handling of young. *Animal Behaviour*, **22**, 679–681.

Lehmann, J., & Feldon, J. (2000). Long-term biobehavioral effects of maternal separation in the rat: Consistent or confusing? *Reviews in the Neurosciences*, **11**, 383–408.

Lehrman, D. S. (1965). Interaction between internal and external environments in the regulation of the reproductive cycle of the ring dove. In F. A. Beach (Ed.), *Sex and behavior* (pp. 355–380). New York: Wiley.

Levine, S. (1957). Infantile experience and resistance to physiological stress. *Science*, **126**, 405.

Levine, S. (2002). Enduring effects of early experience in adult behavior. In: D. W. Pfaff, A.P. Arnold, A. M. Etgen, S.E. Fahrbach, & R. T. Rubin, (Eds.), *Hormones, brain and behavior, Vol. 4* (pp. 535–542). New York: Academic Press.

Lewontin, R. C. (1982). Organism and environment. In H. C. Plotkin (Ed.), *Learning, development, and culture: Essays in evolutionary epistemology* (pp. 151–172). New York: Wiley.

Liu, D., Caldji, C., Sharma, S., Plotsky, P. M., & Meaney, M. J. (2000). Influence of neonatal rearing conditions on stress-induced adrenocorticotropin responses and norepinepherine release in the hypothalamic paraventricular nucleus. *Journal of Neuroendocrinology*, **12**, 5–12.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., et al. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic–pituitary–adrenal responses to stress. *Science*, **277**, 1659–1662.

Lorenz, K. (1937). The conception of instinctive behavior. In C. H. Schiller (Ed. and Trans.), (1957). *Instinctive behavior: The development of a modern concept*. New York: International Universities Press.

Lorenz, K. (1965). *The evolution and modification of behavior*. Chicago, IL: University of Chicago Press.

Lussana, F., Painter, R. C., Ocke, M. C., Buller, H. R., Bossuyt, P. M., & Roseboom, T. J. (2008). Prenatal exposure to the Dutch famine is associated with a preference for fatty foods and a more atherogenic lipid profile. *American Journal of Clinical Nutrition*, **88**, 1648–1652.

Ma, X., Buffler, P. A., Gunier, R. B., Dahl, G., Smith, M. T., Reinier, K., et al. (2002). Critical windows of exposure to household pesticides and risk of childhood leukemia. *Environmental Health Perspectives*, **110**(9), 955–960.

Meaney, M. J. (2001). Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annual Review Neuroscience*, **24**, 1161–1192.

Meaney, M. J., & Szyf, M. (2005). Maternal care as a model for experience-dependent chromatin plasticity? *Trends in Neuroscience*, **28**, 456–463.

Meaney, M. J., Tannenbaum, B., Francis, D., Bhatnager, S., Shanks, N., Viau, V., et al. (1994). Early environmental programming hypothalamic-pituitary-adrenal responses to stress. *Seminars in the Neurosciences*, **6**, 247–259.

Meaney, M. J., Viau, V., Aitken, D. H., & Bhatnagar, S. (1989). Glucocorticoid receptors in brain and pituitary of the lactating rat. *Physiology & Behavior*, **45**, 209–212.

Meitzen, J., Thompson, C. K., Choi, H., Perkel, D. J., & Brenowitz, E. A. (2009). Time course of changes in Gambel's white-crowned sparrow song behavior following transitions in breeding condition. *Hormones and Behavior*, **55**, 217–227.

Mennella, J. A., Ziegler, P., Briefel, R., & Novak, T. (2006). Feeding infants and toddlers study: The types of foods fed to Hispanic infants and toddlers. *Journal of American Diet Association*, **106**, (1 Suppl 1), 96–106.

Michel, G. F. (1986). Experiential influences on hormonally dependent ring dove parental care. *Annals of the New York Academy of Sciences*, **474**, 158–165.

Michel, G. F. (2001). Growth curve analyses are best suited to examine the relation between developmental pathways and selective breeding. *Developmental Psychobiology*, **39**(4), 247–250.

Michel, G. F. (2007). Doing what comes naturally: The role of self generated experience in behavioral development. *European Journal of Developmental Science*, **1**, 152–161.

Michel, G. F. (in press). Behavioral science, engineering, and poetry revisited. *Journal of Comparative Psychology*.

Michel, G. F., & Moore, C. L. (1995). *Developmental psychobiology: An interdisciplinary science*. MIT Press: Cambridge, MA: MIT Press.

Michel, G. F., & Tyler, A. N. (2005). Critical period: A history of the transition from questions of when, to what, to how. *Developmental Psychobiology*, **46**(3), 156–162.

Michel, G. F., & Tyler, A. N. (2007a). Can knowledge of developmental processes illuminate the evolution of parental care? *Developmental Psychobiology*, **49**(1), 33–44.

Michel, G. F., & Tyler, A. N. (2007b). Developing human nature: "Development to" vs. "Development from." *Developmental Psychobiology*, **49**(8), 788–799.

Moore, C. L. (1982). Maternal behavior of rats is affected by hormonal condition of pups. *Journal of Comparative & Physiological Psychology*, **96**, 123–129.

Moore, C. L. (1984). Maternal contributions to the development of masculine sexual behavior in laboratory rats. *Developmental Psychobiology*, **17**, 347–356.

Moore, C. L. (1985). Sex differences in urinary odors produced by young laboratory rats (Rattus norvegicus). *Journal of Comparative & Physiological Psychology*, **99**, 336–341.

Moore, C. L. (1992). The role of maternal stimulation in the development of sexual behavior and its neural basis. *Annals of the New York Academy of Sciences*, **662**, 160–177.

Moore, C. L., & Chadwick-Dias, A. M. (1986). Behavioral responses of infant rats to maternal licking: Variations with age and sex. *Developmental Psychobiology*, **19**, 427–438.

Moore, C. L., Dou, H., & Juraska, J. M. (1992). Maternal stimulation affects the number of motor neurons in a sexually dimorphic nucleus of the lumbar spinal cord. *Brain Research*, **572**, 52–56.

Moore, C. L., & Morelli, G. A. (1979). Mother rats interact differently with male and female offspring. *Journal of Comparative & Physiological Psychology*, **93**, 677–684.

Moore, C. L., & White, R. H. (1996). Sex differences in sensory and motor branches of the pudendal nerve of the rat. *Hormones and Behavior*, **30**, 590–599.

Moore, C. L., Wong, L., Daum, M. C., & Leclair, O. U. (1997). Mother-infant interactions in two strains of rats: Implications for dissociating mechanism and function of a maternal pattern. *Developmental Psychobiology*, **30**, 301–312.

Núñez, J. F., Ferré, P., García, E., Escorihuela, R. M., Fernández-Teruel, A., & Tobeña, A. (1995). Postnatal handling reduces emotionality ratings and accelerates two way active avoidance in female rats. *Physiology & Behavior*, **57**, 831–835.

Parfitt, D. B., Levin, J. K., Saltstein, K. P., Klayman, A. S., Greer, L. M., & Helmreich, D. L. (2004). Differential early rearing environments can accentuate or attenuate the responses to stress in male C57BL/6 mice. *Brain Research*, **1016**, 111–118.

Patisaul, H. P., and Adewale, H. B. (2009). Long-term effects of environmental endocrine disruptors on reproductive physiology and behavior. *Frontiers in Behavioral Neuroscience*, **3**(10), 1–18.

Phoenix, C. H., Goy, R. W., Gerall, A. A., & Young, W. C. (1959). Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology*, **65**, 369–382.

Plotsky, P. M., & Meaney, M. J. (1993). Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress induced release in adult rats. *Molecular Brain Research*, **18**, 195–200.

Plotsky, P. M., Thrivikraman, K. V., Nemeroff, C. B., Caldji, C., Sharma, S., & Meaney, M. J. (2005). Long-term consequences of neonatal rearing on central corticotrophin releasing factor systems in adult male rat offspring. *Neuropsychopharmacology*, **30**, 2192–2204.

Raff, R. A. (1996). *The shape of life*. Chicago, IL: Chicago University Press.

Rovee-Collier, C. (2001). Information pick-up by infants: What is it, and how can we tell? *Journal of Experimental Child Psychology*, **78**, 35–49.

Sapolsky, R. M., Meaney, M. J., & McEwen, B. S. (1985). The development of the glucocorticoid receptor system in the rat limbic brain. III. Negative-feedback regulation. *Brain Research*, **350**, 169–173.

Schlichting, C. D., & Pigliucci, M. (1998). *Phenotypic evolution: A reaction norm perspective*. Sunderland, MA: Sinauer Associates.

Schneirla, T. C. (1966). Behavioral development and comparative psychology. *Quarterly Review of Biology*, **41**, 283–302.

Shapiro, D. Y. (1983). Distinguishing behavioral interactions from visual cues as causes of adult sex change in coral reef fish. *Hormones and Behavior*, **17**, 424–432.

Singer, J. D., & Willett, J. B. (2003). *Applied longitudinal data analysis: Modeling change and event occurance*. New York: Oxford University Press.

Sullivan, R. M., Shokrai, N., & Leon, M. (1988). Physical stimulation reduces the body temperature of infant rats. *Developmental Psychobiology*, **21**, 225–235.

Sullivan, R. M., Wilson, D. A., & Leon, M. (1988). Physical stimulation reduces the brain temperature of infant rats. *Developmental Psychobiology*, **21**, 237–250.

Tyler, A. N. (2006). When is a description not an explanation: A response to Armstrong et al. (2006). *Developmental Psychobiology*, **48**(4), 332–335.

von Uexkuell, J. (1957). A stroll through the world of animals and men. In C. H. Schiller (Ed. & Trans.) *Instinctive behavior* (pp. 5–80). New York: International Universities Press.

Vince, M. A. (1974). Some environmental effects on the activity and development of the avian embryo. In G. Gottlieb (Ed.), *Behavioral embryology, Vol. 1: Studies on the development of behavior and the nervous system* (pp. 285–323). New York: Academic Press.

Walker, C.-D Naef, L., Xu, S., Long, H., Moquin, L., Srivastava, L., et al. 2007. *Perinatal high fat feeding of the mother induces long-term changes in the mesocorticolimbic dopamine system of rat offspring*. paper presented at the 3rd Annual Parental Brain Conference, Boston, MA.

Weaver, I. C., Diorio, J., Seckl, J. R., Szyf, M., & Meaney, M. J. (2004). Early environmental regulation of hippocampal glucocorticoid receptor gene expression: Characterization of intracellular mediators and potential genomic target sites. *Annals of the New York Academy of Sciences*, **1024**, 182–212.

Weaver, I. C., Grant, R. J., & Meaney, M. J. (2002). Maternal behavior regulates long-term hippocampal expression of BAX and apoptosis in the offspring. *Journal of Neurochemistry*, **82**, 998–1002.

West, M. J., King, A. P., & Arberg, A. A. (1988). The inheritance of niches: The role of ecological legacies in ontogeny. In E. M. Blass (Ed.), *Handbook of behavioral neurobiology, Vol. 9: Developmental psychobiology and behavioral ecology* (pp. 41–62). New York: Plenum.

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. New York: Oxford University Press.

Wheelwright, N. T., Swett, M. B., Levin, I. I., Kroodsma, D. E., Freeman-Gallant, C. R., & Williams, H., (2008). The influence of different tutor types on song learning in a natural bird population. *Animal Behaviour*, **75**, 1479–1493.

Williams, H. (2004). Birdsong and singing behavior. *Annals of the New York Academy of Sciences*, **1016**, 1–30.

Wilson, D. A., Fletcher, M. L., & Sullivan, R. M. (2004). Acetylcholine and olfactory perceptual learning. *Learning and Memory*, **11**, 28–34.

Zeigler H. P., & Marler P. (Eds.) (2004). Behavioral neurobiology of birdsong. *Annals of the New York Academy of Sciences*, **1016**.

7

Contemporary Ideas in Physics and Biology in Gottlieb's Psychology

Ty Partridge and Gary Greenberg

Gilbert Gottlieb was among the leading theorists in all of psychology and his imprint on contemporary developmental science generally and to developmental psychobiology and comparative psychology in particular is difficult to overstate. In many ways Gottlieb's early formulations (e.g., Gottlieb, 1973, 1984, 1985) regarding the dynamic multilevel transactions integrating biology and ecology in the course of development were prescient of major advances in the field resulting from empirical findings in molecular genetics and cell development along with conceptual and methodological tools from nonlinear dynamic systems and complex adaptive systems theory. As such, in this chapter, we have tried to demonstrate explicitly these linkages between Gilbert Gottlieb's work and contemporary ideas issuing forth from nonlinear dynamic systems theory and complex adaptive systems theory. In fond appreciation for Gilbert's singular gift of placing the long-standing dispute between geneocentric perspectives and contextual perspectives regarding the role of biology and ecology in development (see Gottlieb (1992) for an excellent example), we also pursue the historical development of the main theoretical points addressed in this essay.

Emergence, Self-Organization, and Hierarchy

It is not unfair to state that since adopting the paradigm of science in the late 19th century psychology has suffered a severe case of physics envy (e.g., Heylighen, Cilliers, & Gershonson, 2007). The early experimentalists such as Wundt and James wanted the new science of psychology to be a natural science, and so emulated the techniques and philosophy of physics. Indeed, behavior was understood by them to

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be as natural a phenomenon as rolling balls down inclined planes was for Galileo. Thus, early scientific psychology was atomistic (structuralism), Darwinian (functionalism), and mechanistic (behaviorism). Late 19th- and 20th-century psychology is now understood to have been materialistic, positivistic, and reductionistic. In this Newtonian world, there is no place for novelty – everything that exists has always existed, though perhaps in different forms (Heylighen et al., 2007). However, just as this approach in physics and the other sciences has failed to live up to its initial promise and has since given way to a more holistic, field oriented, and contextual paradigm (Davies & Gribbin, 1992; Goodwin, 1994; Kauffman, 2000; Sheldrake, 1995), so psychology too has begun to give up its adherence to an old fashioned physics and has made inroads into this newly emerging scientific paradigm (e.g., Chorover, 1990; Lerner, 2002). This new perspective, and its extremely broad application, can be summarized as follows:

> since the 1960s, an increasing amount of experimental data. . . imposes a new attitude concerning the description of nature. Such ordinary systems as a layer of fluid or a mixture of chemical products can generate, under appropriate conditions, a multitude of *self-organisation phenomena* on a macroscopic scale – a scale orders of magnitude larger than the range of fundamental interactions – in the form of spatial patterns or temporal rhythms. . .. [Such states of matter] provide the natural archetypes for understanding a large body of phenomena in branches which traditionally were outside the realm of physics, such as turbulence, the circulation of the atmosphere and the oceans, plate tectonics, glaciations, and other forces that shape our natural environment; or, even, the emergence of self-replicating systems capable of storing and generating information, embryonic development, the electrical activity of the brain, or the behavior of populations in an ecosystem or in economic development. (Nicolis, 1989, p. 316)

While psychology comes late to a full-blown adoption of this new perspective in science, there are seeds of these ideas in the writings of Lloyd Morgan (1923) on emergent evolution, J. R. Kantor on interbehaviorism (1959; Pronko, 1980), and T. C. Schneirla on behavioral levels (Aronson, Tobach, Rosenblatt, & Lehrman, 1972). Contemporary biologists such as Brian Goodwin (1994) and Stuart Kauffman (1993, 1995) have elucidated the linkages between developmental psychobiology and newly emerging concepts of complex adaptive systems and self organization (Prigogine & Stengers, 1984). Indeed, systems thinking is now even playing a role in medicine (Ahn, Tewari, Poon, & Phillips, 2006). In psychology this approach is the hallmark of developmental psychobiology (e.g., Michel and Moore, 1995), developmental systems theory (e.g., Ford & Lerner, 1992; Oyama, Griffiths, & Gray, 2001), and the probabilistic epigenesis of Gilbert Gottlieb (e.g., 1992, 1997), the main focus of this chapter.

There are three crucial linkages among these diverse ideas: the important organizing principle of integrative levels, the idea that there is a tendency towards increased complexity with evolutionary advance, and the contextual nature of

behavioral events. A synthesis of these three ideas leads to a developmental perspective in which behavior is seen to be the result of the fusion of biological and psychosocial factors, by probabilistic epigenetic events rather than by preprogrammed genetic or other biochemical ones (Gottlieb, 1992, 1984; Kuo, 1967). The physics model of this is referred to as "nonlinear dynamic systems theory." Used in psychology it provides a theoretically consistent language with which to describe and analyze behavioral development (Michel & Moore, 1995; Novak, 1996).

Nonlinear dynamics contains a lexicon of concepts pertaining to change processes over time that does not exist in any other known theoretical system. Dynamical models allow us to compare and contrast seemingly unrelated phenomena that often share common dynamical structures. Nonlinear dynamics and complex systems analysis are continuing to help revolutionize our understanding in many of the life sciences. While these ideas are just beginning to find their way into mainstream psychology (Boker, 2001; Damon & Lerner, 2001; Newell & Molenaar, 1998, Sulis & Trofimova, 2000) they are not yet widely accepted, and indeed, much of psychology is still dominated by the reductionistic model – this is certainly true of Evolutionary Psychology (e.g., Buss, 2005; Pinker, 2002), which reduces behavior to brain modules and inherited genetic factors.

The overall situation in the traditional sciences was summarized by Stuart Kauffman (1993, p. 173), a leading figure in the widespread application of these ideas, as follows:

> Eighteenth-century science, following the Newtonian revolution, has been characterized as developing the sciences of organized simplicity, nineteenth-century science, via statistical mechanics, as focusing on disorganized complexity, and twentieth and twenty-first-century as confronting organized complexity.

By the 1980s it was possible to refer to these developments as complexity science. Its fundamental aspects include non-linear dynamics and statistical mechanics, computer science, developmental evolutionary biology, and the application of these ideas to higher levels of behavioral organization (Heylighen et al., 2007).

Hierarchy

A crucial idea is the view that the universe is ordered as a family of hierarchies in which natural phenomena exist in levels of increasing organization and complexity. Indeed, the sciences themselves have been divided into areas of study based on these qualitative changes in complexity of organization, with physics and chemistry addressing the lower levels of complexity and biology, psychology, and sociology addressing higher levels of complexity (see Feibleman, 1954). Gell-Mann (1994) refers to this hierarchical nature of the sciences by raising the question of which

science is the fundamental one, "science A is more fundamental than science B when. . . the laws of science A encompass in principle the phenomena and laws of science B. . ." (p. 109). Novak (1996) refers to this hierarchy as a "continuum of scientific disciplines" (p. 4). The hierarchical nature of phenomena is illustrated in Box 7.1 and summarized by Aronson (1984, p. 66) thus:

> [The levels concept]. . . is a view of the universe as a family of hierarchies in which natural phenomena exist in levels of increasing organization and complexity. Associated with this concept is the important corollary that these successions of levels are the products of evolution. Herein lies the parallel with anagenesis.

Anagenesis recognizes the role played in psychology by the evolution of increasing complex biological forms, especially nervous systems (Greenberg, 1995; Greenberg, Partridge, Weiss, & Haraway, 1999). It is of interest to note that in the mid 20th century it was popular to criticize comparative psychology for not having a theoretical framework (e.g., Hodos & Campbell, 1969)

Box 7.1. Hierarchies

Anthropology	Organism
Sociology	Organ
Psychology	Tissue
Biology	Cell
Chemistry	Organelle
Physics	Molecule
Mathematics	Atom

Gilbert Gottlieb was one of the few to take issue with this: "There is a theory in comparative psychology, and that theory is a hierarchical classification of adaptive behavior by grade [i.e., anagenetic analysis], independent of cladistic (i.e., genetic) relationship" (1984, p. 454); and, "Anagenesis is of course not the only theory in comparative psychology, but it has been a major one since at least as early as the 19th century" (1984, p. 449). Greenberg (1995) provided a summary of the role of anagenesis in comparative psychology.

For our purposes it is important to emphasize that increasing complexity is so pervasive a phenomenon that some have likened it to a second of law of evolution after natural selection (Saunders & Ho, 1976, 1981, 1984). Many evolutionists have adopted this line of thinking, including Stebbins (1969) who suggested that we can recognize at least eight major levels of complexity in the evolution of life (and behavior) and Maynard Smith (Smith and Szathmáry, 1999) who at different times

has identified five or eight levels of complexity, though he associated each with degrees of organization of genetic material. The important point to be made here is that there is a hierarchy of levels of increasing complexity and organization in the evolution of life, and not that there are five or eight such levels. This was recognized earlier by Pringle (1951), who noted that, "The characteristic of living systems which distinguishes them most clearly from the non-living is their property of progressing by the process which is called evolution from less to more complex states of organization" (p. 175). It is worth pointing out that while complexity is not easily defined, it still plays a crucial role in modern evolutionary biology (McShea, 1996). For a thorough treatment of complexity in nature see Chaisson (2001). With respect to the application of complexity to development in psychology the following statement by Arthur (1993, p. 144) is telling: "The writer Peter Matthiessen once said, 'The secret of well-being is simplicity.' True. Yet the secret of evolution is the continual emergence of complexity. Simplicity brings a spareness, a grit; it cuts the fat. Yet complexity makes organisms like us possible in the first place."

Of course we recognize that this is not only a controversial issue, it is as well a contentious one, debate frequently occurring from ideological perspectives (Lewin, 1992). We find the statement by Bonner (1988, pp. 5–6) to best reflect our position:

> There is an interesting blind spot among biologists. While we readily admit that the first organisms were bacteria-like and that the most complex organism of all is our kind, it is considered bad form to take this as any kind of progression. . .. It is quite permissible for the paleontologist to refer to strata as upper and lower, for they are literally above and below each other. . . But these fossil organisms in the lower strata will, in general, be more primitive in structure as well as belong to a fauna and flora of earlier times, so in this sense "lower" and "higher" are quite acceptable terms. . .. But one is flirting with sin if one says a worm is a lower animal and a vertebrate a higher animal, even though their fossil origins will be found in lower and higher strata.

Bonner's book is an exposition on the evolution of biological complexity, a phenomenon he likens to a "law" of evolution.

Schneirla's concept of behavioral levels, described systematically in a paper written with his colleague and student Ethel Tobach (Tobach & Schneirla, 1968), is derived from the concept of integrative levels. This concept served as an organizing theme to explain the full range of behavior across the animal kingdom in a recent book (Greenberg & Haraway, 2002). The behavioral levels are separated into two groups, one at which biological factors dominate behavior and one at which psychological principles become important. The levels originally proposed are:

1. *Taxis*: At this level behavior is under immediate stimulus control, such as in the case of a moth flying towards a light source.

2. *Biotaxis*: At this next, higher, level behavior is influenced not only by the immediate presence of a stimulus but also by the presence of biochemical sequelae from other organisms, that is, by the presence of stimulation which is a concomitant of the presence of other organisms. An example is the sexual attraction of male moths to pheromones secreted by females.
3. *Biosocial*: At this level the social interaction of groups of animals plays an important role in organizing and regulating behavior. Among Schneirla's research contributions was his analysis of the behavior of army ants, whose cyclic activity was seen to be a result of reciprocal social stimulation provided by the enormous number of individuals in an ant colony. One might study the behavior of individual ants fruitlessly to discern the source of their cyclical behavior pattern, which is displayed only when ants are together in large numbers (e.g., Gordon, 1988, 1997).
4. *Psychotaxis*: At this level mediation by past experience enters into the behavioral equation, and behavior is no longer tied only to the immediate presence of a stimulus. Thus, an animal's current behavior may be affected by an earlier history of experiential effects. In their analysis of cat/kitten behavior, Rosenblatt and Schneirla (1962) showed that the relationship between infant and mother is founded in biotactic responses – the kitten orients to the mother by means of tactile and olfactory stimuli – but higher-order phenomena such as learning and reinforcement play an important role in later stages of that relationship.
5. *Psychosocial*: Behavior organized at this level is represented by the complex social bonds and social behaviors which are characteristic of advanced vertebrates. For example, among primates, lasting social bonds result from complex biosocial and biotactic interactions between an infant and a mother such as those involved in rocking, providing of contact comfort (Harlow, 1958), and nursing. Primate social behavior is organized at this level, and we (Greenberg et al., 1999) have proposed further subdividing this level into three separate behavioral grades distinguished by the nature of communication complexity: a communication only, non-language level (e.g., vervet monkeys); a proto-language level (e.g., chimpanzees and bonobos); and a true language level (only *H. sapiens*).

Emergence

The demarcations and transitions between the levels of organization listed in Box 7.1 and the behavioral levels described by Tobach and Schneirla (1968) are nonlinear and probabilistically discontinuous. As the number of components or events at each level increases (i.e., as complexity increases) a critical ratio of component number and component interconnectivity results. At this critical ratio

the system displays molar level stability and micro level instability. In other words, the behavior of individual components within the system is volatile, but the global "structure" of the system as a whole is stable. This property of global stability and internal instability allows these systems, which Kauffman (1993, 2000) refers to as being "poised at the edge of chaos," to be quite adaptable to changing environmental pressures and contingencies making them ideal for flourishing under principles of natural selection. There are thresholds of organizational complexity at which small quantitative increases result in qualitative discontinuities (i.e., levels) resulting in the appearance of new levels. In dynamic systems this is known as a "phase transition."

A phase transition results in a new and more complex level of organization. No new inputs are required. Rather, the new level arises from a reorganization of the old elements and is characterized by a new whole, even though there are no new elements. The new whole demonstrates new properties not apparent prior to the transition. These new properties are said to emerge from the reorganization of the old elements. Emergence is often thought of as some sort of mystical concept. For example, the notion that consciousness emerges from complex neural functioning is understood by some to invoke a "vital" force that is somehow added to the mix. This misunderstanding stems from a confusion in ascribing properties to levels. Even Francis Crick acknowledged this seemingly mystical aspect of emergence (Crick, 1994), though he went on to refer to its scientific meaning.

When asking someone favorable to the notion of emergence about the concept, they often reply that it means "a whole is different from the sum of its parts." While this statement is true, it contributes to the misunderstanding. Language, for example, can be understood to be an emergent property of the dynamic interplay of a number of factors including neocortical size relative to body size, social and cultural complexity, and abstract reasoning ability. Taken improperly, emergence in this context is taken to mean that language would be due to the additive effects of these factors plus the "emergent factor." The emergent factor here would be taken to be an independent property that is added to the mix as the necessary component to produce language and is often ascribed vitalistic properties. To the contrary, however, the emergent property is not a property at the level of individual components of a system. Rather, it is a property of the entire system. In the absence of that system there is no emergent property. Put another way, concepts which are referred to as emergent (i.e., language, social behavior, symbolic thought, etc.) are not entities, but rather are *processes* of collections of entities. Contemporary cognitive scientists understand mental phenomena from this perspective; mind, thus, is conceptualized by some to be an emergent property of the organism's nervous and other critical systems. (Bunge, 1980; Sperry, 1987).

The unique properties of water that arise when hydrogen and oxygen are combined two parts to one and catalyzed with a spark is one common example of emergence. These chemicals simply become water in the right context, that is, the presence of electricity. The emergent properties of water are not separate from, but

are part of, the system, hydrogen-oxygen-electricity. The properties of water are not inherent in the properties of any component of that system. Similarly, chlorine gas is highly toxic and sodium burns when immersed in water. Yet sodium chloride, an essential ingredient in our diet is just that – chlorine and sodium – which take on different properties when organized as common salt (Novak, 1996).

This then is what we mean when we refer to emergent properties (e.g., "A significant aspect of nonequilibrium physics and self-organisation is the emergence of *new levels of description* brought about by the underlying dynamics" Nicolis, 1989, p. 341). Another crucial aspect of what we mean when we talk about emergence is what could be referred to as the emergent event. The concepts of state-space, attractors, and phase transitions provide a useful language for understanding what we mean here. All systems are comprised of organized components. Each component has variable attributes, but for simplicity sake let us assume that each component has only one variable attribute and that attribute varies between only two states (e.g., on and off). We can define a state-space for that system as the total number of combinations of component values. For example a system with only two components, each of which could be either on or off, could only exist in the following states: on–on, on–off, off–on, off–off. This then is the total state space for that system. However, most complex systems do not exist – and are not capable of existing – in every possible state in their state-space. For example, in our simple system "on-on" may not be very likely.

The sub-set of states most frequently occupied by a system are referred to as attractors. To use the water example again, water, as an organized system of hydrogen, oxygen, and electricity, has three attractors: ice, liquid water, and steam. A phase transition takes place when the system jumps from one attractor to another, as when water goes from liquid form to gaseous form (steam). This transition can be referred to as emergent because there is no in-between stage, and the properties of steam and liquid water are qualitatively different. Thus, when we conceptualize language as emergent from increased brain complexity, symbolic reasoning ability, and social complexity, we are concluding that there is a qualitative "leap" from non-language, or proto-language, to "true" language.

As we stated above, while emergence is only now finding its way into the routine lexicon of science, the idea is not new. A good summary of the history of emergent thinking in psychology is provided by Sawyer (2002). He shows that the roots of this line of thinking lie in 19th century organicism – that an organism is more than merely the sum of its parts and that it depends on the structural arrangement of its parts, its organization. And, of course, the holism of the Gestalt psychologists was steeped in emergent thought. [It is of interest to note that Smuts (1926) apparently was the first to use the term holism which he defined in the Gestalt way as wholes being greater than the sum of their parts.] In 1941 the increasing influence of the concept of integrative levels resulted in a symposium at the University of Chicago (Redfield, 1942). Papers presented there included Hyman's "The Transition from the Unicellular to the Multicellular Individual;" Gerard's treatment of "Higher

Levels of Integration;" Jennings', "The Transition from the Individual to the Social Level;" Carpenter's, "Societies of Monkeys and Apes;" and several others. More recently, Goldstein (1999) has discussed the history of emergence as a scientific construct in a new journal, aptly titled, *Emergence*.

Having worked from this perspective for the entirety of our careers, we are quite at home with these ideas. Others still have difficulty accepting the idea of emergence, suggesting that it has an almost "mystical" quality. However, though emergent phenomena appear to be results without apparent causes, "there is nothing mystical, magical or unscientific about it" (Fromm, 2005b). Goldstein (1999, p. 49) provides a workable definition: "Emergence. . . refers to the arising of novel and coherent structures, patterns, and properties during the process of self-organization in complex systems." Even the idea of self-organization troubles some contemporary scientists. But, these ideas are now well accepted in contemporary physics (Davies, 1989). Indeed, the core concept behind self-organization is understood to be that of emergence (Fromm, 2005a, 2005b). It is the application of such concepts to new areas of science and to scientists unfamiliar with them that leads to skepticism.

Einstein himself wrestled with the new ideas of quantum physics, unable to accept that some events are indeterminate, that there must be some as yet undiscovered underlying causes of all events. There is a parallel here with emergence and complex systems as alternatives to reductionistic analyses. Where do these new wholes come from, these newly emerging properties and structures – and how can events self-organize? It was Newton who said he makes no hypotheses about gravity – it just is. And so it is with self-organization. An implication of Big Bang cosmology, is that given enough time, hydrogen and helium become sentient beings (Bryson, 2003; Singh, 2005).

> It has to be stressed that the existence of chaotic outcomes of this kind does not involve an abandonment of causality *in principle*. If we could measure to the degree of accuracy we need then we could model the system, albeit in non-linear terms, and then we could predict what the outcome of changes would be. *In practice* we can't. It is precisely this practical limit – that word "limit" – which seems to set a boundary on science and science derived technology. (Byrne, 1998, p. 19)

We refer the reader to Goldstein's (1999) treatment for a thorough discussion of this aspect of the topic.

Suffice it to say, that for our purposes, applied to psychology, we can identify emergent phenomena by several criteria: they display radical novelty – features not present in the underlying complex system; they display coherence or correlation – they have a unity over time; they exist at the global or macro level and not at all at the underlying micro level; they are dynamical, arising as a result of the dynamic interplay of underlying micro events; and they are ostensive – they really exist and are observable.

The contentious debate between reductionistic and holistic orientations regarding the fundamental nature of scientific explanations is one of the more longstanding debates in the philosophy of science. The concept of emergence as it turns out is perhaps the most important construct in the entire debate, but not in the manner in which we may have thought. As with most longstanding debates the argument between determinism and holism has been characterized by strawman attacks and a difficulty in finding a clear articulation of the exact nature of the conflict. Frequently, the debate has been couched in terms of deterministic vs. probabilistic models of causality. The reductionist approach is allied with a deterministic understanding of causality and the holistic approach applies a more probabilistic interpretation of causality. However, this debate tends to be obfuscating. There are very few adherents to strict determinism still operating in science. Indeed, even the most fundamental of all reductionistic scientific enterprises – the search for a universal theory in particle physics, which would unite our theoretical understanding of the physical universe from the very smallest of scales to the very largest of scales – is firmly entrenched in a probabilistic field calculus.

The other common debate regards the flow of causal information. Reductionist approaches are characterized as arguing that the flow of causality runs from the parts to the whole. Considering brain behavior relationships for example, a reductionistic account could employ a probabilistic causal model and even a complex "causal network" among brain regions, yet functional neural structure will always be considered primal in terms of causal relationships with behavioral functions. In contrast, a holistic approach would incorporate such neural structures into a broader multilevel causal network in which causal interdependencies would flow from both part to whole (i.e., neural organization to behavior) and from whole to part (i.e, behavior to neural organization) – that is, "downward causation" (Campbell, 1990). For example, an individual's behavior is influenced not only by its neurophysiological processes, but also by its societal rules and regulations. This latter type of argument comes closer to reflecting the fundamental differences between reductionistic and holistic approaches to science. However, the concept of emergence, which is the keystone to most holistic approaches, implies a much deeper distinction between these two philosophical approaches. This more central distinction has to do with the computability of causal relations from parts to wholes.

The principle thesis of emergentist theorists and philosophers has been that even with a full knowledge of all the lower order parts and their potential relationships, the laws of the higher order wholes cannot be deduced. For example, emergentists often argue that even armed with the full sequence of the human genome and a full understanding of the multiplicity of regulatory networks involved in protein synthesis – a full understanding of even morphological phenotypes let alone behavioral phenotypes, could not be ascertained. Likewise, even if all of the neural circuits of the human brain could be sketched in a grand schematic and all of the probabilistic rules governing the synaptic flow of information could be catalogued,

emergentists claim that neuroscience would be no better prepared to predict behavior. This has always been the defining claim of emergent holism and for the better part of scientific history it has been the Achilles heel of the position. The central and tacit assumption of the reductionist claim is that if we had a full knowledge of the parts of a system such as the genome or the brain, along with the rules governing their interrelationships then an algorithm could be derived which could be used to predict the macro-behavior of the system – even if only in probabilistic terms. Serving the reductionist argument is a long history of empirical success at doing just this. Indeed, the entirety of the symbiotic relationship between mathematics and science has been based on this assumption. And with this symbiotic relationship reductionistic science has had the advantages of a formalized logical system in which to base its claims. Of course, reductionism has a long and successful history in science. We see merit in agreeing with Dennett's (1995) distinction between two forms of this: *bland* reductionism – the important study of parts, which no scientist would deny; and *greedy* reductionism, which pushes the idea to extremes, that everything can eventually be explained by physics, chemistry, and mathematics.

Emergentist approaches on the other hand have had to argue largely from intuition and empirical observation. However, the exponential growth in computational power across the last two decades has given rise to not only a new domain of science – the study of complex dynamic systems – but also an entirely new formalized logic system. Modern science has largely been built on a calculus comprised of continuous functions. In order to use these functions enormous simplifying assumptions need to be made about the uniqueness of the constituent parts being studied. When studying a system comprised of even a few discrete but interdependent units this branch of mathematics becomes nearly intractable. Consider the difficulty inherent in the three-body problem for example. Newton's laws of gravity work quite well for two bodies, but put at third body into the system and it becomes nearly impossible to solve.

Mayr (1985) has pointed out that while emergence is a key to understanding how complex systems develop from simpler ones, it plays a crucial role in living organisms: "Such emergence is quite universal, occurring of course in inanimate systems, but it nowhere else plays the important role that it does in living systems" (p. 58). While the idea of emergence plays an important role in contemporary science it has been discussed since the late 19th and early 20th centuries (e.g., Morgan, 1901). Karl Popper (1974) pointed out that, "We live in a universe of emergent novelty" (p. 282). As we have pointed out earlier, there is also a downward causation aspect to emergence, consistent with the integrative levels model of science, the hierarchical or layered view of the universe of events (Kim, 1999). As Kim (1999, p. 24) explains this, "life and consciousness, emergent properties out of physicochemical and neural properties respectively, have a causal flow of events at the lower levels, levels from which they emerge. That of course is downward causation." This idea is also consistent with the idea of epigenesis in development, that events and processes

at all levels effect events and processes at all levels – the bidirectional model favored by Gottlieb. Heylighen et al. (2007) suggest that despite almost every property that matters to us is emergent (e.g., beauty, life, status), science ignored emergence and holism for so long because of the success of the Newtonian model.

Wolfram (2002) has argued that computational algorithms provide an alternative symbolic system from which to analyze scientific problems. The primary advantage of the algorithmic approach is that it can model complex interactions among multiple discrete entities in a much more tractable manner than differential calculus. In investigating the broad utility of an algorithmic approach to science Wolfram (2002) has identified what is referred to as the principle of computational irreducibility. Part of the success of differential calculus in science is that it provides scientific laws in the form of a symbolic short-hand. In other words, a mathematical evaluation of the equations allows the scientist to know with a relative degree of certainty what the long range behavior of the system the equation describes will be. For example, using Newton's laws of motion we can calculate the state of our solar system 200 billion years from now rather than having to wait 200 billion years to find out. Unfortunately, the principle of computational irreducibility states that in complex dynamic systems, even if the rules governing local interactions are deterministic and simple, the long-run behavior of the system as a whole can not be determined without running the system – we have to wait and see. Thus, the behavior of the system is just not predictable from a complete knowledge of the system components and their interactions. This is true even in computer simulations where the programmer defines the rules!

We conclude this section with a comment by Goldstein (1999, p. 60):

> In effect, there seems to be no end to the emergence of emergents. Therefore, the unpredictability of emergents will always be one step ahead of the ground won by prediction and, accordingly, emergence will always stay one step ahead of the provisionality argument. As a result, it seems that emergence is here to stay. Of course, this doesn't mean that there will be no great inroads into making the unpredictability of emergence more predictable. Rather, it goes along with the general reframing of the entire issue of predictability in scientific explanation that complexity theory has begun. Similar to the role of the uncertainty principle in quantum physics, the nonlinearity of the complex systems under investigation by complexity theory introduces a degree of unpredictability that even in principle will not completely yield to more and more probing.

Nonlinear Dynamic Complex Systems and the Role of Genes in Behavioral Development

In the foregoing section we have introduced a number of key concepts that distinguished the developmental and systems oriented theoretical perspectives that

have characterized the last 50 years of research in comparative psychology and developmental psychobiology. The implications of this theoretical orientation extend far beyond these two traditional sub-disciplines to be sure. We have consistently made the argument that utilizing the explanatory framework we have outlined above impacts the substantive science of fields ranging from human development, clinical psychology, social psychology, cognitive psychology, and cognitive neuroscience (Greenberg et al., 2004). Indeed, we have made the claim that this perspective represents a general psychology (e.g., Greenberg et al., 1999; Greenberg et al., 2004) and is certainly, along with evolutionary psychology, the only orientation laying claim to be a grand synthesizing theory, bringing all of psychology under a common philosophical, meta-theoretical, and conceptual rubric (Caporael, 2001). Perhaps it is because comparative psychologists, developmental psychobiologists, contemporary developmental psychology, and evolutionary psychology all have as a central focus the integration of phenomena across multiple levels of analysis (i.e., biology, behavior, local ecology, culture) that there is a recognition of the need for a broad synthesizing theory. Additionally, because biology, which in its ultimate reduction is genetics, is central to these fields, it comes as no surprise that the conceptualization of the role of the gene in behavior is the cornerstone manifestation of these competing worldviews.

The Conceptual Foundations of Gene Theory

For thousands of years, at least since the beginnings of agrarian culture, human beings have recognized that the traits of offspring resemble, in a predictable manner, those of their parents and that this relationship holds true for both animal and plant species (Provine, 2001). Indeed, the rise of modern society is largely predicated on this insight which has allowed farmers to produce grains with higher yields, fruits that are less susceptible to local disease and infestations, and higher quality meat products (Diamond, 1997). It is in this agrarian past that modern genetics has its origins (Provine, 2001). Mendel's discovery that this parent-offspring predictability followed reliable statistical laws is one of the landmark discoveries in modern science (Fisher, 1918; Gould, 1976). Yet, the rule-like regularity with which phenotypic traits get passed from one generation to the next has also led to one of the greatest debates in the history of science – the nature-nurture debate. The crux of the dilemma stems from the fact that while patterns of inheritance are reliably observed and can, with statistical estimations extending from Mendel's initial calculations, be predicted with some degree of accuracy; the process or mechanism through which this statistical regularity occurs has remained a mystery (Michel & Moore, 1995). We have deliberately avoided using the term transmitted since it implies a mechanism for which there seems to be little more than presumptive support.

The genesis of contemporary gene theory is found in early scientific attempts to resolve the unknown process which generated these statistical regularities between parent and offspring traits. Contemporary gene theory was born out of an era when the Newtonian mechanical universe was the dominant paradigm of science (or natural philosophy, to which science was then referred). This worldview held that the universe and all of its subsidiary features, such as plant and animal life, functioned via the mechanical interactions of discrete and independent objects, be they atoms, molecules, or as in the present case genes; causes were considered to be singular (i.e., there was one and only one actual cause for an event) and deterministic (see Mazzocchi, 2008). Further, the explanation for most complex events was thought to be found through a process of reducing the objects involved down to their most fundamental and atomistic dimensions and then delineating the deterministic transactions among those atomistic components. Within this philosophical backdrop, contemporary gene theory derived its key principles (see Gottlieb, 2006):

1. Genes must be discrete causal agents "located" in the germ cells. This principle is derived entirely from the Newtonian assumptions of linear, singular, and deterministic causes. Indeed, it was a completely assumed a priori "given." The only empirical observations related to this principle were the basic, observable aspects of sexual reproduction.
2. Genes behaved statistically "as if" they contained independent and unique causal information, which additively combine to form the adult organism; although there were no formal tests of this assumption. The logic being that if our atomistic and additive conceptualization is true then the statistical properties of the organism would follow certain parameters; the statistical properties follow these parameters; therefore our conceptualization of the gene is true. Philosophers of science refer to this logical fallacy as "affirming the consequent."
3. Because traits are predictable from the statistical estimations of Mendel and then later Fisher, both of whom did not include terms representing either development or environmental variation; it was further asserted that the causal information contained in genes was effectively isolated and independent of external influence (either biological or ecological).

These key principles, variations of which are referred to as the central dogma of molecular biology, soon became codified into the methodological operations of quantitative genetics. At its inception, and indeed, until only very recently the gene, seemingly so concrete and definitive a structure, was nothing more than a hypothetical construct in a statistical equation (Burian, 1985). Even with the discovery of the unique and highly functional structure of deoxynucleic acid (DNA) by Watson and Crick (1953), little more empirical light was shown on the subject than simply having a molecule with the kinds of properties through which the hypothetical gene might work. It has only been in the last decade or so that molecular biology

has developed the methodological tools for inducing segments of DNA to synthesize proteins and thereby identify a "biological gene" as opposed to a "statistical gene" or what Moss (2002) refers to as a D-gene (for DNA) rather than a P-gene (for phenotype inferred). It is worth noting that molecular biology has, within just a few short years of reliably observing the action of genes, abandoned the central dogma of genetics as untenable (e.g., Hsieh & Gage, 2004; Pelengaris & Kahn, 2006). Albeit molecular biologists have shifted the locus of causal information up the biological chain to the protein, shifting from genetics to proteiomics. The assumptive logic has remained much the same, but it became clear that genes simply did not function in the manner that many behavior geneticists and evolutionary psychologists still assume to be the case. In fact, work in stem cell biology takes contextual influences on gene synthesis to be foundational (e.g., Hsieh & Gage, 2004).

One of the key, and perhaps most damaging, outcomes of the historical development of the gene concept is that not only did the assumptive base underlying the central dogma become codified into the methodology of the discipline, but this once hypothetical construct became reified in the statistical equations of quantitative geneticists and subsequently divorced from empirical observation. As evidence for this we note that there is not a single published study using a "genetically informed design" in which a biological assessment of any kind has been undertaken. There are no actual genes to be found in the methods section of quantitative behavior genetics studies. Because of this empirical divorce, quantitative behavior geneticists have persisted in their Newtonian belief of the hypothetical gene; even when their molecular biology counterparts have abandoned the idea.

A Developmental Systems Perspective on the Role of Genes in Development

Developmental psychobiology, which has close historical ties to both comparative psychology and experimental embryology, was also impacted by the methodological limitation surrounding a pivotal construct that no one had ever empirically measured. Chiefly, in the absence of an empirical gene, the field could only indirectly infer an alternative view of the role of genes in development. Because of the experimental tradition of developmental psychobiology, the arguments counter to the central dogma of genetics, such as those made by Gottlieb (2007), were derived from a much stronger logical position. A large portion of this entire body of empirical literature is comprised of studies which provide counterfactual evidence to the central tenets of behavioral genetics.

One of the strongest of these arguments against the central dogma of genetics is drawn from experimental findings supporting the norm-of-reaction concept

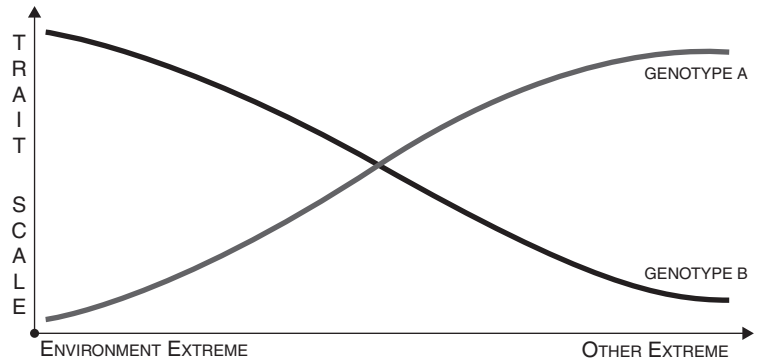

Figure 7.1. Conceptual view of the norm of reaction concept.

(Platt & Sanislow, 1988; Michel & Tyler, 2007). The norm-of-reaction concept is a relatively old concept in the field of embryology which states that phenotypic variability is restricted in a population because the developmental ecology of a population is relatively homogeneous (i.e., normalized) and that under conditions of increased ecological variability there would be an associated increase in phenotypic variability (see Gottlieb, 1992). This is in stark contrast to the reaction range concept which posits genetic limits on phenotypic variability with a limited range of variance due to environmental conditions (see Gottlieb, 1992). A central idea is that there is substantial phenotypic plasticity latent in the genome and that it is the developmental ecology that serves as the limiting or normalizing source of phenotypic variance rather than the genome. An idealized version of this concept is displayed in Figure 7.1.

There are several classic empirical examples of this effect, notable is the difference in wing morphology in several species of Drosophila (e.g., D. Willistori, & D. Melanagaster) (see Riziki, 1954; Dobzhansky & Spassky, 1944). As was the case for the drosophila, differential temperature during embryonic development is a key extra-genetic mechanism for affecting phenotypic outcomes. Temperature has been shown to not only directly impact broad morphological outcomes (Kaplan & Phillips, 2006), but also, molar developmental processes, including heterochronic differences in development (Johnston & Wilson, 2006), as well as intra-cellular processes such as, rates of diffusion, enzyme induced protein activity, and gene expression (see Hochachka & Somero, 2002). Early experimental work on the pluripotency of cell lines also provided counter-factual evidence to the central dogma (see Gottlieb, 1992). Gould (1980) discussed this phenomenon in an article he titled "Hen's Teeth and Horses Toes." Despite eons with neither, relatively simple manipulations of fertilized eggs results in both – the genome is plastic enough to allow this, which is impossible from the reaction range point of view.

Yet, the field was largely in the position of arguing what genes could not logically do rather than what they in fact did; a much less appealing case. There were several

gifted theorists, Gilbert Gottlieb (2007) being among them, who did articulate an alternative to the central dogma, an alternative in which contexts and timing were paramount; where the gene, while important, ought not be supreme though some had called it mighty. This developmental-oriented theoretical perspective, the core principles of which we discuss more fully below, differed from the standard central dogma perspective on a number of levels. On a substantive level, based on empirical observations, genes are considered to be participatory components of a much broader biopsychosocial system. Genes were likely important factors in the development of phenotypic traits; remember there had not been any empirical investigation of a biological gene, but they could not serve as the reductive prime cause. As such, it was inferred that the gene could have no independent causal influence and rather worked through a causal interactions among variables in an integrated hierarchy (see Weiss, 1959). Further, phenotypic development has been shown to be highly probabilistic rather than deterministic, a result of the continual dynamic transactions across development of biological, psychological, and ecological variables.

The Impasse between Genocentric and Developmental Systems Perspectives

On a much deeper level, the theoretical perspective of Gottlieb and his predecessors (e.g., Kuo, 1967; Schneirla, 1957) stood in stark contrast to the Newtonian world view. Theirs was a perspective in which causes were multiply determined and where multiple causal events could lead to the same outcome; a perspective in which the idea of a deterministic cause was untenable; where hierarchical systems, like organisms, were determined by the dynamic patterns of relationships among their parts rather than by the additive attributes of their constituent parts. Change and probabilistic systems were the currency of the universe rather than static deterministic, clockworks. This perspective was born out of a larger scientific zeitgeist ushered in by the quantum dynamics revolution in physics as we pointed out above. Newtonian physics was out and Einstein and Heisenberg were the minds of the new physics. This latter relationship between the philosophical and theoretical revolution in physics and the theoretical framework of early comparative psychology and developmental psychobiology has important implications for contemporary gene theory and the role of development in resolving the long-intractable nature-nurture controversy. The argument between developmentally oriented theorists, such as Gottlieb, and the central dogma oriented perspectives of many behavior geneticists was played out without either side having empirical knowledge of an actual biological gene. So, both sides based much of their conceptualization on indirect inferences. And, while the counterfactual experimental evidence of developmental psychobiologists was particularly condemning

for the central dogma perspective, which was on much weaker logical footing, there was no methodological framework for laying out the developmental and systems-oriented postulates of developmental psychobiology. As such, concepts such as emergence, integrative levels, probabilistic epigenesis, horizontal and vertical coactions, were relegated by opponents to being little more than descriptive heuristics.

Further, the methodological framework of Fisher (1918), Neyman & Pearson (1933), and others working from a Newtonian based model had won out over alternative, more dynamic perspectives such as that of Bayes (1763). Interestingly, the Bayesian perspective failed to gain a foothold in early social science largely because it was computationally intractable at the time. So despite being conceptually tighter and more consistent with modern philosophy of science perspectives it was not widely adopted as an inferential tool. However, with contemporary computing power, the Bayesian perspective is the analytic bedrock of complex systems theory. The result being that the dominant methodology was better suited to the central dogma perspective of behavior genetics, providing the illusion that this perspective had empirical support, despite the weakness of the logic (affirming the consequent) and the lack of measurement of the key construct in question (namely the gene).

Advances in Nonlinear Dynamic Systems Theory and their Relation to the Role of Biology in Behavioral Development

However, because of the ascendancy in physics of quantum theory and growing interest in modeling nonlinear dynamic systems, presumably due to their ubiquity in nature, mathematical, conceptual, and methodological development which was consistent with this perspective continued to develop concurrent with developments in comparative psychology and developmental psychobiology. These lines of scholarship proceeded largely independently and unknown to each other for the greater part of the 20th century. Then, at the end of the 1980s there was a resurgent awareness by scientists in theoretical physics, looking for new phenomena with which to refine their deductive mathematical models and scientists in theoretical biology, developmental psychology, and economics with their inductively arrived at conceptual frameworks, had both reached the same consensus via different logical approaches. Concepts of dynamic complex systems, emergence, self-organization, symmetry breaking, and network causality were being incorporated by both sets of scientific traditions.

As a result of this convergence of ideas, comparative and developmental psychobiologists now have at their disposal a methodology that is commensurate with their core theoretical principles. We find, now that not only do empirical data, largely from experimental embryology and comparative psychology, indirectly

support inferences about the role of integrated biopsychosocial systems in shaping phenotypic outcomes, but our colleagues in theoretical physics and mathematics working with mathematical models of biological systems have deductively demonstrated that these same principles hold widely. Further, there is now a set of methodological tools with the capability of testing many of the developmental systems postulates directly.

The concepts of hierarchy, integrative levels and systems, self-organization, and emergence so central to the orientation that Gottlieb brought to psychology have been rather fully developed over the last quarter century and are being employed with considerable alacrity by molecular biologists, ecologists, and even economists. The larger point here is that through experimental studies of developing organisms, both pre- and post-natal, it became clear to developmental psychobiologists and comparative psychologists that the conceptualization of the gene as held by the central dogma was untenable; it would not explain their empirical findings. As such, this set of experimental disciplines drew from new ideas in physics emerging at the turn of the 20th century for an explanatory heuristic that was more consistent with the findings of the field. Independently, but concurrently, theoretical physicists were continuing to develop a mathematical formalism with which to test hypotheses regarding the dynamics of hierarchically nested systems, complex systems with no centralized controls, etc and also concluded that all systems, be they computational (i.e., bits of information), physical, biological, or social displayed exactly the properties suggested by early theorists in both of these fields – both sets of disciplines; working from different approaches – one primarily inductive, the other primarily deductive – found a convergent set of shared ideas which have profoundly greater explanatory capability and parsimony than those central to genocentric orientations like behavioral genetics and evolutionary psychology.

One of the more important outcomes of this convergence is that we can now specify hypotheses directly corresponding to the key principles of this developmental systems perspective and test them using appropriate methodological tools. As molecular biologists are beginning to recognize "key notions such as emergence, nonlinearity, and self-organization already offer conceptual tools that can contribute to transform and improve science" (Mazzocchi, 2008, p. 13). Linking these core concepts with analytic and methodological tools such as the use of cellular automata (Wolfram, 2002), Bayesian network analyses (Gill & Swartz, 2004), state and phase portraits (Kelso, 2000; Rand, Kapuniai, Crowell, & Pearce, 2001; Van Geert 1998) State Space Grids (Granic & Hollenstein, 2003; Lewis, Lamey, & Douglas, 1999); and nonlinear dynamic systems approaches to longitudinal covariance models (e.g., Boker 2001; Nesselroade, 2006) is where the future of developmental science lies and, Gilbert Gottlieb's legacy will be, at least in part, his essential role in moving the entire field through this conceptual paradigmatic shift on its way to becoming an empirically mature science.

Cellular Automata

So, how can we incorporate these methodologies into the study of developmental psychobiology? As we have stated several times in this chapter, the concept of emergence is perhaps the most central concept in our perspective and yet it is the most analytically troubling. While we are primarily using the concept of emergence to explain how biological factors and ecological factors, through their transactions over development, give rise to complex behaviors, a host of "systems" oriented disciplines are raising similar kinds of questions. Indeed, fields ranging from economics to urban planning to biology are asking the same question; how can local interactions between elements of a system give rise to system level behaviors that are not "encoded" anywhere in the parts of the system. As Wolfram (2002) noted, these systems are comprised of so many interacting pieces that there is no calculus for predicting the state of the system at some point in the future; one must simply watch the system and see where it goes.

However, because of the enormity of reductionistic data on local interactions that exists, we can build relatively complete computer models of the systems we study and watch their dynamics unfold in computational time, which is substantially faster than real time. Doing so does two things, first it affords us the ability to examine the macro-properties of a system and we know that those properties are emergent because they were not encoded in our computer model. Second, it allows us to assign probabilities to eventual system states and see how changes in the initial conditions – the initial state of our computer model – affect those probabilities. And indeed, our colleagues in related systems sciences have been profitably using these models for the last decade. Sylva and Clarke (2002) have recently used cellular automata-based models to identify likely growth patterns in urban areas in Portugal. Economists have successfully used agent-based cellular automata models to predict investor decisions with greater accuracy than more traditional, reductionistic models (Qui, Kandhai, & Sloot, 2007). Evolutionary biologists have predicted emergent speciation patterns using these analytic approaches (Oxman, Alon, & Dekel, 2008). And epidemiologists have used agent-based cellular automata models to successfully predict and elucidate viral outbreak patterns (Young, Stark, & Kirschner, 2008). The one thing that all of these phenomena share with each other as well as with many areas of study within developmental psychobiology, is that they are integrated systems comprised of many variables which are interdependent on each other. There has been sufficient work using this methodology across multiple disciplines that Grimm et al., (2005) in a recent article in *Science* presented an overview of an abstract modeling strategy referred to as Pattern-Oriented Modeling, which has the aim of improving modeling procedures across disciplines. It certainly seems that developmental psychobiology, as envisioned by Gottlieb and his many colleagues and students, is primed to benefit from

adopting this analytical approach which has proven so useful to other systems-oriented disciplines.

Boolean Network Models

Like cellular automata, Boolean network analyses are concerned with understanding the dynamics of integrated systems. In these models, variables are graphically represented by a node (similar to ovals in structural equation models (SEMs)), the state of each variable is often represented as a binary code (i.e., genes can be in state 1-synthesizing, or 0-inactive), and variables in the system are linked graphically by lines which represent, conditional probabilities (similar to regression path coefficients in SEM). These models allow for the analysis of the micro-dynamics of a system. The micro-dynamics include features like, the specific relationships between variables, how the system becomes integrated over time, and the dynamics of the system as a whole, such as becoming canalized. These models have been highly useful in modeling neural systems, computer architecture, genetic regulatory networks, and social systems (e.g., Shmulevich, Dougherty, & Zhang, 2002). Like emergence, the notions of horizontal and vertical coactions are central to the perspective that we share with Gottlieb. Traditional ANOVA and regression-based models were designed to study variable sets that did not have coactions. Yet, these network models are highly consistent mathematical representations of the kinds of coactions within and across scales Gottlieb and colleagues have proposed (Gottlieb, 2004).

One of the cornerstone concepts that we have discussed is that of emergence. An important implication of the concept of emergence is that the level of analysis shifts from the behavior of individual variables to the behavior of the entire system as a whole. To extend this to the developmental psychobiology view of Gottlieb and others, what matters for development is not the particular genes an individual possesses, specific elements of the organism's neurophysiology, or individual aspects of the organism's developmental ecology, but rather the interplay of the entire system of variables over time. David Magnusson and his colleague Lars Bergman have eloquently demonstrated that psychological science as a whole has been dominated by a variable oriented perspective (e.g., Von Eye & Bergman, 2003; Magnusson, 2000). Moreover, traditional measurement and analysis tools of the field do not have the capacity to represent a system level perspective. In short, we cannot study the behavior of integrated systems as a whole because we can not observe the behavior of the system with traditional tools.

State Space and Phase Space

We, as a field then, need to begin to adopt measurement and analysis tools that are tailored to such a systems view. State space and phase space portraits are emerging

analytic tools for evaluating system-level properties and are being increasingly used effectively by related disciplines such as ecology. The most direct analogue to these two assessment tools, of which most developmental psychologist are familiar, is a scatter plot. In a traditional two-variable scatter plot, the x-, and y-axes represent the range of possible values of two random variables. The scatter of points on the plot indicates the locations of all the joint occurrences of corresponding values for the two variables. There are several important pieces of information that can be obtained by examining such a scatter plot. If the entire plot is evenly covered with points, then we know that the two variables have no structural relationship (i.e., a low score on one variable yields no information about the likely value on the other variable). However, if the points on the scatter plot are quite dense along a diagonal line and sparse to non-existent everywhere else, then we can conclude that the two variables have a strong structural relationship. We can also learn if the relationship is linear or nonlinear. State space portraits are simply scatterplots, often on a continuous scale, meaning that rather that the individual points are so closely spaced that they look like continuous lines. An example can be seen in Figure 7.2.

While the scatter plots we are used to in psychology are bi-variate, state space portraits are often high dimensional scatter plots, meaning that the include scores about two or more variables simultaneously. We can learn a great deal about a system by simply examining the state space portrait. State space portraits that display organized patterns imply a highly integrated system, less organized patterns imply a poorly integrated system. For example, a state space portrait that is compacted within a restricted area of the total space available suggests that the system has become canalyzed and is resistant to change – influencing only one variable would likely have little change on the overall system. Embryologists and cell biologists have repeatedly demonstrated morphological canalization across development and one of the key factors in the system is genetic redundancy. We have also learned that highly constrained biological systems as defined by narrow, compact state spaces, are inflexible do not adapt easily to changes in the ecology – in short, they are vulnerable.

The phase space portrait is a companion analysis to the state space portrait. The key distinction is that the phase space portrait incorporates time. Again, think of a bi-variate scatter plot, but instead of having all the points on the scatter plot at the same time, imagine that they are plotted one at a time in a temporally ordered sequence. Further, by including a directional arrow between each set of two points, indicating which point came first, we can see how the behavior of the two variables co-develop over time. The classic example of phase portrait analysis is that of the prey-predator relationship. On one axis the number of prey animals is plotted and on the other is plotted the number of preditor animals. Each point on the graph represents the number of prey and predator animals at a given point in time and with the use of directional arrows connecting the points we can see the dynamics of the prey-predator system. There are a number of things that we can learn about this system by examining the phase portrait. First, we can determine how sensitive the

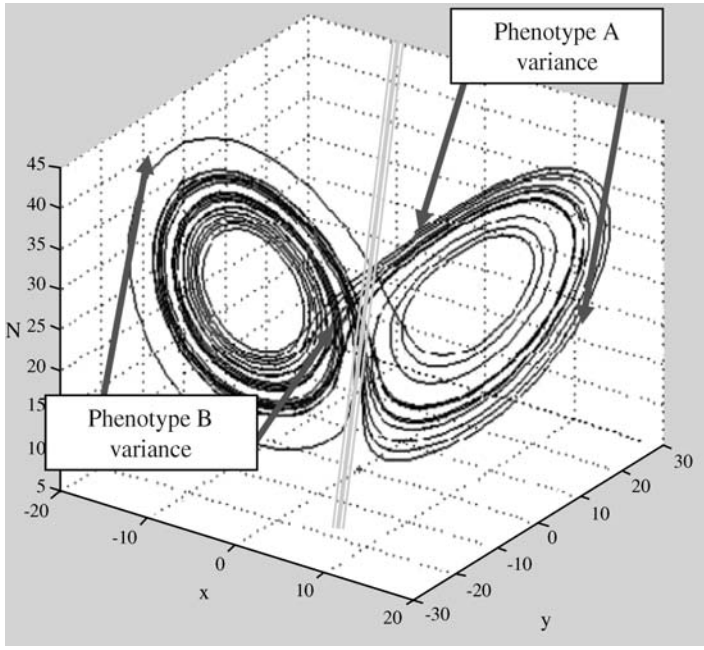

Figure 7.2. Heuristic depiction of range of phenotypic expression using a phase portrait of the Lorenz equations.

Notes: In the figure is a phase portrait of the Lorenz Equations. In this diagram, each point represents a heuristic representation of a phenotypic possibility. A phenotype being a complex variable defined by the entire biopsychosocial attributes of an individual organism. This is in contrast to a phenotypic trait, like eye color or wing pattern, etc.

The x-, y-, and z-axes represent hypothetical measures of the organism's ecology. Simply for illustrative purposes, let's say that the x-axis is a measure of genetic background variance, the y-axis is a measure of environmental context variance, and the z-axis represents variance in developmental history for each organism. The diagonal line in the middle of the figure represents what dynamic systems researchers refer to as the separatrix. As the bioecological system supporting a given phenotype drifts away from this separatrix region the phenotype is relatively robust to variation in either biological or ecological change; however, along the separatrix even minor variations in the bioecology leads to dramatic phenotypic change.

prey and predator animals are to each other. If fluctuations in birth or death rates of a prey species have a relatively small influence on the number of predator animals then we know that this particular system is loosely coupled, and probably relatively stable. However, if small changes in prey birth or death rates have a large impact on the number of predator animals then we know that the prey-predator system is tightly coupled and probably unstable. We also can assess the degree of dynamic homeostasis or regularity of the system. This is a developmentally emergent property of the system as a whole and can not be evaluated by deconstructing the prey-preditor system and analyzing the two species separately. We can calculate

statistical properties of the system as a whole such as the Lyapunov exponent, which provides information on how far out in time the behavior of the system can be accurately predicted. Again, the Lyapunov exponent is an emergent statistical property of the system and cannot be determined by assessing the variability of the individual variables comprising the system.

Over the last decade important advances have been made in adapting state space and phase space portrait analyses to the level of data and measurement methodology common to psychology. Most psychophysiological data can be directly analyzed using the aforementioned techniques (e.g., eeg). Esther Thelen and her colleagues (e.g., Thelen and Smith, 1998) used state and phase portrait analytic approaches to revolutionize the field of motor and perceptual development in infancy. For the present, data at the behavioral level do not meet the requirements of these approaches directly. Yet, important work adapting these analytic tools to be better suited to traditional psychological data has substantially bridged that gap (e.g., Lewis et al., 1999; Granic & Hollenstein, 2003). For example, Lewis et al. (1999) characterized the developmental dynamics of two dimensions of early childhood socioemotional development; intensity of distress and attention to mother. The former is an index of emotional reactivity and the latter is an index of the child's attempt to regulate negative emotion via maternal contact. Using a two dimensional ordinal state space grid yielded unparalleled insight into the dynamics of infant emotional development – insight that was not feasible with more traditional statistical analyses.

A full description of these methods is beyond the scope of this chapter. Rather the aim here is to provide a brief description of a few of the analytic methods developed to study the properties of nonlinear dynamic systems, how these methods have been incorporated into related disciplines, and, most importantly, that the phenomena for which these methods were designed to assess are conceptually identical to many of the central concepts, which have, as a result of the work of Gilbert Gottlieb, become foundational in contemporary developmental psychobiology.

Gilbert Gottlieb, Probabilistic Epigenesis and Developmental Systems Theory

"Because a great deal of psychology is implicitly reductionist [e.g., cognitive neuroscience, behavioral genetics, evolutionary psychology], emergence has remained largely unexplored by mainstream psychologists" (Sawyer, 2002, p. 24). However, the ideas discussed above, gleaned from current thinking in physics and biology, play a crucial role in the psychology of Gilbert Gottlieb. He was a pioneer in the late-20th-century growth of developmental systems theory, a biopsychosocial point of view which is a synthesis of both developmental biology and developmental psychology. The main ideas of this now important perspective in psychology are that genes, and other biological processes, do not have primacy in

explanations of behavior development. Rather, there is a bidirectional interaction or fusion between external environmental and internal biological and physiological events and process – development, from this perspective, is a process of emergence. Indeed, the ideas we have discussed above – self-organization, complexity, and emergence form the basis of Gottlieb's dynamic systems approach in which development is understood to be a continual, epigenetic process of emergence (Sawyer, 2002; Smith, 2006).

Despite the success of development system theory, we agree with Sawyer (2002, p. 24) that,

> The mainstream of contemporary psychology seems to hold to a view of science which is atomist and reductionist; many psychologists believe that psychology will ultimately be unified with biology. Some of the most rapid growth in psychology today has been in the most extremely reductionist paradigms: cognitive neuroscience, behavioral genetics, and evolutionary psychology.

However, Gottlieb has suggested that few psychologists, and in fact many biologists, are simply unaware of recent developments in biology that render the standard accepted models as no longer valid: "While this fact is not well known in the social and behavioral sciences, it is surprising to find that it is also not widely appreciated in biology proper. . .[!]" (Gottlieb, 2001a, p. 47). He was not alone in this assessment as even a molecular biologist has noted (Strohman, 1997). This point is underscored by Mazzocchi (2008, p. 11): "the reductionist approach can no longer cope with both the enormous amount of information that comes from the so-called '-omics' sciences and technologies – genomics, proteomics, metabolomics and so on – and the astonishing complexity that they reveal."

We prefer to understand this failure to recognize these facts as reflecting part of the sociology of science in which new ideas are seen as threatening (Barber, 1961; Barnes, Bloor, & Henry, 1996) and thus ignored, or, perhaps, awaiting a new paradigm that can accommodate them (Strohman, 1997). Indeed, the bidirectional nature of gene action was acknowledged by Hull as early as 1972 who also noted it to be ignored by his contemporaries, "we have, until now, all but ignored the effect of the environment on genetic reactions. The same molecular structures behave differently under different circumstances" (Hull, 1972, p. 498). This, of course, is a key message in virtually all of Gottlieb's later writings.

Gottlieb's approach to psychology embodied four orienting ideas:

1. Behavior (as well as biology) is largely the result of developmental processes, virtually from the moment of conception to death.
2. Behavioral neophenotypes refer to the drastic changes in behavioral development that can arise from significant alterations in the contextual conditions of usual organismic development. Gottlieb cites as an example Kuo's experiment in which the previously thought instinctive sexual behavior of male dogs

was virtually reversed by controlling the experiential history of the animals. Essential to the creation of neophenotypes are the timing, duration, and quality of contextual alteration.

3. Norm of reaction (discussed above) which is contrasted with the concept of the reaction-range. Largely as the result of mainstream evolutionary thinking, which takes a neo-Haeckelian approach, it has been assumed that an organism's genotype sets narrow limits on the range of phenotypic expression. This line of thinking is referred to as the reaction-range concept. In contrast, the norm of reaction concept assumes that there are no presupposed limits on phenotypic expression. In this conception, genes are limited to a necessary role but of a more limited ontogenetic significance than they have typically been granted.
4. Non-obvious experiential precursors of behavior. Being a developmentalist, and given his appreciation of the role of epigenetic processes in behavioral development, Gottlieb was, of course, against the idea that behavior was in any way instinctive, that is, programmed by the genes, a popular idea among ethologists and even psychologists at the time he began his career. Rather, Gottlieb believed that experiences formed the basis of all behaviors, though those experiences were often non-obvious. "As for non obvious experiences, who could have dreamed that squirrel monkeys' innate fear of snakes derives from their earlier experience with live insects (Masataka, 1994)? Or that chicks perceiving meal worms as edible morsels is dependent on their having seen their toes move (Wallman, 1979)" (Gottlieb, 2001b, p. 2)? Of course, the search for these developmental precursors of behavior is arduous (Lerner, 2004). It is not always easy to find such experiences; that is what renders them non-obvious. Kuo (1967), for example, examined 3,000 (!) developing chicken eggs in examining the prenatal influences of the post-hatching pecking behavior. Can what goes on in a developing egg be any more non obvious?

Probabilistic Epigenesis

Epigenesis is among the older concepts in developmental biology (see Woodger, 1929) standing as an ontological reaction against preformationism; a debate that has been characterized as part of "one of the most important and difficult antitheses involving. . . a large part of biological science" (Woodger, 1929, p. 334). This concept was incorporated into psychology from its earliest days as an emerging scientific discipline (e.g., Gessell, 1928), but the use of the epigenesis concept in psychology was also enveloped in an ontological debate between deterministic views of epigenetic processes, such as those characterized by Gessell, Freud, Erikson, and later Ainsworth; and a probabilistic view of epigenesis. In both cases, development is characterized as a continual process of increasing biological

and behavioral complexity as a function of physical and psychological reorganization. As articulated by Bretherton and Ainsworth (1980, p. 316) deterministic epigenesis "implies that an organism has some basic ground plan of development in which different issues gain ascendancy at particular stages in development." In stark contrast to this view is that of probabilistic epigenesis. Probabilistic epigenesis recognizes that there is no ground plan and that at each stage of development the future path is understood to be the result of the dynamic interplay of a complex array of biological and environmental factors. According to this view, while development occurs within an environment, that environment is not benign, but is rather the source of important influences on the course of development. Every biological and behavioral feature, then, at every point in development, is the result of a functional product of the dynamic relationship between the organism and all features of its environment. Thus, in contrast to the deterministic version of epigenesis, the "rules" governing the process of change from low complexity to high complexity are neither "stored" in a specific place such as the genome, nor are they independent of the developmental process. Rather, the "rules" of development are diffusely spread across the entire developmental system and are a product of the developmental process itself. In other words, the "cause" of development is the action of developing. This probabilistic epigenetic viewpoint is emerging as *"the"* organizing principle of developmental cell biology in fields ranging from embryonic stem cell biology (Hsieh & Gage, 2004) to oncology (Feinberg, Ohlsson et al. 2006).

Probabilistic epigenesis has been described in a variety of ways, but none so well put as that by Moltz (1965, p. 44):

> An epigenetic approach holds that all response systems are synthesized during ontogeny and that this synthesis involves the integrative influence of both intraorganic processes and extrinsic stimulative conditions. It considers gene effects to be contingent on environmental conditions and regards the genotype as capable of entering into different classes of relationships depending on the prevailing environmental context. In the epigeneticist's view, the environment is not benignly supportive, but actively implicated in determining the very structure and organization of each response system.

Behavior is understood here to be not predetermined by biology, but rather a result of the organism's past, present, and physiology. Behavior continues to grow and develop from fertilization to death, and remains somewhat plastic or flexible throughout life. There are no guarantees as to how an organism will turn out behaviorally, since the interaction of the three sets of factors influencing organisms may change as a result of unknown and unpredictable factors. Development is thus "probabilistic" rather than predetermined to develop in one way or another. The organism-environment exists as a fused unit, the influences between them being bidirectional (Gottlieb, 1992, 1997).

In one of his earliest theoretical papers (actually written in 1965, although not published until later), Gottlieb distinguished between predetermined and probabilistic epigenesis, the former firmly on the unbending nature side of the nature-nurture issue. Thus, according to predetermined epigenesis "sensory stimulation does not influence or determine the course of behavioral development in any significant way" (Gottlieb, 1970, p. 112). This approach to behavior understands it to be the outcome of biology (or nature), with very little influence at all of experience (or nurture) (Gottlieb, 2001a). Gottlieb's own work (1973), as well as that of others (e.g., Kuo, 1967; Smotherman & Robinson, 1988) has shown that to be simply wrong.

The alternative position explicated by and favored by Gottlieb, is that of probabilistic epigenesis in which "behavioral development of individuals within a given species does not follow an invariant or inevitable course, and, more specifically, that the sequence and outcome of individual behavioral development is probable. . . rather than certain" (2001a, p. 43). Indeed, this is seen now to hold not just for behavior, but "is recognized in many quarters as a defining feature of development" (Gottlieb, 2003, p. 341).

Two lessons that remained with Gottlieb from his graduate education were the importance of prenatal development and the bidirectional nature of structure-function relations (2001a). He never denied that genetics played a role in structural and functional development, just that other factors were involved as well ["genes are part of the developmental system" (2003, p. 345)], and that structure-function relations were bidirectional. He was, in fact, among the first to demonstrate that sensory stimulation enhanced gene expression in the duck embryo. Indeed, at the time of that research, "there was only one other study in the literature implicating exteroceptive influences on genetic activity" (Gottlieb, 2001a, p, 46). However; over the past half-dozen years an enormous amount of empirical work has demonstrated that the activity of genes is nearly completely contextually determined. In a recent *Nature: Genetics*, review of epigenetic processes in cell development, seven distinct post-translational processes were identified that influence gene activity (Spvakov & Fisher, 2007). These multiple processes are just aspects of the "pillars of epigenetics" which include DNA methylation, histone modifications, and RNA interference. In a separate review, Hsieh and Gage (2004) again review a wide array of epigenetic processes, ranging from chromatin structure, and noncoding RNA, to extracellular signaling systems, all of which influence genetic activity associated with cell differentiation and development. Additionally, the forefront of behavioral teratology research has begun to focus on extra-organismic factors which influence these complex epigenetic regulatory processes linked to patterns of gene expression. The converging findings from these multiple disciplines demonstrate strong support for the bi-directional processes or coactions both vertically and horizontally as Gottlieb consistently argued.

Comparative psychology is concerned with the development and evolution of behavior. The field has historically been considered to be the study of non-human organisms. Indeed, one contemporary and popular view (Blumberg & Wasserman, 1995; Hirsch, 1987; Wasserman, 1997) suggests that we return to our roots – that of the study of mental continuity among the animal groups (Romanes, 1885). Comparative psychology thus envisioned is limited to the study of animal cognition. However, we understand comparative psychology much more broadly, to be the study of origins of *all* behavior, a *general* psychology if you will. Thus, we understand and conceptualize comparative psychology to be the study of the evolution and development of behavior of all organisms. As we have suggested above, the discipline has received criticism for a lack of guiding theory and for misunderstandings of evolutionary theory; again, our discussion above has laid this criticism to rest.

More importantly, comparative psychology in particular, and indeed, psychology in general, has been identified as a biological science, its principles in essence reducible to those of biology, physiology, and even further, to chemistry and physics. While we have argued that psychology is a mature and unique science with principles of its own we also recognize and appreciate the importance that principles of the other sciences play in our understanding behavioral origins (Greenberg, Partridge, & Ablah 2008; Greenberg & Haraway, 2002). Of course, psychology has included the study of genetic, hormonal, and neural factors that impact on behavioral origins, if not from its scientific beginnings then surely from the early part of the 20th century. We have tried to show in this chapter the manner in which contemporary ideas in other areas of mainstream science, particularly molecular biology and physics, impact the understanding of the approach to psychology championed by Gilbert Gottlieb and his intellectual predecessors.

As is the case with the many influential scientists, Gottlieb was ahead of his time in recognizing the relationships of ideas from other disciplines, and their utility, in formulating his own unique understandings of behavioral origins. This was true of his appreciation of embryological events at a time when the literature in this area was extremely meager, as pointed out recently by Rosenblatt (2007), himself a major player in the line of thought associated with Gottlieb and the other so-called "New York epigeneticists" such as T. C. Schneirla, Daniel Lehrman, Lester Aaronson, and Ethel Tobach (e.g., Aronson, Tobach, Lehrman & Rosenblatt, 1970). While appreciating the uniqueness of the science of psychology, these individuals saw it as a natural science, consistent and compatible with the principles of all of the natural sciences. It comes as no surprise therefore, that the picture we have painted of contemporary ideas in physics and biology are now seen to play an important, though contributing, role in psychology and the origins of behavior. Gottlieb passed away in 2007, and while he left behind an important corpus of work, we can only wonder what directions he might have pursued had he lived only a little longer.

References

Ahn, C. A., Tewari, M., Poon, C.-S., & Phillips, R. S. (2006). The limits of reductionism in medicine: Could systems biology offer an alternative? *PloS Medicine*, **3**(6), 1–5.

Aronson, L. R. (1984). Levels of integration and organization: A re-evaluation of the evolutionary scale. In G. Greenberg, & E. Tobach (Eds.), *Evolution of behavior and integrative levels* (pp. 57–81). Hillsdale, NJ: Erlbaum.

Aronson L. R., Tobach E., Lehrman D. S., & Rosenblatt J. S. (Eds.) (1970). *Development and evolution of behavior: Essays in memory of T. C. Schneirla*. San Francisco: W. H. Freeman.

Aronson L. R., Tobach E., Rosenblatt J. R., & Lehrman D. H. (Eds.). (1972). *Selected writings of T. C. Schneirla*. San Francisco: Freeman.

Arthur, W. B. (1993). Why do things become more complex? *Scientific American*, **268**(5), 144.

Barber, B. (1961). Resistance by scientists to scientific discovery. *Science*, **134**, 596–602.

Barnes, B., Bloor, D., & Henry, J. (1996). *Scientific knowledge: A sociological analysis*. Chicago: University of Chicago Press.

Bayes, T. (1763). An essay toward solving a problem in the doctrine of chances. *Philosophical Transactions of the Royal Society of London*, **53**, 370–418.

Blumberg, M. S., & Wasserman, E. A. (1995). Animal mind and the argument from design. *American Psychologist*, **50**(3), 133–144.

Boker, S. M. (2001). Differential structural equation modeling of intraindividual variability. In L. M. Colllins, & A. G. Sayer (Eds.), *New methods for the analysis of change* (pp. 5–27). Washington DC: APA Press.

Bonner, J. T. (1988). *The evolution of complexity: By natural selection*. Princeton, NJ: Princeton University Press.

Bryson, B. (2003). *A short history of nearly everything*. New York: Broadway Books.

Bunge, M. (1980). *The mind-body problem*. Oxford: Pergamon.

Burian, R. M. (1985). On conceptual change in biology: The case of the gene. In D. J. Depew, & B. H. Weber (Eds.), *Evolution at a crossroads: The new biology and the new philosophy of science* (pp. 21–42). Cambridge, MA: MIT Press.

Buss D. M. (Ed.). (2005). *The handbook of evolutionary psychology*. Hoboken, NJ: John Wiley.

Byrne, D. (1998). *Complexity theory and the social sciences: An introduction*. London: Routledge.

Campbell, D. T. (1990). Levels of organization, downward causation, and the selection-theory approach to evolutionary epistemology. In G. Greenberg, & E. Tobach (Eds.), *Theories of the evolution of knowing* (pp. 1–17). Hillsdale, NJ: Erlbaum.

Caporael, L. R. (2001). Evolutionary psychology: Toward a unifying theory and a hybrid science. *Annual Review of Psychology*, **52**, 607–628.

Chaisson, E. J. (2001). *Cosmic evolution: The rise of complexity in nature*. Cambridge, MA: Harvard University Press.

Chorover, S. L. (1990). Paradigms lost and regained: Changing beliefs, values, and practices in neuropsychology. In G. Greenberg, & Tobach E. (Eds.), *Theories of the evolution of knowing* (pp. 87–106). Hillsdale, NJ: Erlbaum.

Crick, F. (1994). *The astonishing hypothesis*. London: Simon & Schuster.

Damon, W., & Lerner, R. M. (2001). *Theoretical models of human development*. New York: John Wiley.

Davies P. (Ed.). (1989). *The new physics*. Cambridge: Cambridge University Press. (Reprinted 1994).

Davies, P., & Gribbin, J. (1992). *The matter myth*. New York: Simon & Schuster.

Dennett, D. C. (1995). *Darwin's dangerous idea: Evolution and the meanings of life*. London: Penguin.

Diamond, J. (1997). *Guns, germs, and steel: The fates of human societies*. New York: W. W. Norton and Co.

Dobzhansky, T. H., & Spassky, B. (1944). Genetics of natural populations. XI Manifestation of genetic variance in drosophila pseudoobscura in different environments. *Genetics*, **29**, 270–290.

Feibleman, J. K. (1954). Theory of integrative levels. *British Journal for the Philosophy of Science*, **5**, 59–66.

Feinberg, A. P., Ohlsson, R., & Henikoff, S. (2006). The epigenetic progenitor origin of human cancer. *Nature Review: Genetics*, **7**(1): 21–33.

Fisher, R. A. (1918). The correlations between relatives on the supposition of Mendelian inheritance. *Transactions of the Royal Society of Edinburgh*, **52**, 399–433.

Ford, D. H., & Lerner, R. M. (1992). *Developmental systems theory: An integrative approach*. Newbury Park, CA: Sage.

Fromm, J. (2005a). Ten questions about emergence. Retrieved March 16, 2010, from http://arxiv.org/abs/nlin.AO/0509049.

Fromm, J. (2005b). Types and forms of emergence. http://arxiv.org/abs.nlin.AO/0506028.

Gessell, A. (1928). Maturation and infant behavior patterns. *Psychological Review*, **36**, 307–319.

Gill, P. S., & Swartz, T. B. (2004). Bayesian analysis of directed graphs data with applications to social networks. *Journal of the Royal Statistical Society Series C*, **53**(2), 249–260.

Gell-Mann, M. (1994). *The quark and the jaguar: Adventures in the simple and the complex*. London: Little, Brown and Company.

Goldstein, J. (1999). Emergence as a construct: History and issues. *Emergence*, **1**, 49–72.

Goodwin, B. (1994). *How the leopard got its spots: The evolution of complexity*. New York: Scribner's.

Gordon, D. (1988). The development of flexibility in the colony organization of harvester ants. In G. Greenberg, & E. Tobach (Eds.), *Evolution of social behavior and integrative levels* (pp. 197–203). Hillsdale, NJ: Erlbaum.

Gordon, D. (1997). Task allocation and interaction in social insect colonies. In G. Greenberg, & E. Tobach (Eds.), *Comparative psychology of invertebrates: The field and laboratory study of insect behavior* (pp. 125–134), New York: Garland.

Gottlieb, G. (1970). Conceptions of prenatal behavior. In L. R. Aronson, E. Tobach, D. S. Lehrman, & J. S. Rosenblatt (Eds.), *Development and evolution of behavior: Essays in memory of T. C. Schneirla* (pp. 111–137). San Francisco: W. H. Freeman.

Gottlieb, G. (1973). Introduction to behavioral embryology. In G. Gottlieb (Ed.), *Studies on the development of behavior and the nervous system. Volume 1. Behavioral embryology* (pp. 3–45). New York: Academic Press.

Gottlieb, G. (1984). Evolutionary trends and evolutionary origins: Relevance to theory in comparative psychology. *Psychological Review*, **91**, 448–456.

Gottlieb, G. (1985). Anagenesis: Theoretical basis for the ecological void in comparative psychology. In T. D. Johnston, & A. T. Pietrewicz (Eds.), *Issues in the ecological study of learning* (pp. 59–72). Hillsdale, NJ: Erlbaum.

Gottlieb, G. (1992). *Individual development and evolution: The genesis of novel behavior*. New York: Oxford University Press.

Gottlieb, G. (1997). *Synthesizing nature-nurture: Prenatal roots of instinctive behavior*. Mawah, NJ: Earlbaum.

Gottlieb, G. (2001a). A developmental psychobiological systems view: Early formulation and current status. In S. Oyama, P. E. Griffiths, & R. D. Gray (Eds.), *Cycles of contingency: Developmental systems and evolution* (pp. 41–54). Cambridge, MA: MIT Press.

Gottlieb, G. (2001b). The relevance of developmental-psychobiological metatheory to developmental neuropsychology. *Developmental Neuropsychology*, **19**, 1–9.

Gottlieb, G. (2004). Normally occurring environmental and behavioral influences on gene activity: From central dogma to probabilistic epigenesis. In C. G. Coll, E. L. Bearer, & R. M. Lerner (Eds.), *Nature and nurture: The complex interplay of genetic and environmental influences on human behavior and development* (pp. 85–106). Mahwah, NJ: Erlbaum.

Gottlieb, G. (2006). Developmental neurobehavioral genetics: Development as explanation. In B. C. Jones, & P. Moremede (Eds.), *Neurobehavioral genetics: Methods and applications* (2nd ed., pp. 17–28). Boca Raton, FL: CRC Press.

Gottlieb, G. (2007). Developmental epigenesis. *Developmental Science*, **10**(1), 1–11.

Gould, S. J. (1976). Grades and clades revisited. In R. B. Masterson, W. Hodos, & H. Jerison (Eds.), *Evolution, brain, and behavior: Persistent problems* (pp. 115–122). New York: Wiley.

Gould, S. J. (1980). Hen's teeth and horses toes. *Natural History*, **89**(3), 24–28.

Granic, I., & Hollenstein, T. (2003). Dynamic systems methods for models of developmental psychopathology. *Development and Psychopathology*, **15**, 641–669.

Greenberg, G. (1995). Anagenetic theory in comparative psychology. *International Journal of Comparative Psychology*, **8**, 31–41.

Greenberg, G., & Haraway, M. M. (2002). *Principles of comparative psychology*. Boston: Allyn & Bacon.

Greenberg, G., Partridge, T., Weiss, E., & Haraway, M. M. (1999). Integrative levels, the brain, and the emergence of complex behavior. *Review of General Psychology*, **3**, 168–187.

Greenberg, G., Partridge, T., Weiss, E., & Pisula, W. (2004). Comparative psychology: A new perspective for the 21st century. Up the spiral staircase. *Developmental Psychobiology*, **44**, 1–15.

Greenberg, G., Partridge, T., & Ablah, E. (2007). The significance of the concept of emergence for comparative psychology. In D. Washburn (Ed.), *Primate perspectives on behavior and cognition* (pp. 81–98). Washington, DC: American Psychological Association.

Grimm, V., Revilla, E., Berger, U., Jeltsch, F., Mooij, W. M., Railsback, S. F., et al. (2005). Pattern-oriented modeling of agent-based complex systems: Lessons from ecology. *Science*, **310**, 987–991.

Harlow, H. H. (1958). The nature of love. *American Psychologist*, **13**, 673–685.

Heylighen, F., Cilliers, P., & Gershenson, C. (2007). Complexity and philosophy. In J. Bogg, & R. Geyer (Eds.), *Complexity, science and society*. Oxford: Radcliffe.

Hirsch, J. (1987). Special issue: Comparative psychology – past, present, and future. *Journal of Comparative Psychology*, **101**, 219–291.

Hochachka, P. W., & Somero, G. N. (2002). *Biochemical adaptation: Mechanism and process in physiological evolution*. New York: Oxford University Press.

Hodos, W., & Campbell, C. B. G. (1969). *Scala naturae:* Why there is no theory in comparative psychology. *Psychological Review*, **76**, 337–350.

Hsieh, J., & Gage, F. H. (2004). Epigenetic control of neural stem cell fate. *Current Opinion in Genetics and Development*, **14**, 461–469.

Hull, D. L. (1972). Reduction in genetics – biology or philosophy? *Philosophy of Science*, **39**, 491–499.

Kaplan, R. H., & Phillips, P. C. (2006). Ecological and developmental contest of natural selection: Maternal effects and thermally induced plasticity in the frog *Bombina Orientalis*. *Evolution*, **60**(1), 142–156.

Johnston, I. A., & Wilson, R. S. (2006). Temperature-induced developmental plasticity in ectotherms. In S. J. Warburton (Ed.), *Comparative developmental physiology: Contribution tools and trends* (pp. 124–138). New York: Oxford University Press.

Kantor, J. R. (1959). *Interbehavioral psychology: A sample of scientific system construction*. Bloomington, IN: Principia Press.

Kauffman, S. A. (1993). *The origins of order: Self-organization and selection in evolution*. New York: Oxford University Press.

Kauffman, S. A. (1995). *At home in the universe: The search for the laws of self-organization and complexity*. New York: Oxford University Press.

Kauffman, S. (2000). *Investigations*. Oxford: Oxford University Press.

Kelso, J. A. S. (2000). Principles of dynamic pattern formation and change for a science of human behavior. In L. R. Bergman, R. B. Cairns, L. Nilsson, & L. Nystedt (Eds.), *Developmental science and the holistic approach* (pp. 33–48). Mahwah, NJ: Earlbaum.

Kim, J. (1999). Making sense of emergence. *Philosophical Studies*, **95**, 3–36.

Kuo, Z. Y. (1967). *The dynamics of behavior development*. New York: Random House.

Lerner, R. M. (2002). *Concepts and theories of human development. 3rd edition.*Mahwah, NJ: Erlbaum.

Lerner, R. M. (2004). Genes and the promotion of positive human development: Hereditarian versus developmental systems perspectives. In C. G. Coll, E. L. Bearer, & R. M. Lerner (Eds.), *Nature and nurture: The complex interplay of genetic*

and environmental influences on human behavior and development (pp. 1–33). Mahwah, NJ: Erlbaum.

Lewin, R. (1992). *Complexity: Life at the edge of chaos*. New York: Macmillan.

Lewis, M. D., Lamey, A. V., & Douglas, L. (1999). A new dynamic systems method for the analysis of early socioemotional development. *Developmental Science*, **2**(4), 457–475.

Magnusson, D. (2000). The individual as the organizing principle in psychological inquiry: A holistic approach. In L. R. Bergman, R. B. Cairns, L. Nilsson, & L. Nystedt (Eds.), *Developmental science and the holistic approach* (pp. 33–48). Mahwah, NJ: Erlbaum.

Masataka, N. (1994). Effects of experience with live insects on the development of fear of snakes in squirrel monkeys, *Saimiri sciurens*. *Animal Behaviour*, **46**, 741–746.

Mayr, E. (1985). How biology differs from the physical sciences. In D. J. Depew, & B. H. Weber (Eds.), *Evolution at a crossroads: The new biology and the new philosophy of science* (pp. 44–63). Cambridge, MA: MIT Press.

Mazzocchi, F. (2008). Copmlexity in biology. Exceeding the limits of reductionism and determinism using complexity theory. *EMBO Reports*, **9**(1), 10–14.

McShea, D. W. (1996). Metazoan complexity and evolution: Is there a trend? *Evolution: International Journal of Organic Evolution*, **50**, 477–492.

Michel, G. F., & Moore, C. L. (1995). *Developmental psychobiology: An interdisciplinary science*. Cambridge, MA: MIT Press.

Michel, G. F., & Tyler, A. N. (2007). Developing human nature: Development to vs. development from? *Developmental Psychobiology*, **49**(8), 788–799.

Moltz, H. (1965). Contemporary instinct theory and the fixed action pattern. *Psychological Review*, **72**, 27–47.

Morgan, C. L. (1901). *Introduction to comparative psychology*. London: Scott.

Morgan, C. L. (1923). *Emergent evolution: The Gifford lectures*. London: Williams and Norgate.

Moss, L. (2002). From representational preformationism to the epigenesis of openness to the world? Reflections on a new vision of the organism. *Annals of the New York Academy of Science*, **981**, 219–230.

Neyman, J., & Pearson, E. S. (1933). On the problem of the most efficient tests of statistical hypotheses. *Philosophical Transactions of the Royal Society*, **231**, 289–336.

Nesselroade, J. R. (2006). Quantitative modeling in adult development and aging: Reflections and projections. In C. S. Bergman, & S. M. Boker (Eds.), *Methodological issues in aging* (pp. 1–18). Mahwah. NJ: Routledge.

Newell, K. M., & Molenaar, P. C. M. (1998). *Applications of nonlinear dynamics to developmental process modeling*. Mahwah, NJ: Erlbaum.

Nicolis, G. (1989). Physics of far-from-equilibrium systems and self-organisation. In P. Davies (Ed.), *The new physics* (pp. 316–347). Cambridge: Cambridge University Press. (Reprinted 1994).

Novak, G. (1996). *Developmental psychology: Dynamical systems and behavior analysis*. Reno, NV: Context Press.

Oxman, E., Alon, U., & Dekel, E. (2008). Defined order of evolutionary adaptations: experimental evidence. *Evolution*, **62**(7), 1547–1554.

Oyama S., Griffiths P. E., & Gray R. D. (Eds.). (2001). *Cycles of contingency: Developmental systems & evolution*. Cambridge, MA: MIT Press.

Pelengaris, S., & Kahn, M. (2006). *The molecular biology of cancer*. Malden, MA: Blackwell Publishing.

Pinker, S. (2002). *The blank slate: The modern denial of human nature*. New York: Penguin.

Platt, S. A., & Sanislow, C. A. (1988). Norm-of-reaction: Definition and misinterpretation of animal research. *Journal of Comparative Psychology*, **102**(3), 254–261.

Popper, K. R. (1974). Scientific reduction and the essential incompleteness of all science. In F. J. Ayala, and T. Dobzhansky (Eds.), *Studies in the philosophy of biology* (pp. 259–284). Berkeley, CA: University of California Press.

Prigogine, I., & Stengers, I. (1984). *Order out of chaos: Man's new dialogue with nature*. New York: Bantam Books.

Pringle, J. W. S. (1951). On the parallel between learning and evolution. *Behaviour*, **3**, 174–215.

Pronko, N. H. (1980). *Psychology from the standpoint of an interbehaviorist*. Belmont, CA: Wadsworth.

Provine, W. B. (2001). *The origins of theoretical population genetics*. Chicago, IL: University of Chicago Press.

Qiu, G., Kandhai, D., & Sloot, M. A. (2007). Understanding the complex dynamics of stock markets through cellular automata. *Physical Review E*, **75**, 046116.

Rand, J. D., Kapuniai, L. E., Crowell, D. H., & Pearce, J. (2001). Nonlinear analysis of 30-second sleep stage epochs. In W. Sullis, & I. Trofimova (Eds.), *Nonliniear dynamics in the life and social sciences* (pp. 195–203). Washington, DC: IOS Press.

Redfield, R. (1942). *Levels of integration in biological and social systems*. Lancaster: Cattell Press.

Riziki, T. M. (1954). Desoxyribose nucleic acid in the symbiotic microorganisms of the cockraoch, *Blattella Germanica*. *Science*, **120**(3105), 35–36.

Romanes, G. J. (1885). *Mental evolution in animals*. New York: Appleton.

Rosenblatt, J. S. (2007). Gilbert Gottlieb: Intermediator between psychology and evolutionary biology. *Developmental Psychobiology*, **49**(8), 800–807.

Rosenblatt, J. S., & Schneirla, T. C. (1962). The behavior of cats. In E. S. E. Hafez (Ed.), *The behaviour of domestic animals* (pp. 453–488). London: Ballière, Tindall & Cox.

Saunders, P. T., & Ho, M.-W. (1976). On the increase in complexity in evolution. *Journal of Theoretical Biology*, **63**, 375–384.

Saunders, P. T., & Ho, M.-W. (1981). On the increase of complexity in evolution II. The relativity of complexity and the principle of minimum increase. *Journal of Theoretical Biology*, **90**, 515–530.

Saunders, P. T., & Ho, M.-W. (1984). The complexity of organisms. In J. W. Pollard (Ed.), *Evolutionary theory: Paths into the future* (pp. 121–139). New York: Wiley.

Sawyer, R. K. (2002). Emergence in psychology: Lessons from the history of non-reductionist science. *Human Development*, **45**, 2–28.

Schneirla, T. C. (1957). The concept of development in comparative psychology. In D. B. Harris (Ed.), *The concept of development: An issue in the study of human behavior* (pp. 78–108). Minneapolis: University of Minnesota Press.

Sheldrake, R. (1995). *Seven experiments that could change the world*. New York: Riverhead Books.

Shmulevich, I., Dougherty, E. R., & Zhang, W. (2002). From Boolean to Probabilistic Boolean Networks as Models of Genetic Regulatory Networks. *Proceedings of the IEEE*, **90**(11), 1178–1192.

Sylva, E. A., & Clarke, K. C. (2002). Calibration of the SLEUTH urban growth model for Lisbon and Porto, Portugal. *Computers, Environment, and Urban Systems*. **26**(6), 525–552.

Singh, S. (2005). *Big bang: The origin of the universe*. New York: Harper.

Smith, L. B. (2006). Movement matters: The contributions of Esther Thelan. *Biological Theory*, **1**, 87–89.

Smith, J. M., & Szathmáry, E. (1999). *The origins of life: From the birth of life to the origin of language*. Oxford: Oxford University Press.

Smotherman, W. P., & Robinson, S. R. (Eds.). (1988). *Behavior of the fetus*. Caldwell, NJ: The Telford Press.

Smuts, J. C. (1926). *Holism and evolution*. New York: Macmillan.

Sperry, R. M. (1987). Structure and significance of the consciousness revolution. *Journal of Mind and Behavior*, **8**, 37–65.

Stebbins, G. L. (1969). *The basis of progressive evolution*. Chapel Hill: University of North Carolina Press.

Strohman, R. C. (1997). The coming Kuhnian revolution in biology. *Nature Biotechnology*, **15**, 194–200.

Sulis W., & Trofimova I. (Eds.). (2000). *Nonlinear dynamics in life and social sciences*. Amsterdam: IOS Press.

Thelen, E., & Smith, L. B. (1998). Dynamic systems theories. In W. Damon, & R. M. Lerner (Eds.), *Handbook of child psychology: Volume 1: Theorectical models of human development* (5th ed.). (pp. 563–634). Hoboken, NJ, US: John Wiley & Sons Inc.

Tobach, E., & Schneirla, T. C. (1968). The biopsychology of social behavior of animals. In R. E. Cook, & S. Levin (Eds.), *The biological basis of pediatric practice*. New York: McGraw-Hill.

Van Geert, P. (1998). A dynamic systems model of basic developmental mechanisms: Piaget, Vygotsky, and beyond. *Psychological Review*, **105**(4), 634–677.

Von Eye, A., & Bergman, L. (2003). Research strategies in developmental psychopathology: Dimensional identity and the person-oriented approach. *Developmental Psychopathology*, **15**, 553–580.

Wallman, J. (1979). A minimum visual restriction experiment: Preventing chicks from seeing their feet affects later rewsponses to mealworms. *Developmental Psychobiology*, **12**, 391–397.

Wasserman, E. A. (1997). Animal cognition: Past, present, and future. *Journal of Experimental Psychology: Animal Behavior Processes*, **23**, 123–135.

Watson, J. D., & Crick, F. H. C. (1953). A structure for deoxyribose nucleic acid. *Nature*, **171**, 737.

Weiss, P. (1959). Cellular dynamics. *Review of Modern Physics*, **31**, 11–20.

Wolfram, S. (2002). *A new kind of science*. Champaign, IL: Wolfram Media.

Woodger, J. H. (1929). *Biologicalal principles: A critical study*. London: Routledge and Kegan Paul.

Young, D., Stark, J., & Kirschner, D. (2008). Systems biology of persistent infection: Tuberculosis as a case study. *Nature Reviews: Microbiology*, **6**, 520–528.

Part III

Empirical Studies of Behavioral Development and Genetics

8

Behavioral Development during the Mother-Young Interaction in Placental Mammals

The development of behavior in the relationship with the mother

Jay S. Rosenblatt

Introduction

The invitation to contribute a chapter to the volume dedicated to celebrating the contributions of Gilbert Gottlieb to the field of behavioral development and evolution has been a strong motivating force in what I have written. Gilbert pioneered the analysis of behavioral development in a precocial bird, the mallard, in which he showed that the duckling's relationship with the parent began even before hatching. In responding to its own prehatch vocalizations it prepared itself to respond to the parental call after hatching. His concept of probablistic epigenesis led him to consider how behavioral development could be the leading edge of evolutionary change through the creation of behavioral neophenotypes (Rosenblatt, 2007a, 2007b). Extending these concepts, it is quite evident in the study of behavioral development among placental mammals that during evolution the young have achieved fine-tuned behavioral adaptations to their mothers. These adaptations are quite different depending upon the evolutionary background of the mother and the nature of her offspring at birth. This chapter will consider the behavioral development of altricial, precocial, and semi-precocial young among placental mammals within the behavioral framework provided by the mother.

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In my research and my writings I have been concerned separately with the organization of maternal behavior, its sensory, neural, and hormonal determinants, and with the nature of behavioral development chiefly among altricial newborn kittens and rats. In the present chapter I want to explore the behavioral interactions between the mother and her offspring, chiefly from the point of view of how these contribute to the development of the young. For the mother this relationship promotes her inclusive fitness, for the offspring it ensures its life. It is evident that the interactions between the mother and young are based upon underlying physiological mechanisms however the emphasis in this chapter is on the behavioral interactions themselves.

It is generally agreed that among the mammals behavioral development during the period from birth (and even before birth) to weaning and for a short adolescent period has a deep and lifelong influence. This is the period when the development of the young is most under the influence of the mother (and the father and siblings in many species) and the environment that she provides. The nature of the relationship between the mother and her young is one of mutual dependence: the mother depends on the young to maintain her maternal behavior once it has been initiated and the young depend upon the mother for their survival and growth (Rosenblatt, 1992; Stern, 1997). We shall describe a universal pattern of relationship among mothers and their young in which the mother bears the larger load early in the relationship and, as the young grow and develop and are able to take care of themselves her solicitous care declines. The decline occurs at different rates among the different kinds of mammals as for example in altricial species such as the rat and cat and many other small mammals, in sheep, and other ungulates with precocial newborn (i.e., goats, horses, deer) and in the primates with semi-precocial newborn. In the final section of this chapter we will consider several ways in which this relationship has been characterized with respect to the selective factors that have shaped it.

In this chapter our concern will be the young and how they develop their behavior towards the mother and the environment she provides for them including their siblings. Corresponding to the different kinds of young, there are different sensory bases for the mother's behavior towards the young. Broad, Curley, and Keverne (2006) have reviewed the physiological and behavioral bases in the mother for the formation of the initial bond and its elaboration in the newborn of species with altricial, precocial, and semi-precocial young. They have shown that among altricial rodents and precocial ungulates the sensory basis for mother-infant bonding is largely olfactory and to some extent auditory and in semi-precocial primates it is mainly visual and auditory.

The first section of this chapter characterizes mother-young interactions during the suckling period in altricial, semi-precocial, and precocial species. In the next section the behavioral development of the young is divided into four areas each concerning a different aspect of their development. The first describes how the young initiate and develop the suckling relationship. The second concerns how the

young begin to use the mother as an orientation center, in addition to her function as a source of nutriment, providing them with security and psychological anchoring in the environment. The third section describes the growing ability of altricial young mainly to remain at or to find their way to familiar and secure places represented by a sibling huddle or a home site both of which have been conditioned by the mother's odors. The fourth section describes play behavior, which occurs initially under the aegis of the mother, during which the young organize behavioral sequences, learn about their environment, and interact socially with age mates (Bekoff, 1972; Burghardt, 1998).

Mother-Young Interactions during the Suckling Period

Altricial Species

The cat typifies the course of the mother-young interaction among species with altricial young. The mother initiates nursing during the first 3 weeks by approaching the young in the home site, awakening them and lying down, presenting her nipple region to them (Rosenblatt, Turkewitz, & Schneirla, 1961; Schneirla, Rosenblatt, & Tobach, 1963). Kittens grasp nipples and suckle until they fall asleep and the mother either sleeps with them or leaves them. The mother licks them to eliminate and lies with them to warm them. As the kittens mature, they initiate suckling and this increases from the 3rd to 5th week. The mother initially cooperates with their efforts to grasp nipples by lying down but after the 5th week the mother tries to evade suckling initiated by the kittens by climbing to a shelf out of reach of the kittens (Rosenblatt et al., 1961). Kittens begin to feed from dishes at the end of the 4th week and no longer suckle after the 2nd month.

A similar pattern is seen in the rat in which mothers initiate nursing exclusively during the first 2 weeks by entering the nest and arousing their pups, hovering over them as they grasp the nipples and settling over them in a high crouch which facilitates nipple grasping (Cramer, Thiels, & Alberts, 1990; Rosenblatt, 1965; Stern & Johnson, 1989; Thiels, Alberts, & Cramer, 1990). The mother begins to evade the pups' suckling approaches outside the nest and at the end of the 3rd week she presses her ventrum against the floor or a wall in response to the pups' suckling approaches (Cramer et al., 1990). This pattern is also seen in the mouse (König & Mark, 1987; Weber & Olsson, 2008) and the puppy (Fuller and Du Buis, 1962; Pal, 2008; Rheingold, 1963; Scott, 1958, 1962).

The situation in the rabbit is somewhat different because the mother is only present in the nest to interact with her young during 3 minutes each day when she nurses the young (González-Mariscal & Rosenblatt, 1996). The relationship ends abruptly when the mother no longer returns to the nest to nurse her young at 31 days after parturition. At 5 days of age the pups respond to her odor by

lifting their heads to suckle. Still later at 10 days of age, in response to the odor, they await her arrival at the nest opening and immediately attach to nipples when she enters the nest: they take the initiative in suckling earlier and earlier in the feeding relationship (Hudson & Distel, 1983; Ivanitskii, 1962; Kindemann, Gervais, & Hudson, 1991).

Semi-Precocial Species

In the rhesus monkey, characteristic of many anthropoid primates, the infant is initially carried by the mother in a ventro-ventro position enabling it to grasp her nipples but by the 7th month ventro-ventro carrying declines. The mother initiates nursing during the first few weeks by picking up the infant and when the infant attempts to grasp a nipple it is nearly always accepted (Hinde & Simpson, 1975; Hinde & White, 1974). The initiation of nursing by the mother declines sharply beginning around the 3rd month. Infants initiate nearly all of the ventro-ventro contacts by the 4th month but their efforts to gain a nipple are often prevented and rejections by the mother increase over this same period (Simpson, Simpson, & Howe, 1986).

In the chimpanzee, after parturition, the mother carries the infant against her breast supporting it with her arms and thighs (Goodall, 1968; van Lawick-Goodall, 1969). Contact between the infant and the mother is based entirely on the mother's initiative in cradling the infant against her body as she sits or lies and when moving from place to place: the infant grasps the mother's hair with her support. During its early contacts with the mother the infant chimpanzee suckles at intervals. It initiates the suckling bouts, by locating the nipple with side-to-side movements of the head, later directly grasping the nipple. By the 2nd week the infant is able to cling unsupported for short periods and by 3 months of age it is able to support itself against the mother's body with the mothers help. The mother guides the infant to ride on her back from 5 to 7 months of age (van Lawick-Goodall, 1969).

Infant chimpanzees initially crawl about on the mother's body and later they begin to leave the mother but she restrains them until between 3.5 and 5 months when the infants break contact with the mother for the first time sitting next to her. Mothers do not play an active role in weaning behavior. Until 4–5 years of age, mother and young always travel together but this begins to decline at 5–7 years of age and by 8 years and older, they hardly ever travel together.

Since human mothers initiate nursing sessions with their infants, evidence of the shift to infant initiated or termination of suckling, as occurs in all other primates, is not easily obtained. Mothers increasingly depend upon signs of restlessness or discomfort to initiate feeding their infants which suggests infants are taking the initiative at least in a diffuse way. Some evidence of the infant's initiative in the decline in suckling is found among mothers who stop breastfeeding their

infants during the first year. Those who stop at the end of 2 months report that their infants are not satisfied by breast milk alone, and among those that stop breastfeeding after the 3rd month, self-weaning is indicated in statements that the infant began to bite or lost interest in nursing or began to wean itself (Li, Fein, Chen, & Grummer-Strawn, 2008).

Among humans Blauvelt and McKenna, (1961) have provided a detailed description of human infant nipple attachment during the first 24 hours. In cradling the infant on her shoulder the mother provides a sequence of tactile stimulation that induces the infant to rotate its head towards her bringing it into contact with her breast where lip stimulation elicits nipple grasping. Infants are also responsive to breast odors in their orienting to and mouthing of their mother's nipple and Doucet, Soussignan, Sagot, & Schaal (2007) have shown these derive from the nipple, the areola, and from milk (Varendi, Porter, & Winberg, 1994).

Precocial Species

Among sheep, goats, and many other ungulates (i.e. horses, cows, deer) the initial nursing takes place shortly after parturition. During the first 2 hours the mother forms an individual bond with her own newborn based on its individual odor. While lambs remain close to the mother when she grazes, kids are often hidden for the first few days and visited by the mother several times a day for feeding. On the 3rd day the mother leads the kid away from its hiding place and from then on it follows as she leads it (Collias, 1956; Poindron, Nowak, Lévy, Porter, & Schaal, 1993).

Once mutual recognition is established, the mother permits only her own lamb to suckle. Lambs approach their mothers to suckle by passing in front of her so that she can smell their anal region before allowing them to suckle but if a lamb is attempting to suckle from a strange mother it approaches her from the rear to avoid detection (Poindron & Le Neindre, 1980). The bond formed between the lamb and its mother is evident during the first two months in their close proximity even while the lamb is grazing; they are responsive to one another's vocalizations and there is a rapid reunion when separation grows too large (Collias 1956; Hinch, Lescrivain, Lynch, & Elwin, 1987). During the 2nd month the distance of the lamb from the mother in pasture increases from 2 m to 4 m and during the 3rd month the distance increases to 10 m. Nursings, which are frequent during the first week, decline by 50% by the 4th week. The mother no longer approaches the young; the young approach her when she calls and it is the mother who terminates nursing rather than the lamb or kid. When lambs were tethered mothers moved to the lamb at 30 m during the first two or three weeks but thereafter they did not go to them (Hinch et al., 1987). Weaning takes place between 4 and 6 months when the mother prevents the young from nursing and the amount of milk provided the young decreases (Arnold, Wallace, & Maller, 1979). As Poindron et al. (1993) note, the

young lose interest in the mother and as Collias (1956) notes the first play behaviors among the kids is seen at 9 days and increases thereafter. Similar interaction between the mother and offspring has been described in the Japanese Black cattle (Hirata et al. 2003) in which during an early period of close association, "mutual independence of mother and young rapidly develops in the first 30–50 days after parturition." (Hirata et al., 2003, p. 161)

In the roe deer during the first week the mother initiates nursing by approaching the fawn and either sniffing or licking it or standing nearby reaching toward it with her head (Espark, 1969). There is a change in their relationship after the first week. The young initiate suckling by making high-pitched calls searching for the mother and walking to her nearby. Nursing is terminated by the fawn but after that time feedings are increasingly terminated by the mother. The mother licks the fawn continuously during the first 3 or 4 weeks but she licks it only sporadically after that time and later she no longer licks the fawn.

Analysis of Suckling Development

Altricial Species

Among kittens suckling is initiated during parturition or shortly afterward. During their initial suckling kittens learn to prefer individual pairs of nipples on the first or second day which they suckle 70% or more of their feedings during the entire suckling period despite changes in the mother's behavior during nursing (Ewer, 1959; Rosenblatt, 1971). Learning nipple preferences is an individual phenomenon since the rate of learning is not affected by how many kittens are in the litter.

Kittens use olfaction to approach the mother for suckling but use mainly tactile stimuli to locate and attach to the nipple; anosmic kittens during the first two weeks are unable to find the nipple and those that do find it do not grasp it to suckle (Larson & Stein, 1984; Shuleikina-Turpaeva, 1986). Anesthetizing the kitten's lips prevents it from localizing the nipple although it searches the mother's fur all over her body. When two kittens are reared together in a brooder they do not suckle from separate nipples because the brooder surface cannot compete with the sibling's fur for nuzzling as the female's fur obviously does (Guyot, Bennett, & Cross, 1980).

Using a simplified model of the mother's ventrum consisting of a brooder mounted with flanges that varied either in surface texture or odor in which only one of two nipples provided milk, nipple localization was studied in kittens isolated from the mother (Rosenblatt, 1971, 1983). Over a two-day training period newborn kittens learned to choose one or the other of the flanges associated with the nipple that provided milk. Kittens rapidly established a path that brought them

to the correct flange and nipple and when elements of the path were removed or altered their performance was disrupted briefly but they soon relearned the new path (Rosenblatt, 1983). Their behavior suggests that after they learned which flange and nipple provided milk they learned a path to the nipple that included several elements, the floor, the brooder, the flange and the nipple. It is likely that similar learning of a path to the mother's nipples is learned by kittens very soon after birth.

In their studies of early learning of nipple-grasping in puppies, Stanley, Bacon, and Fehr (1970) found that puppies also learned to choose one of two surface textures of a path leading to the nipple to obtain milk in a brooder. When the soft texture was the one they favored, they established a path to the nipple rapidly and when the path was changed and the texture was now coarse they slowly learned the new path to the nipple.

Further developments in kitten suckling arise from the growing ability of kittens to walk and to use vision to orient to distant objects (Beaver, 1980; Rosenblatt, 1979; Sireteanu, 1985). At the end of the 3rd week kittens are able to use vision to respond to the mother when she is nearby. This is shown by the finding that kittens made anosmic are able to find the mother and locate their preferred nipple, grasp it and suckle during the 3rd week (Shuleikina-Turpaeva, 1986). Once they reach the mother the kittens nuzzle in her fur and if she is standing she accommodates them by lying down in a nursing position. From then on kitten approaches to the mother for suckling are adjusted to the mother's behavior and depend largely on whether the mother makes herself available and lies down after kittens have begun to suckle while she is standing. Kittens watch the mother as she moves around the cage before making an attempt to approach her and grasp their preferred nipples.

Kittens depend upon experience with the mother to be able to adapt to changes in her nursing behavior. Kittens that are raised in isolation from the mother and littermates over the period from 25 to 45 days, reared with a brooder provided with a nipple which they suckled to obtain milk, then returned to the mother and siblings, are unable to suckle from the mother (Rosenblatt, 1971; Rosenblatt et al., 1961). They make contact with her but even when their siblings suckle they do not approach the mother to suckle. Kittens need to be in continuous contact with the mother in order to be able to adjust to changes in her nursing behavior and these kittens were absent during the period of rapid change in her nursing after 25 days of age. It is not that they cannot remember that they suckled from her previously because kittens that are returned to their brooder after a long absence of 25 days suckle from it within 10 to 25 minutes. Unlike the situation with the mother, no changes in the mode of suckling are required to resume suckling on the brooder since any changes that do occur in suckling are based solely on the kitten's behavior.

In the rat the initial nipple grasping is in response to amniotic fluid and saliva deposited by the mother on the ventrum during parturition (Blass, 1990; Blass et al., 1979; Teicher & Blass, 1976, 1977). Prenatal swallowing of this fluid

[or an experimentally provided lemon scent, Pedersen & Blass (1982)] contributes to its attraction for the newborn, combined with the sensory stimulation the newborn receives during parturition.

The mother initiates suckling by the young by entering the nest and assuming a high crouch (Stern & Johnson, 1989). The pups crawl under her ventrum and grasp the nipple from below while on the back, a motor pattern that in already present prenatally in the fetus (Blass & Teicher, 1980; Eilam & Smotherman, 1996; Polan & Hofer, 1999; Polan, Milano, Eljuga, & Hofer, 2002; Stern & Johnson, 1989). Maternal anal licking of pups to stimulate elimination before and during suckling contributes to their response to nipple odor (Sullivan, Brake, Hofer, & Williams, 1986; Sullivan, Hofer, & Brake, 1986). Over the next week, the pups crawl forward towards her when the mother enters the nest, she lies down on her side, and now the pups grasp nipples while crawling forward in a prone position (Eilam, Goffman, & Smotherman, 1999; Prechtl, 1952). During their initial suckling newborn rat pups that responded to amniotic fluid develop an appetitive response to the nipple covered with their own saliva and to the odor and taste of the milk, Responses to these stimuli are retained during subsequent feedings and serve as stimuli for operant learning of responses to additional characteristics of the mother encountered during suckling (Arias & Chotro, 2006; Brake, 1981; Cheslock, Sanders, & Spear, 2004; Johanson & Hall, 1979; Johanson, Hall, & Polefrone, 1984). As their ability to crawl increases they learn to crawl to the mother at a distance to grasp a nipple and suckle (Amsel, Burdette, & Letz, 1976; Kenny & Blass, 1977). With the decline in the mother's initiation of nursing, and the disappearance of the nest in the third week, rat pups take the initiative in approaching the mother to suckle at a distance outside the nest site. Initially their approach to the mother is based mainly on olfactory and tactile stimulation but after their eyes open, around 14 days of age, they begin to leave the nest area and approach her to suckle, very likely based on vision (Rosenblatt, 1965; Telle, 1966 cited in Blass, 1990). It is during this period that the mother begins to actively evade their suckling approaches (Cramer et al., 1990).

Weaning in rat young occurs over the period from Day 15 to Day 25 when the young begin eating solid food and gradually terminate their suckling over the next 2 weeks. As they increase their food intake they take less milk from the mother than she is able to produce and after Day 20 her milk supply declines and they receive even less milk (Thiels, Cramer, & Alberts, 1988).

Precocial Species

Newborn lambs initiate suckling shortly after parturition by searching and locating the teat of the mother who licks the lamb and stimulates it to stand with its head facing her udder and teat (Vince, 1992, 1993; Vince, Lynch, Mottershead, Green, & Elwin, 1985). Visual stimulation plays the major role in the lamb's location of the

nipple but auditory stimuli emitted by the mother and tactile stimuli from the udder and teat cooperate to elicit teat grasping.

Among sheep, as noted earlier, the mother learns the odor of her lamb during the first two hours after parturition and she allows only her lamb to suckle from her. Lambs learn their mother's odor by 24 hours however if they are prevented from suckling during the first 2 to 6 hours, they require more than 24 hours to develop the preference. Normally lambs suckle and receive colostrums during the first several hours: obtaining colostrum during this period is an important component of the early experience that enables lambs to develop their attachment to the mother and this is supplemented by the gastric fill that colostrums produce in the newborn (Nowak, 2006).

Suckling in Semi-Precocial Species

In a review of the onset of maternal behavior at parturition and shortly afterward in a large variety of non-human primates, Rosenblatt (1991) was unable to find evidence that mothers aid the newborn, clinging to her ventral fur, to locate and attach to a nipple to suckle either at parturition or afterward. Supported by the mother, the infants of many species, nuzzle the mother's ventrum, locate and attach to a nipple.

Learned nipple preferences, usually the left nipple, have been reported in the infants of many primates including chimpanzees, gorillas, and humans, in many Old World monkeys, and in twins of New World monkeys (e.g., common marmoset) infants that adopt opposite nipple preferences (see review, Hopkins & De Lathouwers, 2006).

Learning has been reported in the early nursing adjustment of human infants. In fact, one of the earliest reports of learning in young animals was reported by Gunther (1961), a pediatrician, who observed that infants experiencing obstructed breathing during attempted suckling on an inadequately formed breast on the first day reacted negatively and this was carried over to the next several days before normal suckling could be established. Schaal et al., (1980) have shown that infants learn to respond to the specific odors of their mother during nursing and can turn to her before nursing actually begins when she approaches.

Newborn human infants also learn to orient to their mother's voice during nursing. Infants are normally nursed on the left breast then shifted to the right breast and they develop a preference for this order of being nursed. Bottle-fed infants, on the other hand, are fed only on the left side by most mothers. As a consequence of these different experiences bottle-fed and breast-fed infants respond differently to the sound of their mother's voice during nursing and when she is not nursing. Bottle fed infants always turn to right regardless of whether the mother's voice comes from the left or the right but breast-fed infants turn to the left or to the right depending the source of the mother's voice.

The Mother as an Orientation Center for the Young

In addition to her role as provider of nutrients, the mother functions as orientation center for the newborn and developing young. She does this by providing stimulation that attracts the young and calms them, and by determining many of the characteristics of their developmental environment. In altricial young the developmental environment consists of siblings and the nest or home site where nursing takes place and the young huddle.

The most thorough studies of the effects of immediate and long-term (i.e., 24 hours) separation from the mother have been done in the rat by Hofer and his associates (Hofer, 1984, 1994); significant studies have been done in several species of New World monkeys (Harlow, 1961; Hinde, 1974; Kaufman, 1974).

There is an immediate effect when young of many species are separated from their mother often consisting of vocalizations and either an increase or an inhibition of activity which can rapidly be ameliorated by restoring the mother or a surrogate (i.e., sibling, maternal odor). In infant rats separation from the mother and placement in an unfamiliar environment induces loud vocalization, an increase in activity, and reduced heart rate – evidence of severe disturbance (Richardson, Siegel, & Campbell, 1988; Siegel, Richardson, & Campbell, 1988). An anesthetized mother placed in the unfamiliar environment is able to calm the infant and increase its heart rate and this occurs in infants reared apart from the mother from shortly after birth (Hofer, Shair, & Murowchick, 1989). While they are in contact with the mother many important physiological functions are being regulated. The "regulators" have been identified and in each instance they are aspects of the mother's behavior, in addition to providing milk, that have these effects on the young when they are in contact with the mother (Hofer, 1984, 1994). In effect, therefore, by suckling and seeking contact with the mother the young are enabled to regulate these important physiological functions.

Among the young of a variety of mammals ranging from rats and cats, to sheep and goats, and a large variety of primates the close physical association between the young and the mother testifies to the mother's attractiveness to the young in addition to the nursing she provides. In altricial species the young are attracted by the thermal, tactile, and olfactory properties that elicit their approach responses and by her readiness to adapt her behavior to their limited behavioral capacities, corralling them under and against her ventrum (Hall & Oppenheimer, 1987; rat, Polan et al., 2002; rabbit, Serra & Nowak, 2008; Val-Laillet & Nowak, 2008). Moreover, the mother licks the young and this stimulates their approaches to her and aids in their elimination functions. Olfactory stimulation is particularly effective in attracting young to the mother, in part because of its association with suckling, but also because of its association with thermal and tactile stimulation (Alberts 1978; Rosenblatt, 1983). Harlow & Zimmerman (1959) have shown that

the tactile and visual characteristics of the artificial "mother" in rhesus monkeys need not be associated with suckling to attract young.

Among precocial and semi-precocial species the young are able to stand and walk at birth and therefore they are able to approach and contact the mother shortly after parturition and in the weeks and months that follow they view the mother as the center of their world and orient their behavior in relation to her (agouti, Galef & Clark, 1976; horse, Heiter & Vicente, 2008; common marmoset, Ingram, 1977; sheep and goats, Collias, 1956; Hersher, Richmond, & Moore, 1963; Hinch et al., 1987; Nowak, 2006; Vince, 1992, 1993, Vince et al., 1985; rhesus monkey, Harlow, 1974; Harlow & Zimmerman, 1959; Hinde, 1974; Hinde & Spencer-Booth, 1969; langurs, Jay, 1963; humans, Rheingold & Eckerman, 1979).

In sheep recognition of the mother is especially important since mothers permit only their own lambs to suckle (Lévy, Keller, & Poindron, 2004; Sèbe, Nowak, Poindron, & Aubun, 2007). While ewes recognize their lambs by their odors, the young initially recognize their mothers by their vocalizations. Maternal vocalizations before and after parturition contribute to the lamb's recognition of its mother beginning at 48 hours and for several months during which the mother remains the orientation center for the lamb while it is grazing. The lamb tends to remain within one meter of the mother for 75% of the time. Mothers also have a tendency to follow the lamb as it grazes but this ends at 2.5 months, nevertheless the lamb still remains near her and this behavior survives a 2-month separation between them (Galeana, Orihuela, Aguirre, & Vázquez, 2007; Hinch et al., 1987).

In the guinea pig maternal licking and lying on her side are important components that initially attract the precocial young to the mother. From the beginning the mother remains the orientation center for the growing young and separation from her evokes distress vocalization at a high level (e.g. 500 vocalizations per 5 min) over the initial five weeks and is not absent until the young are three months of age (Pettijohn, 1979).

Rheingold & Eckerman (1970) have reviewed the use of the mother as an orientation center among the young of many primate species, including humans, as they begin to explore their social and physical environment. Langur and baboon young begin to leave the mother at 2 months reaching a distance of 2.5 feet at three months of age. Human infants begin to leave the mother at 7 months but remain close to her until 10 months when they wander 20 feet from her.

In the common marmoset the mother, father, and adolescents and sub-adults provide the caretaking for the infant during the period from birth to 10 weeks when weaning occurs (Ingram, 1977). In this species infants are carried by the three types of caretakers about equally for the first 6 weeks; only the mother nurses the infant but all three serve as orientation centers during this period. After the 4th week the infant takes the initiative in remaining within 15 cm of either parent during the weaning period in spite of the fact that during this period its attempts to climb on to the parents are rejected.

In rhesus monkey infants there is a non-nursing/suckling relationship over the first year (Hinde & Spencer-Booth, 1969). Until 6 months of age infants cling to the mother without suckling about a quarter of the time. They increasingly leave the mother at 2 months but they continue to approach the mother throughout the first year.

The artificial mothers on which rhesus infants were raised by Harlow (1961) and Harlow & Zimmerman (1959) functioned as orientation centers especially when infants were frightened. The comforting mother under these conditions was the cloth mother in preference to the wire mother, whether or not it was the mother which "fed" the infant. Since the cloth mother that did not provide nursing was equally preferred in the frightening situation to the one that did provide milk, suckling is not necessarily the basis for establishing the cloth mother as the preferred orientation center. In the absence of the mother in the fearful situation rhesus infants froze in a crouched position (Harlow & Zimmerman, 1959).

Huddling and Home Site Orientation

Huddling and home site orientation have in common that the individual young, separated from its siblings or its home site uses thermal, tactile, olfactory, and visual stimuli to reestablish contact with them and to return to its home site.

Huddling is characteristic of altricial young that live in a nest with siblings. In these species periods of suckling and thermoregulatory contact with the mother alternate with periods when the mother is absent and huddling functions as a supplementary means of thermoregulation for the pups (Alberts, 1978; Alberts & Gubernick, 1983). The huddle is a social group that results from the actions of individual pups responding to the insulating properties of the huddle: under cold conditions pups seek the huddle but under warm conditions they remain apart from it (Alberts & Gubernick, 1983).

In orienting to and huddling with other pups, young initially respond to their tactile and thermal characteristics but after the second week they respond to odors that have been deposited on the pups by the mother (Kojima & Alberts, 2009). Altman, Sudarshan, Das, McCormick, & Barnes (1975) showed that rat pups can also find their way to their huddling siblings from distances and along paths that suggest they used visual cues as well as olfactory cues that are present on the floor surfaces. Pups do not begin to orient to siblings located at a distance in the home site until the end of the 2nd week or the middle of the 3rd week. Only after their eyes have opened on Day 14 do they increase the frequency and speed of reaching the home site and the huddle.

The majority of species of ungulates and primates do not utilize fixed nest sites for their young but the young either follow the mother, are carried by her or cling

to her therefore, the mother is, in effect, the equivalent of a mobile nest site. (See previous section).

Orientation to the home site (i.e., nest or nursing site) among nesting species, young rats, mice, hamsters, and kittens has been studied as aspects of the young animal's ability to return to a familiar location that contains the mother's odors and may be warmer than the surrounding areas. Home site orientation was studied in rat pups by Galler (1979), Johanson, Turkewitz, & Hamburgh, (1979) and Sczerzenie & Hsiao (1977) starting before the pups' eyes open around Day 14. As early as 3 days of age, pups orient to the home site by pivoting until they point in its direction (Altman & Sudarshan, 1975). Pups are tested for home site orientation by being placed alone in their home cages where they have to crawl to the home site from an adjacent corner at a distance of 11 inches and a diagonally opposite corner at a distance of 17 to 20 inches. Between 6 and 14 days of age, nearly all of the pups reach the home and remain there (Johanson et al., 1979) but fewer reach the home from the diagonal corner (Galler, 1979). After eye-opening rat pups are able to orient to the home site containing huddling pups from even longer distances along routes that are unlikely to provide olfactory paths to the home site. Nearly all of the pups reach the home site by 18 days of age (Altman et al., 1975). In a variation of the procedure, home cage odors were used to motivate rat pups 6 to 15 days of age to adopt one of two paths that led to the home cage which was distinguished either by its position (right or left) or by the rough or smooth floor texture (Bulut & Altman, 1974). Six- and 10-day old pups learned the route in about 7 days and 15–day pups learned it in 5 days.

Home site orientation tests that reveal the homing behavior of rat pups during the 3rd week also show the beginning of the pups' tendency to wander outside the home site or to remain outside it (Galler, 1979; Johanson et al., 1979). The increase in activity outside the nest area after the 18th day coincides with the pups' approaches to the mother outside the nest (Rosenblatt, 1965) and with pups beginning to take solid food and to initiate play-fighting (Alberts & Leimback 1980; Cramer et al., 1990; Thiels et al., 1990).

Aspects of home site orientation, including responsiveness to thermal and odor gradients of stimulation leading young to approach the sources, have been studied in mice, hamsters (Leonard, 1974), puppies (Crighton & Pownall, 1974; Welker, 1959), and rabbits (Hull & Hull, 1982). In young precocial hares that congregate at their natal site each day to suckle from the mother who returns to feed them, Stavy, Goldblatt, & Terkel (1984) found a preference to orient to their own odor to guide their return to the nursing site for the daily nursing during the 30-day period that normally precedes weaning.

Home site orientation occurs in kittens where it has received more study than in any other mammal (Luschekin & Shuleikina, 1989; Mermet, Coureaud, McGrane, & Schaal, 2007; Rosenblatt, 1965, 1971, 1972, 1974, 1976; Rosenblatt, Turkewitz, & Schneirla, 1961, 1969). Beginning around the 4th day kittens placed alone in an adjacent corner of their home cage, emptied of mother and kittens, crawl to the

home site, a distance of 35 inches, by following an odor gradient, and on arriving there they come to rest (Luschekin & Shuleikina, 1989; Rosenblatt, 1971). The warmth of the home cage shortly after mother and pups have been removed aids in inducing the kittens to remain in the home site once they arrive there (Freeman & Rosenblatt, 1978a). When the odor gradient is interrupted by removing a floor panel and replacing it with a washed panel, kittens crawl in the direction of the home site but stop at the border of the new panel and come to rest there. After the floor panel has been in place for 24 hr and odors are deposited on it by the mother and siblings, kittens enter it as before and come to rest in the home site. The odors used by kittens have not been identified but mothers have individually specific odors to which their own kittens orient in crawling to the home site but which alien kittens are not able to use to return to the home (Freeman and Rosenblatt, 1978b).

At the early age of 6 days, kittens placed in a further corner diagonally across from the home site simply pivot in place without adopting a path to the home site. Placed there after the 12th day, kittens begin to crawl to the home site via a path through the adjacent corner. Later, around the 14th to 18th day, they straighten this path and crawl or began to walk directly to the home site from the diagonal corner (Luschekin & Shuleikina, 1989; Rosenblatt, 1965). As they walk towards their home site kittens periodically sample the floor by nose-tapping presumably to receive olfactory stimulation from the floor to aid in detecting the odor gradient guiding them to their home site.

In contrast to their behavior in the home cage, kittens placed in each of the corners of a cage similar to their home cage but without maternal and sibling odors (i.e., an open field), briefly pivot in place then they remain immobile for the entire test and this behavior does not change until around 18 days of age when they begin to explore the cage floor by walking and nose-tapping. Frederickson & Frederickson (1977) have shown that this exploratory behavior in an open field increases in kittens from 21 to 42 days.

The disturbance caused by removing kittens from their home site is expressed not only in their movements but in their vocalizations. Kitten vocalization begins immediately when they are lowered into contact with the cage floor surface and detect that they are not in the home site, and it increases in intensity throughout the 2- or 3-minute tests. The increase is less in the nearby adjacent corner than in the more distant diagonal corner and is loudest in the cage without maternal odors where it is maximal. As kittens in the home cage begin to crawl towards the home site, however, vocalizations diminish in loudness and when they reach the home site vocalizations terminate. Being in the home site calms the kittens and they soon fall asleep. In the cage either without maternal odors or in a large room, 2-week old kittens placed at a distance from the home and mother, increase the frequency and intensity of their vocalization during the entire test (Rheingold & Eckerman, 1970).

The sensory basis of home site orientation in the kitten has been studied by Luschekin & Shuleikina (1989). Making kittens anosmic with intranasal infusions of zinc sulfate eliminates the early appearance of orientation from the adjacent to the

home site but by using visual stimulation from 16 and 24 days of age 60% of the kittens are able to reach the home site from the adjacent corner.

Home site orientation begins to disappear in kittens after day 18 when they leave the home site and wander in the cage. They show an attraction to approach areas of the cage at a distance which they see from the home site. If their eyes are sealed on day 18 they remain in the home site after reaching it and even up to the age of 30 days do not leave it to wander around the cage (Luschekin and Shuleikina, 1989).

Under more natural conditions when the mother is present in the cage, she emits purring sounds during nursing and short tonal sounds when kittens are removed. When these sounds were played from the home site to 15- to 30-day old kittens they returned to the home site from the adjacent and diagonal corners.

Play Behavior in Young Animals

Play is a behavior of young animals that occurs during a specified period in their early behavioral development. The behavior patterns of play and the ages when play occurs differ in different species. Play peaks at 20 days in house mice, 10–15 weeks in kittens, 3–4 weeks in pronghorn, 10–20 months in the Olive baboon (Byers, 1998), and after 6 years of age in orangutans (van Andrichem, Utami, Wich, van Hooff, & Sterck, 2006). Kittens begin social play at nearly 4 weeks of age (Rosenblatt et al., 1961). The behaviors that characterize play differ particularly among the small mammals, the ungulates, and the primates in which the young differ greatly in their behavior and motor patterns. What is common to play behavior in the young of all species is that it almost always occurs between animals of similar age and developmental status except in cases when play is directed at the mother (Nunes, Muecke, Sanches, Hoffmeier & Lancaster, 2004), it involves species-characteristic motor patterns and accompanying emotional responses, and it undergoes changes during the period of play.

The phenomenon of play has interested many theorists because at the time it is performed it has no obvious goal and therefore its immediate function and motivation are not apparent (Pellis & Pellis, 1998). In the absence of any immediate function of play a more distant function has been sought as practice for later predatory behavior and as a socializing influence (Panksepp, 1980) and this, in turn, has fueled speculation of its adaptive value in the evolution of mammals (Burghardt, 1998). Burghardt (1998) has provided the most exhaustive consideration of the possible motivational basis for play behavior, which he views as linked to appetitive and innate responses of early ethological theory. Poirier & Smith (1974), noting its presence in the mammals but in few other orders, propose that during play "the most highly developed 'learning animals' . . . experiment with behavioral patterns, learning in the process" (Poirier & Smith, 1974, p. 276). Bateson (2005) believes that play has a leading role in evolutionary modification.

He has proposed that "The existence of a phenotype, acquired by learning, sets an end-point against which phenotypes that develop in other ways must be compared" (Bateson, 2005, p. 29). He proposes the special nature of play is that "aspects of play can, indeed, increase the total sum of spontaneously developing behavioural structures that serve to solve complex problems" (Bateson, 2005, p. 31).

Play arises in kittens, as noted earlier, at the end of the 4th week (28 days) shortly after they first become capable of mature walking, running, jumping, and climbing (Rosenblatt et al., 1961) and in rat pups during the 3rd week (18 days) when they have developed the same abilities (Panksepp, 1980; Rosenblatt, 1965). Until then behavior in both species has been centered on their suckling approaches to the mother, huddling together with their siblings in the home region or nest, with few excursions outside the home region and few opportunities to make contact with inanimate objects except nesting material, food pellets and food in dishes, or their wider physical environment. As Stamps (1995, p. 49) has pointed out "play involving basic motor patterns (e.g., running, jumping, climbing, abrupt changes in or direction) occurs before young animals begin to move at high speed around barriers and obstacles in their natal home range .... Eventually many infants (*monkeys*) develop complicated serial motor patterns." It has been remarked that play is the first offspring behavior not performed in relation to the mother, but in fact the earliest objects of playful behavior in kittens is the mother's face and later her tail. Play is, however, the first behavior not related to obtaining milk and thermal and tactile comfort from the mother.

Animals seem to know when it is appropriate to play and when more serious business is at hand. Adolescent rat pups placed in an unfamiliar cage, engage in play but if one pup has been induced to show maternal behavior through a sensitization procedure and is given pups to guard it attacks its former play partner (Kalinechev, pers. comm.). When the serious business of obtaining milk from the mother in kittens is terminated earlier during weaning than normally, they increase the time spent in joint play activity (Martin & Bateson, 1985). When their milk intake is reduced they increase their efforts to suckle but do not show the normal increase in their joint play even though they increase their object play when the mother makes herself unavailable to them (Bateson, Mendl, & Feaver, 1990). Similarly, Harlow & Zimmerman (1959) found that rhesus monkey infants played with each other if the cloth mother to which they had become attached and whose presence made them secure was present but not when the wire mother, which they did not become emotionally attached to, was present. Play occurs when all the basic needs of the young for food, warmth, and safety have been satisfied and the young have an excess of energy that needs to be discharged.

Play among kittens is necessarily a joint activity between at least two kittens of approximately the same age and stage of behavioral development. As noted above, no overall incentives or goals are achieved by play therefore play is believed to be without motivation but not without energy input. It is likely, however, that fleeting intentions and goals arise in the course of joint play behavior and determine

particular actions and also in object play kittens are attracted to specific features of an object and direct their behavior to them. West (1974) has described the development of social play among kittens from its early beginning at 2 weeks, and she has traced the increase in its duration from the 4th week to its decline at the 15th to 19th week. During play kittens have the first opportunity to integrate their emerging motor behavior with sensory stimuli and to educate the CNS about these integrations. Byers (1998, p. 214) notes that "this period of experience-dependent synapse development (*in the cerebellum*) coincided closely with the age distribution of play." Sivyi (1998) goes further in stating "Any benefit which an animal gains from engaging in play behavior is likely to leave some evidence of that benefit in either neuronal structure (e.g., increased dendritic complexity ... or increased number of post-synaptic receptors) or in neuronal functioning (e.g. enhanced pre-synaptic release or increased sensitivity of receptors)" (Bekoff & Byers, 1998, p. 222). Kittens exhibit a wide range of social behaviors during play in which they alternate with their playmates in attacking and retreating, leading and following, sparring and being on bottom and being on top. Kittens, during play, are highly excited with rapidly shifting emotional responses.

The various behaviors employed in play emerge at different ages among kittens. Belly-up and stand up emerge at 3 weeks of age, all the remaining play behaviors appear between 5 and 6 weeks of age (West, 1974). At 6 weeks pouncing, side-stepping, belly-up, and stand-up predominate, and at 12 weeks belly-up and stand-up predominate. These behaviors are all performed at close range: they involve face to face interaction and mutual, often reciprocal behavioral exchanges. At a later age West found that kittens exhibit horizontal leaps off the floor and initiate chasing one another in alternation, covering more ground than earlier. There is a further stage when actual contact between kittens changes and they signal one another at a distance to initiate play. One kitten signals its intention to play by making a move towards another kitten, watching the reaction to determine its next move which is based on this reaction and play activity continues in this manner.

Only kittens that have been playing together, usually siblings, respond to these abbreviated or "intention" movements. When kittens that had been reared alone in isolation from their mother and siblings, during either 23 to 44 days or 2 to 44 days when play is developing among kittens, were returned to their mothers and siblings they were highly disturbed by the playful approaches of their siblings and hissed at them and at the mother when she approached them to investigate (Rosenblatt et al., 1961). They could perform many playful behaviors with objects that attracted their attention while in isolation but they could not play with other kittens.

Kittens depend on the responsiveness of their age-mates to practice the full range of their motor responses. Although object play develops in kittens around the same time as joint kitten play it lacks many of the features of joint kitten play because the object does not move on its own, does not reciprocate the kitten's actions and does initiate actions except when stimulated by the kitten – its movements are mechanical and not within the range of movements made by other kittens.

Play among puppies is initiated around 5 weeks and increases to a maximum at 8 weeks then declines and ends by the 12th week. Play is divided by Bekoff into social play, agonistic (fighting) play and pseudo-sexual play (Bekoff, 1972). Social play occurs throughout the period of play but agonistic play only begins in the 5th week and pseudo-sexual play in the 6th week. Pal (2008) suggests that play decreases at 8 weeks of age because a stable social hierarchy has developed in the litter as a function of agonistic interactions.

Among Belding's ground squirrels juvenile social play consists of the species-typical behaviors of wrestling, tackling, boxing, and mounting and play copulations; non-social play consists of running and climbing (Nunes et al., 2004). Object play is virtually absent in these young. Play arises when juveniles first appear above ground emerging from their underground nests near the time of weaning at 4 weeks of age. It increases over 2 weeks then declines by the end of the 3rd week of play. Play occurs primarily among siblings; there are individually preferred partners among siblings with whom they play four times longer than other siblings. Tests were used to measure improvement in motor skills over the period of play and the amount of play, especially with a larger number of male partners, resulted in significant improvement in motor skills. Object play did not improve over this period.

In view of their initial advanced sensorimotor abilities, among the precocial young of ungulates and semi-precocial young of primates, play behavior has an additional important function beyond sensorimotor learning. They have already performed many of the play behaviors in other contexts before they begin to play. Play has social functions, for example, becoming familiar with age-mates and other member of the social group and establishing hierarchical relationships (Burghardt, 1998). Both ungulates and primates form permanent social groups with hierarchical organizations. Young are introduced into the social group through their association with their mother and play has an important function in this process. Play is a developmental aspect of social behavior and is both a product of the primate "social brain" and contributor to its education (Bateson, 1994; Dunbar & Schutz, 2007).

Gomendio (1988) described the developmental sequence of play in Cuvier's gazelle. Social play between peers becomes the predominant form of play after individual play at an early age declines. Object play is infrequent at all ages, but locomotor play predominates at early ages and play fighting and sexual play become the dominant forms between 4 and 6 months. Sexual play is often directed at adult males and females but is not reciprocated.

The play behavior of young rhesus monkeys from the 3rd to the 6th month was studied by Tartabini (1991). Play consists of chases, wrestling, and rough and tumble behavior. Mothers appear to control infant play indirectly by releasing males to play at an earlier age than females (3–4 months). After that age play occurs more in males than in females and similar aged siblings are preferred for play partners.

Behavioral Development in the Evolutionary Context of the Mother-Young Relationship

The establishment of three basic types for placental mammals: 1) Small and medium sized terrestrial, sub-terrestrial, and aerial species with mainly altricial young; 2) Small to large arboreal and terrestrial species with semi-precocial young; and 3) Large terrestrial and aquatic species with precocial young, dates back more than 80 million years to before the Cretaceous–Tertiary boundary (Kemp, 2004). We can assume, therefore, that the modes of behavioral development of the young associated with their different initial status and trajectories have been stabilized during evolution.

Different ways of characterizing the basic pattern of relationship between the mother and the young have been proposed that imply different selective factors in the evolution of the relationship. Alberts and Gubernick (1983) view it as a symbiotic relationship in which each of the partners obtains from the other what it needs to function: the mother receives stimulation from the young to maintain her maternal responsiveness and her ability to lactate (Rosenblatt, 1992; Stern, 1997) and the young receive sustenance and warmth from the mother and are protected by her. This view places emphasis on the early phase of the relationship, and implies that there has been mutual selection of adaptive responses, but it does not provide a basis for the dissolution of the relationship. Galef (1981) has described the relationship as parasitic. The young parasitize the mother obtaining the warmth, sustenance, and the protection they require and when they outgrow their need for these resources, having developed the ability to maintain themselves, they wean themselves from the mother. However, the relationship between mother and young is quite different from the usual characterizations in biology of symbiosis and parasitism which are relationships between two different species each carrying entirely different genomes.

In the mother-young relationship the young carry a large complement of the mother's genome, therefore, care of her young must be viewed in the context of the mother's inclusive fitness. Because the young also carries the father's genome, the young's inclusive fitness differs from the mother's. Trivers (1974) has proposed that this situation creates a conflict between the interests of the mother in maximizing her inclusive fitness and those of the young in maximizing their own inclusive fitness. Although as Trivers has pointed out the conflict of interests is present from conception (Crespi & Semenuik, 2004; Haig, 1996) it only becomes a source of contention during the weaning period when, according to Trivers, it is in the interest of the mother's inclusive fitness that she terminate her maternal care as early as practicable and it is in the young's interest that it continue to obtain as much of the mother's resources as it can to promote its own inclusive fitness, a situation that has been labeled "weaning conflict."

Crawford and Balon (1996) have been critical of Trivers's theory from the point of view of life history theory. They are concerned with the developmental, structural, physiological and behavioral processes in the parents and the coordinated processes in the young, in response to ecological demands that produce novel and adaptive patterns of parent-offspring interaction. Trivers (1974) is concerned with the features of any parent-offspring interaction which best predict the potential for evolutionary retention and further elaboration of the interaction pattern. That is, he is concerned with natural selection and the how it acts, recognizing, however, that the effects of this selection radiate throughout all organismic functions and systems. From the point of view of Crawford and Balon, Trivers's view is too narrow and selective, and from the point of view of Trivers, their view is too inclusive and imprecise.

Bateson (1994) also has been critical of the weaning conflict concept (he labels it "squabbling" a more neutral term) from two points of view. It has received little experimental support and there is considerable evidence against weaning necessarily involving discord between mother and young. Squabbling may occur as an effort of the young to escape the mother's control as often as it occurs to obtain additional resources from the mother, or there may be no squabbling at weaning (rats: Thiels et al., 1988; vervet monkeys, Hauser & Fairbanks, 1988; gelada baboons, Barat, Dunbar, & Dunbar, 1995; rhesus monkeys, Gomendio, 1991). Bateson (1994) has provided alternate conditions under which the mother withholds resources from the young or provides them. These conditions are related to her own reproductive status and the resources available to her which affect her ability to maintain her current young and to bear additional offspring.

Secondly, he has introduced behavioral considerations absent from the original formulation of weaning conflict theory in which the focus was to present an evolutionary theory of principles of parental investment and inclusive fitness. Trivers assumed that behavioral decisions between the mother and young were derived from the weaning conflict and much of the research on mother-young interactions has been based on this assumption. As Bateson has noted, conflict has not been found as expected during the period of weaning; it is often uneventful and is usually a cooperative enterprise. The mother is ready to adopt a more passive role in the relationship with the withdrawal of offspring stimuli that maintained her maternal responsiveness and lactation and with the occurrence of behavior that is annoying to the mother (e.g., playing, suckling approaches, climbing on her). The young, on their part, have found more interesting features of their world to engage than the mother, and their capacity to maintain themselves enables them to obtain resources from the environment familiar to them during play and through learning guided by the mother. In many species there is a continuing relationship with the mother as the young become integrated into the social group but it is quite different from their relationship to her during their early development.

Our review has shown that under the influence of the mother's nurturance the young gradually develop the capacities for independent feeding, social

responsiveness, and maneuvering in their environment as starting points for further development in adulthood. Weaning conflict is not necessarily manifest in a behavioral conflict between the mother and her young and when it does occur it is for reasons deriving from the behavioral interaction itself. There are many reasons why conflicts between mothers and their offspring occur: they may occur because of excessive mothering as well as maternal neglect and also because of over demanding or prematurely independent offspring.

References

Alberts, J. R. (1978). Huddling by rat pups: Multisensory control of contact behavior. *Journal of Comparative and Physiological Psychology*, **92**, 220–230.

Alberts, J. R., & Gubernick, D. J. (1983). Reciprocity and resource exchange: A symbiotic model of parent offspring relations. In L. A. Rosenblum & H. Moltz (Eds.), *Symbiosis in parent–offspring interactions*. New York: Plenum Press.

Alberts, J. R., & Leimback, M. P. (1980). The first foray: Maternal influences in nest aggression in the weaning rat. *Developmental Psychobiology*, **13**, 417–429.

Altman, J., & Sudarshan, K. (1975). Postnatal development of locomotion in the laboratory rat. *Animal Behaviour*, **23**, 896–920.

Altman, J., Sudarshan, K., Das, G. D., McCormick, N., & Barnes, D. (1975). The influence of nutrition on neural and behavioral development: III. Development of some motor, particularly locomotory patterns during infancy. *Developmental Psychobiology*, **4**, 97–114.

Amsel, A., Burdette, D. R., & Letz, R. (1976). Appetitive learning, patterned alternation, and extinction in 10-d-old rats with non-lactating suckling as reward. *Nature*, **262**, 816–818.

Arias, C., & Chotro, M. G. (2006). Amniotic fluid can act as an appetitive unconditioned stimulus in preweanling rats. *Developmental Psychobiology*, **49**, 139–149.

Arnold, G. W., Wallace, S. R., & Maller, R. A. (1979). Some factors involved in natural weaning processes in sheep. *Applied Animal Ethology*, **5**, 43–50.

Barat, L., Dunbar, R. I. M., & Dunbar, P. (1995). Mother-infant contact as contingent behaviour in gelada baboons. *Animal Behaviour*, **49**, 805–810.

Bateson, P. (1994). The dynamics of parent-offspring relationships in mammals. *Trends in Ecology and Evolution*, **9**, 399–403.

Bateson, P. (2005). The role of play in the evolution of great apes and humans. In A. D. Pelligrini, & P. K. Smith (Eds.), *The nature of play: Great apes and humans*. New York: Guilford.

Bateson, P., Mendl, M., & Feaver, J. (1990). Playing in the domestic cat is enhanced by rationing of the mother during lactation. *Animal Behaviour*, **40**, 514–525.

Beaver, B. V. (1980). Sensory development in *Felis Catus*. *Laboratory Animals*, **14**, 199–201.

Bekoff, M. (1972). The development of social interaction. Play and metacommunication in mammals: An ethological perspective. *Quarterly Review of Biology*, **47**, 412–434.

Bekoff, M., & Byers, J. A. (Eds.). (1998). *Animal play: Evolutionary, comparative, and ecological perspectives*. Cambridge: Cambridge University Press.

Blass, E. M. (1990). Suckling: Determinants, changes, mechanisms, and lasting impressions. *Developmental Psychology*, **26**, 520–533.

Blass, E. M., Kenny, J. T., Stoloff, M., Bruno, J. P., Teicher, M. H., & Hall, W. G. (1979). Motivation, learning, and memory in the ontogeny of sucking in albino rats. In N. E. Spear & B. A. Campbell (Eds.), *Ontogeny of learning and memory*. Hillsdale, NJ: Erlbaum.

Blass, E. M., & Teicher, M. H. (1980). Suckling. *Science*, **210**, 15–22.

Blauvelt, H., & McKenna, J. (1961). Mother-neonate interaction: Capacity of the human newborn for orientation. In B. M. Foss (Ed.), *Determinants of infant behavior*. London: Methuen.

Brake, S. C. (1981). Suckling infant rats learn a preference for a novel olfactory stimulus paired with milk. *Science*, **211**, 506–508.

Broad, K. D., Curley, J. P., & Keverne, E. B. (2006). Mother-infant bonding and the evolution of mammalian social relationships. *Philosophical Transactions of the Royal Society B, Biological Sciences*, **361**, 2199–2214.

Bulut, F. G., & Altman, J. (1974). Spatial and tactile discrimination learning in infant rats motivated by homing. *Devlopmental Psychobiology*, **7**, 465–473.

Burghardt, G. M. (1998). The evolutionary origins of play revisited: Lessons from turtles. In M. Bekoff & J. A. Byers (Eds.), *Animal play: Evolutionary, comparative, and ecological perspective*. Cambridge: Cambridge University Press.

Byers, J. A. (1998). Biological effects of locomotor play: Getting into shape, or something more specific. In M. Bekoff & J. A. Byers (Eds.), *Animal play: Evolutionary, comparative, and ecological perspectives*. Cambridge: Cambridge University Press.

Cheslock, S. J., Sanders, S. K., & Spear, N. E. (2004). Learning during the newborn's first meal: Special resistance to retroactive interference. *Developmental Science*, **7**, 581–598.

Collias, N. (1956). The analysis of socialization in sheep and goats. *Ecology*, **37**, 228–239.

Cramer, C. P., Thiels, E., & Alberts, J. R. (1990). Weaning in rats. 1. Maternal-Behavior. *Developmental Psychobiology*, **23**, 479–493.

Crawford, D. T., & Balon, E. K. (1996). Cause and effect of parental care in fishes: An epigenetic perspective. *Advances in the Study of Behavior*, **25**, 53–107.

Crespi, B., & Semenuik, C. (2004). Parent-offspring conflict in the evolution of vertebrate reproductive mode. *American Naturalist*, **163**, 635–653.

Crighton, G. W., & Pownall, R. (1974). The homeothermic status of the neonatal dog. *Nature*, **251**, 142–144.

Doucet, S., Soussignan, R., Sagot, P., & Schaal, B. (2007). The "smellscape" of mother's breast: Effects of odor masking and selective unmasking on neonatal arousal, oral, and visual responses. *Developmental Psychobiology*, **49**, 129–138.

Dunbar, R. I. M., & Schutz, S. (2007). Evolution of the social brain. *Science*, **317**, 1344–1347.

Eilam, D., Goffman, D., & Smotherman, W. P. (1999). How the neonatal rat gets to the nipple. II. Changes during development. *Developmental Psychobiology*, **34**, 217–226.

Eilam, D., & Smotherman, W. P. (1996). How the neonatal rat gets to the nipple: Common motor modules and their involvement in the expression of early motor behavior. *Developmental Psychobiology*, **32**, 57–66.

Espark, Y. (1969). Mother-young relations and development of behaviour in roe deer (*Capreolus capreolus* L.). *Viltrey*, **6**, 461–540.

Ewer, R. (1959). Sucking behavior in kittens. *Behaviour*, **15**, 146–152.

Frederickson, C. J., & Frederickson, M. H. (1977). Developmental changes in open-field behavior in the kitten. *Developmental Psychobiology*, **12**, 623–628.

Freeman, N. C. G., & Rosenblatt, J. S. (1978a) The interrelationship between thermal and olfactory stimulation in the development of home orientation in newborn kittens. *Developmental Psychobiology*, **11**, 437–457.

Freeman, N. C. G., & Rosenblatt, J. S. (1978b) Specificity of litter odors in the control of home orientation among kittens. *Developmental Psychobiology*, **11**, 459–468.

Fuller, J. L., & Du Buis, E. M. (1962). The behavior of dogs. In E. S. E. Hafez (Ed.), *The behavior of domestic animals*. London: Balliere & Cox.

Galeana, L., Orihuela, A., Aguirre, V., & Vázquez, R. (2007). Mother-young spatial association and its relation with proximity to a fence separating ewes and lambs during enforced weaning in hair sheep (*Ovis aries*). *Applied Animal Behaviour Science*, **108**, 81–88.

Galef, B. G. (1981). The ecology of weaning: Parasitism and the achievement of independence by altricial mammals. In D. J. Gubernick, & P. H. Klopfer (Eds.), *Parental care in mammals*. New York: Plenum Press.

Galef, B. G., & Clark, M. M. (1976). Non-nurturient functions of mother-young interaction in the Agouti (*Dayprocta punctata). Behavioral Biology*, **17**, 255–262.

Galler, J. R. (1979). Home orientation in nursling rats: The effects of rehabilitation following intergenerational malnutrition. *Developmental Psychobiology*, **12**, 499–508.

Gomendio, M. (1988). The development of different types of play in gazelles: Implications for the nature and functions of play. *Animal Behaviour*, **36**, 825–836.

Gomendio, M. (1991). Parent/offspring contact and maternal investment in rhesus macaques. *Animal Behaviour*, **42**, 993–1005.

González-Mariscal, G., & Rosenblatt, J. S. (1996). Maternal behavior in rabbits. A historical and multidisciplinary perspective. *Advances in the Study of Behavior*, **25**, 333–360.

Goodall, J. (1968). The behaviour of free-living chimpanzees in the Gombe Stream Reserve. *Animal Behaviour Monographs*, **3**, 165–311.

Gunther, M. (1961). Infant behaviour at the breast. In B. M. Foss (Ed.), *Determinants of infant behavior*. London: Methuen.

Guyot, G. W., Bennett, T. L., & Cross, H. A. (1980). The effect of social isolation on the behavior of juvenile domestic cats. *Developmental Psychobiology*, **13**, 317–329.

Haig, D. (1996). Placental hormones, genomic imprinting, and maternal-fetal communication. *Journal of Evolutionary Biology*, **9**, 357–380.

Hall, W. G., & Oppenheimer, R. W. (1987). Developmental psychobiology: Prenatal, perinatal, and early postnatal aspects of behavioral development. *Annual Reviews in Psychology*, **38**, 91–128.

Harlow, H. F. (1961). The development of affectional patterns in infant monkeys. In B. M. Foss (Ed.), *Determinants of infant behavior*. London: Methuen.

Harlow, H. F. (1974). Induction and alleviation of depressive states in monkeys. In N. F. White (Ed.), *Ethology and psychiatry*. Toronto: University of Toronto Press.

Harlow, H. F., & Zimmerman, R. R. (1959). Affectional responses in the infant monkey. *Science*, **130**, 421–432.

Hauser, M. D., & Fairbanks, L. A. (1988). Mother-offspring conflict in vervet monkeys: Variation in response to ecological conditions. *Animal Behavior*, **36**, 802–813.

Heiter, F., & Vicente, L. (2008). Maternal care and foal social relationships in a herd of Sorraia horses: Influence of maternal rank and experiences. *Applied Animal Behaviour Science*, **113**, 189–205.

Hersher, L., Richmond, J. B., & Moore, A. U. (1963). Maternal behavior in sheep and goats. In H. L. Rheingold (Ed.), *Maternal behavior in mammals*. New York: John Wiley.

Hinch, G. N., Lescrivain, J. J., Lynch, E., & Elwin, R. L. (1987). Changes in maternal- young associations with increasing age of lambs. *Applied Animal Behaviour Science*, **17**, 305–318.

Hinde, R. A. Mother/infant relations in Rhesus Monkeys. In N. F. White (1974). (Ed.) *Ethology and psychiatry*. Toronto: University of Toronto Press.

Hinde, R. A., & Simpson, M. J. (1975). Qualities of mother-infant relationships in monkeys. *Ciba Foundation Symposium*, **33**, 39–67.

Hinde, R. A., & Spencer-Booth, Y. (1969). The effect of social companions on mother- infant relationships in Rhesus monkeys. In D. Morris (Ed.), *Primate ethology*. New York: Doubleday & Company.

Hinde, R. A., & White, L. E. (1974). Dynamics of a relationship: Rhesus mother-infant ventro-ventro contact. *Journal of Comparative and Physiological Psychology*, **86**, 8–23.

Hirata, H., Nakagawa, M., Funakoshi, H., Iwamoto, T., Otozu, W., & Kiyota, D. et al. (2003). Mother-young distance in Japanese Black cattle at pasture. *Journal of Ethology*, **21**, 161–168.

Hofer, M. A. (1984). Relationships as regulators: A psychobiologic perspective on bereavement. *Psychosomatic Medicine*, **46**, 183–197.

Hofer, M. A. (1994). Early relationships as regulators of infant physiology and behavior. *Acta Pediatrica Supplement*, **397**, 9–18.

Hofer, M. A., Shair, H. N., & Murowchick, E. (1989). Isolation distress and maternal comfort responses of 2-week-old rat pups reared in social isolation. *Developmental Psychobiology*, **22**, 553–566.

Hopkins, W. D., & De Lathouwers, M. (2006). Left nipple preferences in infant *Pan paniscus* & *P. troglodytes*. *International Journal of Primatology*, **27**, 1653–1662.

Hudson, R., & Distel, H. (1983). Nipple location by newborn rabbits: Behavioural evidence for pheromonal guidance. *Behaviour*, **85**, 260–275.

Hull, J., & Hull, D. (1982). Behavioral thermoregulation in newborn rabbits. *Journal of Comparative and Physiological Psychology*, **96**, 143–147.

Ingram, J. C. (1977). Interactions between parents and infants, and the development of independence in the common marmoset (*Callithrix Jacchus*). *Animal Behaviour*, **25**, 811–827.

Ivanitskii, A. M. (1962). The morphological investigation of development of conditioned alimentatary reaction in rabbits during ontogenesis. *Works Higher Nervous Activity, Physiology, Series*, **4**, 126–141.

Jay, P. (1963). Mother-infant relations in langurs. In H. L. Rheingold (Ed.), *Maternal behavior in mammals*. New York: John Wiley.

Johanson, I. B., & Hall, W. G. (1979). Appetitive learning in 1-day old pups. *Science*, **205**, 419–421.

Johanson, I. B., Hall, W. G., & Polefrone, J. M. (1984). Appetitive conditioning in neonatal rats: Conditioned ingestive responding to stimuli paired with oral infusions of milk. *Developmental Psychology*, **17**, 357–381.

Johanson, I. B., Turkewitz, G., & Hamburgh, M. (1979). Development of home orientation in hypothyroid and hyperthyroid rat pups. *Developmental Psychobiology*, **13**, 331–342.

Kaufman, I. C. (1974). Mother/infant relations in monkeys and humans: A reply to Professor Hinde. In N. F. White (Ed.), *Ethology and psychiatry*. Toronto: University of Toronto Press.

Kemp, T. S. (2004). *Origin and evolution of mammals*. Oxford: Oxford University Press.

Kenny, J. T., & Blass, E. M. (1977). Sucking as incentive to instrumental learning in preweanling rats. *Science*, **196**, 898–899.

Kindemann, U., Gervais, R., & Hudson, R. (1991). Rapid odor conditioning in newborn rabbits: Amnesic effect of hypothermia. *Physiology and Behavior*, **50**, 457–480.

Kojima, S., & Alberts, J. R. (2009). Maternal care can rapidly induce an odor-guided preference in rat pups. *Developmental Psychobiology*, **51**, 95–105.

König, B., & Mark, H. (1987). Maternal care in house mice. *Behavioral Ecology and Sociobiology*, **20**, 1–9.

Larson, M. A., & Stein, B. E. (1984). The use of tactile and olfactory cues in neonatal orientation and localization of the nipple. *Developmental Psychobiology*, **17**, 423–436.

Leonard, C. M. (1974). Thermotaxis in golden hamster pups. *Journal of Comparative and Physiological Psychology*, **86**, 458–469.

Lévy, F., Keller, A., & Poindron, P. (2004). Olfactory regulation of maternal behavior in mammals. *Hormones and Behavior*, **46**, 284–302.

Li, R., Fein, S. B., Chen, J., & Grummer-Strawn, L. M. (2008). Why mothers stop breastfeeding: Mothers' self-reported reasons for stopping during the first year. *Pediatrics*, **122**, S69–S76.

Luschekin, V. S., & Shuleikina, K. V. (1989). Some sensory determinants of home orientation in kittens. *Developmental Psychobiology*, **22**, 601–616.

Martin, P., & Bateson, P. (1985). The influence of experimentally manipulating a component of weaning on the development of play in domestic cats. *Animal Behaviour*, **33**, 511–518.

Mermet, N., Coureaud, G., McGrane, S., & Schaal, B. (2007). Odour-guided social behaviour in newborn and young cats: An analytical survey. *Chemoecology*, **17**, 187–199.

Nowak, R. (2006). Suckling, milk and the development of preferences toward maternal cues by neonates: From early learning to filial attachment. *Advances in the Study of Behavior*, **36**, 1–58.

Nunes, S., Muecke, E.-M., Sanches, Z., Hoffmeier, R. R., & Lancaster, L. T. (2004). Play behavior and motor development in juvenile Belding's ground squirrels (*Spermophilus beldingi*). *Behavioral Ecology and Sociobiology*, **56**, 97–105.

Pal, S. K. (2008). Parental care in free-ranging dogs, *Canis familiaris*. *Applied Animal Behavior Science*, **90**, 31–47.

Panksepp, J. (1980). The ontogeny of play in rats. *Developmental Psychobiology*, **14**, 327–333.

Pedersen, P. E., & Blass, E. M. (1982). Prenatal and postnatal determinants of the 1st suckling episode in the albino rat. *Developmental Psychobiology*, **15**, 349–356.

Pellis, S. M., & Pellis, V. C. (1998). Structure-function interface in the analysis of play fighting. In M. Bekoff, & J. A. Byers (Eds.), *Animal play: Evolutionary, comparative, and ecological perspectives*. Cambridge: Cambridge University Press.

Pettijohn, T. (1979). Attachment and separation distress in the infant guinea pig. *Developmental Psychobiology*, **12**, 73–81.

Poindron, P., & Le Neindre, P. (1980). Endocrine and sensory regulation of maternal behavior in the ewe. *Advances in the Study of Behavior*, **11**, 75–119.

Poindron, P., Nowak, R., Lévy, F., Porter, R. H., & Schaal, B. (1993). Development of exclusive mother-young bonding in sheep and goats. *Reviews in Reproductive Biology*, **15**, 311–364.

Poirier, F. E., & Smith, E. O. (1974). Socializing functions of primate play. *American Zoologist*, **14**, 275–287.

Polan, H. J., & Hofer, M. A. (1999). Maternally directed orienting behaviors of newborn rats. *Developmental Psychobiology*, **34**, 269–279.

Polan, H. J., Milano, D., Eljuga, L., & Hofer, M. A. (2002). Development of a rat's maternally directed orienting behaviors from birth to day 2. *Developmental Psychobiology*, **40**, 81–103.

Prechtl, H. R. F. (1952). Angeborne Bewungungweisen jungen Katzen. *Experientia*, **8**, 220–221.

Rheingold, H. L. (1963). Maternal behavior in the dog. In H. L. Rheingold (Ed.), *Maternal behavior in mammals*. New York: John Wiley.

Rheingold, H., & Eckerman, C. (1970). The infant separates himself from his mother. *Science*, **168**, 78–83.

Rheingold, H., & Eckerman, C. (1979). Familiar social and nonsocial stimuli and the kitten's response to a strange environment. *Developmental Psychobiology*, **4**, 71–89.

Richardson, R., Siegel, M. A., & Campbell, B. A. (1988). Effect of maternal presence on the fear response to an unfamiliar environment as measured by heart-rate in rats as a function of age. *Developmental Psychobiology*, **21**, 613–633.

Rosenblatt, J. S. (1965). The basis of synchrony in the behavioral interaction between the mother and her offspring in the laboratory rat. In B. F. Foss (Ed.), *Determinants of Infant Behavior III*. London: Methuen.

Rosenblatt, J. S. (1971). Suckling and home orientation in kittens: A comparative developmental study. In E. Tobach, L. R. Aronson, & E. Shaw (Eds.), *Biopsychology of development*. New York: Academic Press.

Rosenblatt, J. S. (1972). Learning in newborn kittens. *Scientific American*, **227**, 18–25.

Rosenblatt, J. S. (1974). Some features of early behavioural development in kittens. In N. F. White (Ed.), *Ethology and psychiatry*. Toronto: University of Toronto Press.

Rosenblatt, J. S. (1976). Stages in the early development of altricial newborn of selected species of nonprimate mammals. In P. P. G. Bateson, & R. A. Hinde (Eds.), *Growing points in ethology*. Cambridge: Cambridge University Press.

Rosenblatt, J. S. (1979). The sensorimotor and motivational basis of early behavioral development of selected altricial mammals. In N. E. Spear, & B. A. Campbell (Eds.), *Ontogeny of learning and memory*. Hillsdale, NJ: Erlbaum.

Rosenblatt, J. S. (1983). Olfaction mediates developmental transition in the altricial newborn of selected species of mammals. *Developmental Psychobiology*, **16**, 346–375.

Rosenblatt, J. S. (1991). A psychobiological approach to the maternal behaviour among the primates. In P. Bateson (Ed.), *The development and integration of behaviour*. Cambridge: Cambridge University Press.

Rosenblatt, J. S. (1992). Hormone behavior relationships in the regulation of maternal behavior. In J. Becker, D. Crews, & S. M. Breedlove (Eds.), *Behavioral endocrinology*. Cambridge: MIT Press.

Rosenblatt, J. S. (2007a) A tribute to Gilbert Gottlieb: The evolution of an evolutionist. *European Journal of Developmental Psychology*, **1**, 194–199.

Rosenblatt, J. S. (2007b) Gilbert Gottlieb: Intermediator between psychology and evolutionary biology. *Developmental Psychobiology*, **49**, 800–807.

Rosenblatt, J. S., Turkewitz, G., & Schneirla, T. C. (1961). Early socialization in the domestic cat as based on feeding and other relationships between female and young. In B. M. Foss (Ed.), *Determinants of infant behavior*. London: Methuen.

Rosenblatt, J. S., Turkewitz, G., & Schneirla, T. C. (1969). Development of home orientation in newly born kittens. *Transactions of the New York Academy of Sciences*, **89**, 685–700.

Schaal, B., Montagner, H., Hertling, E., Bolzoni, D., Moyse, A., & Quichon, R. (1980). Les stimulations olfactives dans les relations entre l'enfant et la mère. *Reproduction, Nutrition et Dévelopment*, **20**, 843–858.

Schneirla, T. C., Rosenblatt, J. S., & Tobach, E. (1963). Maternal behavior in the cat. In H. L. Rheingold (Ed.), *Maternal behavior in mammals*. New York: John Wiley.

Scott, J. P. (1958). Critical periods in the development of social behavior in puppies. *Psychosomatic Medicine*, **20**, 42–54.

Scott, J. P. (1962). Critical periods in behavioral development. *Science*, **138**, 949–958.

Sczerzenie, V., & Hsiao, S. (1977). Development of locomotion toward home nesting material in neonatal rats. *Developmental Psychobiology*, **8**, 315–321.

Sèbe, F., Nowak, R., Poindron, P., & Aubun, T. (2007). Establishment of vocal communication and discrimination between ewes and their lamb in the first two days after parturition. *Developmental Psychobiology*, **49**, 375–386.

Serra, J., & Nowak, R. (2008). Olfactory preference for own mother and litter in 1-day- old rabbits and its impairment by thermotaxis. *Developmental Psychobiology*, **50**, 542–553.

Shuleikina-Turpaeva, K. V. (1986). Sensory organization of alimentary behavior in the kitten. *Advances in the Study of Behavior*, **16**, 1–37.

Siegel, M. A., Richardson, R., & Campbell, B. A. (1988). Effects of home nest stimuli on the emotional response of preweanling rats to an unfamiliar environment. *Psychobiology*, **16**, 236–242.

Simpson, M. I. A., Simpson, A. E., & Howe, S. (1986). Changes in the rhesus monkey mother-infant relationship through the first four months of life. *Animal Behaviour*, **34**, 1528–1539.

Sireteanu, R. (1985). The development of visual acuity in very young kittens. A study with forced-choice preferential looking. *Vision Research*, **25**, 781–788.

Sivyi, S. M. (1998). Neurobiological substrates of play behavior: Glimpses into the structure and function of mammalian playfulness. In M. Bekoff, & J. A. Byers (Eds.), *Animal play: Evolutionary, comparative, and ecological perspectives*. Cambridge: Cambridge University Press.

Stamps, J. (1995). Motor learning and value of familiar space. *American Naturalist*, **146**, 41–51.

Stanley, W. C., Bacon, W. E., & Fehr, C. (1970). Discriminate instrumental learning in neonatal dogs. *Journal of Comparative and Physiological Psychology*, **70**, 335–343.

Stavy, M., Goldblatt, A., & Terkel, J. (1984). Home odor preferences in young hares (*Lepus capensisis syriacus*): Effects of age and maternal presence. *Developmental Psychobiology*, **18**, 125–139.

Stern, J. M. (1997). Offspring-induced nurturance: Animal-human parallels. *Developmental Psychobiology*, **31**, 335–343.

Stern, J. M., & Johnson, S. K. (1989). Ventral somatosensory determinants of nursing behavior in Norway rats. I. Effects of variations in the quality and quantity of pup stimuli. *Physiology and Behavior*, **47**, 993–1011.

Sullivan, R. M., Brake, S. C., Hofer, M. A., & Williams, C. L. (1986). Huddling and independent feeding of neonatal rats can be facilitated by a conditioned change in behavioral state. *Developmental Psychobiology*, **19**, 625–635.

Sullivan, R. M., Hofer, M. A., & Brake, S. C. (1986). Olfactory-guided orientation in neonatal rats is enhanced by a conditioned change in behavioral state. *Developmental Psychobiology*, **19**, 615–623.

Tartabini, A. (1991). Social play behaviour in young rhesus monkeys Macaca Mulatta at three different ages: From the 3rd to the 6th month. *Behavioural Processes*, **24**, 185–192.

Teicher, M. H., & Blass, E. M. (1976). Suckling in newborn rats. Eliminated by nipple lavage reinstated by pup saliva. *Science*, **193**, 422–425.

Teicher, M. H., & Blass, E. M. (1977). First suckling response of the newborn albino rat: The roles of olfaction and amniotic fluid. *Science*, **198**, 1635–1636.

Thiels, E., Alberts, J. R., & Cramer, C. P. (1990). Weaning in rats: II. Pup behavior patterns. *Developmental Psychobiology*, **23**, 495–510.

Thiels, E., Cramer, C. P., & Alberts, J. R. (1988). Behavioral interactions rather than milk availability determine decline in milk intake of weanling rats. *Physiology and Behavior*, **42**, 507–515.

Trivers, R. L. (1974). Parent-offspring conflict. *American Zoologist*, **14**, 249–262.

Val-Laillet, D., & Nowak, R. (2008). Early discrimination of the mother by rabbit pups. *Applied Animal Behavior Science*, **111**, 173–182.

van Andrichem, G. G. J., Utami, S. S., Wich, S. A., van Hooff, J. A. R. A. M., & Sterck, E. H. M. (2006). The development of wild immature Sumatra orangutans (*Pongo abelii*) at Ketambe. *Primates*, **47**, 300–309.

van Lawick-Goodall, J. (1969). The behaviour of the chimpanzee. In G. Kurth, & I. Eibl-Eibesfeldt (Eds.), *Hominisation und Verhalten*. Stuttgart: Gustav-Fischer.

Varendi, H., Porter, R. H., & Winberg, J. (1994). Does the newborn baby find the nipple by smell? *Lancet*, **344**, 989–990.

Vince, M. A. (1992). The newly born lamb's pattern of activity before, during, and after the first sucking bout. *Applied Animal Behavior Science*, **33**, 27–33.

Vince, M. A. (1993). Newborn lambs and their dams: The interaction that leads to sucking. *Advances in the Study of Behavior*, **22**, 239–268.

Vince, M. A., Lynch, J. J., Mottershead, B. E., Green, G., & Elwin, R. (1985). Sensory factors involved in immediately postnatal ewe/lamb bonding. *Behaviour*, **90**, 60–84.

Weber, E. M., & Olsson, I. A. S. (2008). Maternal behaviour in *Mus musculus* sp.: An ethological review. *Applied Animal Behaviour Science*, **14**, 1–22.

Welker, W. I. (1959). Factors influencing aggregation of neonatal puppies. *Journal of Comparative and Physiological Psychology*, **52**, 376–380.

West, M. (1974). Social play in the domestic cat. *American Zoologist*, **14**, 391–403.

9

Amniotic Fluid as an Extended Milieu Intérieur

Scott R. Robinson and Valerie Méndez-Gallardo

Introduction

La fixité du milieu intérieur est la condition d'une vie libre et indépendante. ("The constancy of the internal environment is the condition for a free and independent life.") (Claude Bernard, 1878; 1974)

In 1854, the great French physiologist Claude Bernard introduced the concept of *milieu intérieur* to biology. This idea advanced the (then) radical proposal that the many functions of physiology in the body served the common purpose of maintaining a stable, life-sustaining environment in the face of fluctuating and challenging conditions in the external world. Although largely ignored in the 19th century, the concept proved very influential to the work of physiologists in the early 20th century, most notably Walter Cannon (1932), who is credited with coining the term and promoting the general concept of *homeostasis*. Homeostasis is the property of a system, and of all living systems, to regulate conditions within the system to maintain constancy in a few, critical variables. Homeostatic regulation within organs, endocrine and neural systems, and behavior has come to be a central organizing principle in modern functional biology, with implications for fields as diverse as genetics, developmental science, and behavioral neuroscience.

The work of naturalists of the 19th century also gave rise to similar concepts of complex networks of interacting biotic and abiotic factors in the environment. This conception of interlocking relationships among animals, plants, fungi, microorganisms, and the geochemical features of an environment eventually grew into the

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modern science of ecology (originally named by Ernst Haeckel in 1866). As homeostasis is applied to individual animals, the idea of ecosystem applies to the biogeochemical cycles and web of relationships that result in stable organism-environment systems. Unlike typical explanations of homeostatic mechanisms, however, ecosystems are not dependent on any central regulator or controller; balance is achieved as an emergent process of the interactions among the parts of the system.

In developmental science, the perspective that change and increasing complexity during the development is multicausal, contingent, and highly interactive also can be traced to roots in the experimental embryology of the 19th century. But the advent of gene theory in the 20th century seemed for many to provide a singular cause – a central controller or regulator – that could account for the intricacies of development. Many who now identify themselves as developmental psychobiologists or developmental systems theorists dispute this conception of development and recognize that the outcome of development is not preformed in the genome (Gottlieb, 1998; Johnston & Edwards, 2002; Oyama, Griffiths, & Gray, 2001). Genes provide an important resource necessary for developmental change, but exert their influence only through interactions with the organism and its environment. One of the cardinal principles of a developmental systems perspective is that organic form, whether anatomy or behavior, emerges in a process of epigenesis, not as a stipulated outcome of a pre-existing blueprint. The complex network of neurons and synapses that constitutes the nervous system does not develop in a vacuum, merely unwrapped from a preformed cocoon. Neither is it the product of scribblings upon a blank slate by an instructive environment. Rather, it is interaction – among the organism in its present state, the orchestrated products of multiple, interdependent genes, the legacies of its nongenetic inheritance, and the resources and constraints of its immediate environment – that lies at the root of organic development at all levels of analysis.

A race car can travel very fast. But are we to attribute the cause of this speed to the engine, or the tires, to the drive train, or the aerodynamic design of the body that reduces drag? The question is meaningless, because no single factor determines the performance of the car. The "essence" of speed is not to be found in any single design feature, but in the harmonious interaction of the engine and drive-train and tires and body, and a hundred other parts. Of course, we also would have no difficulty in recognizing the contributions of different parts. If one set of tires were replaced with another, we could determine on the test track which tires enabled the best performance of a particular car under a given set of driving conditions. A different car, on a different track, under different weather conditions, could exhibit optimal performance with different tires. Thus, the speed of the car is determined by multiple, interacting causal factors.

In the parallel situation of development, we are faced with an outcome – the phenotype of the organism – that is many orders of magnitude more complex than the race car. Studies that have demonstrated heritable influences on behavior, or even identified specific genes, further our understanding of development by calling

attention to important developmental resources that contribute to particular behavioral phenotypes. But showing that the *FOXP2* gene is reliably associated with developmental verbal dyspraxia (a form of specific language impairment) (Lai, Fisher, Hurst, Vargha-Khadem, & Monaco, 2001) or that knockout mice lacking the *fosB* gene exhibit profound deficits in maternal behavior (Brown, Ye, Bronson, Dikkes & Greenberg, 1996) is not evidence that *FOXP2* is the cause of grammar or that *fosB* is the cause of nurturing (Fisher, 2006; Johnston & Edwards, 2002). The challenge for developmental science is to identify resources involved at all levels of analysis – genes, neural activity, behavior, environment – that *co*-act to produce change and guide the path of growth and differentiation toward predictable developmental outcomes (Blumberg, Freeman, & Robinson, 2010; Gottlieb, 1997; Spencer et al., 2009).

The unifying theoretical principles of physiology and ecology – such as homeostasis and ecological niche – have been borrowed and applied to good effect in developmental psychobiology to understand problems in the early development of behavior. Concepts such as ontogenetic adaptation, ontogenetic niche, resource recycling, and homeostatic regulation within the mother-infant dyad, sibling group or age cohort now are well-recognized and widely accepted dimensions of the interplay between developing organisms and their environment, whether in the womb, nest, huddle, or flock (Alberts & Cramer, 1988; West, King, & Arberg, 1988). Yet in few organisms is the seemingly simple distinction between organism and environment more blurred than in the fetus.

In placental mammals, the fetus develops during an extended residence in a house constructed jointly by its mother and itself. Although the tissues of the fetal environment may be of embryonic or maternal origin, nearly all of the raw materials ultimately derive from the mother. In this regard, the fetus is ecologically equivalent to a parasite, such as the gall fly that stimulates its host plant to produce a mass of tissues that provide protection and nourishment for the developing larva. But in the host-parasite relationship, there is rarely a difficulty in distinguishing which tissue is host, and which is parasite. In the maternal-fetus dyad, the placenta represents so intricate a weaving of embryonic and maternal tissue that we can scarcely identify where one ends and the next begins. The chemical, mechanical, and thermal environment of the fetus is virtually co-extensive with that of the mother. And the immediate physical and sensory environment of the fetus, which consists of the fluid that surrounds it during gestation, is inextricably the joint product of mother and fetus. Unlike the common misconception of the prenatal environment as static and unchanging, where the fetus grows in a vegetative existence, research has revealed the intrauterine world to be dynamic and continually changing, presenting the fetus with a complex array of sensory stimuli in multiple modalities (Lecanuet, Fifer, Krasnegor & Smotherman, 1995; Smotherman & Robinson, 1988b). Recognizing the complexity of the prenatal environment, and the intimate relationship between the fetus and its surroundings, presents the developmental researcher with a significant conundrum in under-

standing how that environment contributes to normal neurobehavioral development.

The First Environment: The Behavioral Relevance of Amniotic Fluid

The prenatal environment can be conceptualized as a series of concentric envelopes or barriers that restrict and regulate exposure of the fetus to the external environment. Most distal are features of the pregnant mother: the abdominal wall, peritoneal space, internal organs and uterus. The uterus consists of an outermost membrane (perimetrium), a layer of smooth muscle that serves as the muscles of labor and a source of spontaneous contraction during gestation (myometrium), and a thick, vascularized inner layer that provides a locus for implantation and the placental interface between mother and fetus (endometrium). The fetus is joined to the placenta by the umbilical cord, which comprises one vein and two arteries that carry oxygen and nutrients to the fetus and waste products back to the mother. Within the uterus, the fetus is surrounded by extraembryonic membranes, most notably the chorion and amnion, which together constitute the amniotic sac that creates a space around the fetus. This amniotic space is filled with the *liquor amnii*, also known as amniotic fluid (AF). In species that bear multiple offspring, each fetus develops within its own compartment, separated from siblings by AF and the amniotic sac, with a separate placental attachment to the uterus. The exception to this rule is some monozygotic twins, about two-thirds of which share a common placenta (monochorionic) and 1–2% share the same amniotic space (monoamniotic) (Bomsel-Helmreich & Al Mufti, 2005). Thus in rats, which typically give birth to a dozen or more offspring and can deliver litters of up to 30 pups, the prenatal environment consists of an array of smaller microenvironments and not a single, homogenous milieu.

AF and the Fetus: A System of Dynamic Regulation

AF represents perhaps the most complex, dynamic, and variable feature of the prenatal environment. AF begins as a filtrate from maternal plasma, and therefore is derived from chemical components in maternal blood that diffuse across the placenta. These may include basic nutrients, oxygen, other constituents of the maternal diet, hormones, alcohol and drugs, anesthetics and antibiotics, environmental toxins, heavy metals and other teratogenic chemicals, and other biological molecules. Permeability of the placenta to various compounds is strongly affected by placental anatomy, which present barriers of different thickness that can impede transfer. The most intimate connections between fetal and maternal circulation are

found in rodents and primates, which have a hemochorial placenta; the thickest barriers are found in ungulates, such as sheep, which have an epitheliochorial placenta. Transfer across the placenta also is affected by the overall size and surface area of the placenta, rates of blood flow in both maternal and fetal circulation, concentration gradients, and chemical characteristics of the compound. As a rule of thumb, compounds with a molecular weight less than 5,000 daltons can cross the placenta by simple diffusion, eventually entering the amniotic compartment (Blackburn, 2007). Other biologically important compounds are transmitted more efficiently via facilitated diffusion (e.g., glucose, iron, ascorbic acid) or active transport (e.g., ATP, sodium, calcium). But placental discontinuities that permit direct contact between maternal and fetal blood can allow much larger compounds, including antibodies, viruses, and whole cells, to pass to the fetus.

Compounds transferred across the placenta are only one source of the chemical composition of AF. Within the uterus, AF also originates in the physiology of the placenta, umbilical cord, embryonic membranes, and the skin, lungs, and kidneys of the fetus (Abbas & Tovey, 1960; Manning, 1995; Tam & Chan, 1977; Wirtschafter & Williams, 1957). Early in gestation, the water content and composition of AF is determined mainly from placental transfer and AF composition is similar to fetal plasma. Later in gestation, fetal behavior and physiology plays a dominant role as the fetus actively swallows and inhales AF and urinates into AF. Fluid volume is not determined solely as a passive equilibrium of diffusion and secretion. It is now broadly accepted that the fetus plays an important role in regulating its own fluid volume through the behaviors of swallowing and micturition (Ross & Brace, 2001; Ross & Nijland, 1997). Fetal swallowing is the major source of water loss and turnover in the fetus, although a significant volume of AF is removed by transfer from the amniotic cavity into the circulatory vessels within the fetal membranes (Gilbert, Newman, Eby-Wilkens & Brace, 1996). Fetal micturition is the major source of AF in late gestation, with additional contributions from excretion of oral, nasal, tracheal and pulmonary fluids (Gilbert & Brace, 1993). Thus, the composition of AF is the combined product of maternal diet and physiology, and the physiology and behavior of the fetus. The result of this interplay is that the composition of AF is both complex and dynamic, regulated yet variable, containing a broad assortment of phospholipids, carbohydrates, proteins, and bioactive molecules, including hormones that can influence the development of neighboring fetuses in the womb (Meisel & Ward, 1981; Ryan & Vandenbergh, 2002; vom Saal & Bronson, 1980), as well as metabolites and cellular debris that can aid the clinician in diagnosing the fetus's developmental status (Manning, 1995; Mauri & Volpe, 1994; Underwood, Gilbert, & Sherman, 2005).

AF provides a medium for fetal behavior, a fluid space in which fetal movements can occur. Evidence from fetuses with neurologic abnormalities suggests that AF reduces the effort required for fetal movement. For example, fetuses with major myelomeningocele can express normal movement *in utero*, but exhibit weakness

and motor impairment after delivery (Manning, 1995). Too little AF during gestation (oligohydramnios) can result in movement restriction (Sival, Visser, & Prechtl, 1990), which is associated with a syndrome of developmental abnormalities. Fetuses that experience a lack of movement (akinesia) for a protracted period can develop various malformations, such as microstomia and micrognathia (small mouth and jaw), thin, brittle skin, and facial dysmorphia. Movement restriction also can result in immobilized joints and altered bone growth (Moessinger, 1983), malformations of the extremities, such as clubfoot or malrotation of the hand (Manning, 1995), or spinal flexion (Albuquerque et al., 2002). Because AF is both swallowed and inspired by the fetus (Bradley & Mistretta, 1973; El-Haddad, Desai, Gayle, & Ross, 2004; Lev & Orlich, 1972; Marsh, King, & Becker, 1963; Ross & Nijland, 1997), it also contributes to the physical development of the mouth and palate, the gastrointestinal tract, and the lungs (Moessinger, 1983; Nimrod, Varela-Gittings, Machin, Campbell, & Wesenberg, 1984). Movement is important for normal fetal development, and regulation of AF volume is an important influence on fetal motor activity.

AF and the Perinate: Attraction and Preference

Because AF represents the immediate environment where the fetus develops prenatally, a number of investigators have questioned whether prenatal experience would lead to recognition of AF as a familiar, and even preferred, sensory stimulus. Research has confirmed that newborn mammals of several species express an attraction to AF and show a preference for the odor of AF over other novel or familiar odors, or no odor at all. Even though exposure to AF is restricted to the prenatal period and the brief period of labor and delivery, it still can exert effects on postnatal behavior for days after birth. For example, rats, lambs, piglets, and human newborns can detect the odor of AF just minutes to hours after birth. Lambs tested 1 hour after birth showed a preference for AF by orienting longer to a cotton pad moistened with the odor of AF than to one with distilled water (Schaal, Orgeur, & Arnould, 1995). Newborn piglets similarly oriented preferentially toward birth fluids or sow's milk over water (Parfet & Gonyou, 1991). Human neonates tested 2 days after birth oriented their nose to the odor of AF for a longer period than to a control stimulus of distilled water (Schaal, Marlier, & Soussignan, 1995). Studies such as these demonstrate that newborns prefer the odor of AF over the alternative of no odor. But newborns not only show a preference for AF over a control stimulus, they also are capable of recognizing and preferentially orienting toward their own AF compared to AF collected from a different, non-familiar pregnancy. This capacity has been documented in rats (Hepper, 1987), lambs (Schaal, Orgeur, et al., 1995), and human newborns (Schaal, Marlier, et al., 1995). This ability to discriminate between familiar versus unfamiliar AF has been suggested as one basis for the olfactory recognition of kin (Hepper, 1987; Robinson & Smotherman, 1991),

which has been documented in many mammalian species (Hepper, 1991; Tang-Martinez, 2001).

Another example of the attractive properties of AF suggests that it is important for the newborn infant to find and attach to the nipple. Newborns of a number of mammalian species depend on olfactory cues to locate the nipple. Some of these cues are specialized chemical signals produced by the lactating mother and secreted in the vicinity of the nipple, such as the waxy exudate in the inguinal region of lactating ewes (Vince & Ward, 1984), the nipple search pheromone on the ventrum of rabbits (Hudson & Distel, 1983; Schaal et al., 2003), and perhaps the exudate of areolar skin glands of the human breast (Schaal, Doucet, Sagot, Hertling, & Soussignan, 2006). However, in other cases, external olfactory cues are behaviorally applied to the nipple to facilitate nipple search and attachment by the newborn. Like many other mammals during parturition, rats engage in extensive licking directed at themselves and the newborn offspring during delivery, which has the effect of distributing AF over the ventrum of the mother. In a study conducted by Teicher and Blass (1977), washing the nipples of rats after labor resulted in no nipple attachment by their offspring. But attachment to the nipple was reinstated after AF was painted on the nipples of an anesthetized rat. AF may play a similar role in the human newborn. In a study conducted only minutes after birth, human newborns were placed in a prone position on their mother's chest. One breast was treated with AF collected during delivery, while the other remained untreated. Results showed that significantly more newborns selected a breast treated with AF over an untreated breast (Varendi, Porter, & Winberg, 1996). Given the widespread practice of cradling the infant to the chest after delivery, transfer of AF to the area of the breasts, thereby aiding nipple search, may be as natural for human mothers as self-directed licking is for rats.

The findings described above suggest that AF is recognized by the newborn, and can direct crucial aspects of infant behavior, such as finding the nipple, during the first minutes or hours after birth. However, some research suggests that the behavioral significance of AF may extend well beyond the perinatal period, and that AF may represent a characteristic that helps prepares the fetus for life outside the womb. For instance, Benoist Schaal has suggested that there is both chemical and behavioral continuity between AF and breast milk (Schaal, 2005). In a study conducted with breast-fed infants tested in a two-choice head turning paradigm, 2-day-old infants did not show a preference between AF and their mother's colostrum. In contrast, 4-day-olds showed a distinct preference by orienting more toward colostrum (Marlier, Schaal, & Soussignan, 1998b). However, in a different study where bottle-fed infants (i.e. infants fed formula milk) were tested in a similar paradigm, 2-day-old and 4-day-old infants both preferred AF over formula milk (Marlier, Schaal, & Soussignan, 1998a). These findings imply that infants shift from a preference for AF to a preference for maternal milk after more extensive suckling experience, but that experience with infant formula is inadequate to promote the shift away from AF. Another inference that may be drawn from these results is that

both AF and colostrum share chemical characteristics that may result in olfactory similarity (they are not discriminated by 2-day-olds), but AF and infant formula do not (Schaal, 2005).

AF and the Infant: Consequences of Prenatal Learning

Although the odor preference for AF demonstrated by newborn rats, sheep, and humans may suggest that prenatal sensory experience results in recognition of familiar odor characteristics in AF, attraction to AF could easily be the result of unlearned responsiveness to specific chemical constituents in AF, analogous to the nipple search pheromones identified in sheep (Vince & Ward, 1984) and rabbits (Schaal et al., 2003). To test the hypothesis of fetal learning, a variety of experimental approaches have been developed over the past three decades to manipulate the chemosensory environment of the fetus and to evaluate the behavioral consequences after birth. These studies have been the focus of a number of earlier reviews (Chotro, Arias, & Laviola, 2007; Hepper, 1996; Robinson & Smotherman, 1991; Schaal & Orgeur, 1992; Smotherman & Robinson, 1987b, 1998), so will be summarized only briefly here.

Prenatal olfactory experience has been indirectly manipulated by introducing novel flavor compounds into the maternal diet or intubating a solution of the flavor into the mother's stomach during pregnancy, or by directly injecting the flavor into the AF of individual fetuses during gestation. The robust finding of such studies has been that prenatal exposure to novel flavor cues produces lasting olfactory memories that are retained after birth to influence general orientation, behavioral activity, and ingestive preferences of newborn, juvenile and adults. Table 9.1 provides a summary of relevant studies, including methods used for prenatal flavor exposure and behavioral assessment after birth. This learning phenomenon appears to be supported by mere exposure to the novel taste or odor cue during gestation, without the experimenter-controlled unconditioned stimuli, rewards, or punishments characteristic of classical and operant conditioning, and has been referred to operationally as sensory exposure learning (Robinson & Smotherman, 1991; Schaal, 2005).

Indirect exposure learning is well illustrated by an early study by Hepper (1988). Female rats were fed a clove of garlic as part of their diet during days E15 to E21 of gestation (birth occurs on embryonic day 22 of gestation, or E22, in rats). Their offspring then were tested in a two-choice shuttle-box at 12 days after birth. Pups prenatally exposed to garlic spent more time over a dish containing garlic than at the opposite end of the box, over a dish containing onion (Hepper, 1988). A similar finding was reported in humans after mothers ingested anise-flavored sweets and cookies during the last 2 weeks of gestation. Infants prenatally exposed to anise in their mother's diet showed a clear preference for anise odor, displaying positive hedonic responses and orientation toward the odor, unlike infants lacking prenatal

Table 9.1. Studies of fetal exposure learning, based on prenatal chemosensory exposure and postnatal behavioral assessment

Species	*Prenatal exposure*			*Postnatal testing*				*Citation*
	Stimulus presented	*Method of exposure*	*Age of exposure*	*Stimulus presented*	*Age of testing*	*Method of assessment*	*Results*	
Rat (*Rattus norvegicus*)	Apple juice	Direct injection into AF	Day 20	Apple juice vs. water	Day 60 after birth	Two-bottle preference drinking test	Greater intake of apple juice	Smotherman, 1982
	Citral	Direct injection into AF	Day 20	Citral vs. nothing	Day 1 after birth	First nipple attachment	Pups attached to nipple coated with citral	Pedersen, & Blass, 1982
	Garlic	Females fed garlic	Day 15 to Day 21 of gestation	Garlic vs. onion	Day 12 after birth	Double-choice paradigm: Pups moved toward a side	Preference for garlic	Hepper, 1988
	Ethanol	Direct injection into AF	Day 21 of gestation	Ethanol and lemon	Day 8 after birth	Odor preference test and intake test	Preference for ethanol odor and greater intake	Chotro, & Molina, 1990
	Ethanol	Maternal intragastric intubation	Day 17 to Day 20 of gestation	Ethanol, water, sucrose, quinine, and sucrose mixed with quinine	Day 14 after birth	Intake test	Greater intake of ethanol and sucrose mixed with quinine	Domínguez, López, & Molina, 1998

(*Continued*)

	Ethanol	Maternal intragastric intubation	Day 17 to Day 20 of gestation	Ethanol, sucrose + quinine, water	Day 14 after birth	Taste reactivity test and intake test	More ingestive responses and greater intake of ethanol	Arias, & Chotro, 2005
Rabbit (*Oryctolagus cuniculus*)	Juniper berries	Females fed juniper berries	From day 15 of pregnancy until day 28 after birth	Juniper berries vs. lab chow or water	Day 28 after birth	Feeding preference test	Preference for juniper berries after prenatal and postnatal exposure	Bilkó, Altbäcker, & Hudon, 1994
	Juniper berries	Females fed juniper berries	From mid gestation	Juniper berry odor vs. nothing	Day 1 after birth before first suckling	Double-choice olfactory test in an arena	Preference for the odor of juniper berries	Semke, Distel, & Hudson, 1995
	Black cumin	Females fed food and water mixed with cumin	From day 17 of pregnancy until day 4 after birth	Placenta, colostrum, garlic, and cumin	Between day 1 and day 3 after birth	Two-choice olfactory test and oral activation test (searching and grasping)	Preference for cumin in all tests	Coureaud, Schaal, Hudson, Orgeur, & Coudert, 2002
Domestic dog (*Canis lupus familiaris*)	Aniseed	Females fed diet containing aniseed	Last 20 days of gestation	Aniseed vs. water, vanilla vs. water	Day 1 after birth	Head turning preference test	Pups oriented their head toward the odor of aniseed	Wells, & Hepper, 2006

Table 9.1 (*Continued*)

Species	*Prenatal exposure*			*Postnatal testing*				*Citation*
	Stimulus presented	*Method of exposure*	*Age of exposure*	*Stimulus presented*	*Age of testing*	*Method of assessment*	*Results*	
Human	Anise	Mothers fed anise flavored food	Week 39 and 40 of gestation	Anethole (pure anise flavor) diluted in paraffin oil vs. paraffin oil	Day 1 and Day 4 after birth	Oral and facial responses and Head orientation	Less negative facial expressions and head orientation toward anise	Schaal, Marlier, & Soussignan, 2000
	Carrot juice	Mothers drank carrot juice	Last trimester of pregnancy and first 2 months of lactation	Cereal with carrot juice vs. cereal with water	5 mo after birth	Negative facial responses and mother's reports	Less negative facial expression for carrot cereal	Mennella, Jagnow, & Beauchamp, 2001
Pig (*Sus domestica*)	Garlic and Anise	Females fed diet containing garlic or anise	Last month of gestation and during lactation	Garlic and Anise in food vs. normal food	Days 3 and 10 after weaning	Feed intake	Greater feed intake by piglets weaned at 6 weeks and after prenatal exposure	Langendijk, Bolhuis, & Laurenssen, 2007
Lamb (*Ovis aries*)	Oregano essential oil	Females fed diet containing oregano essential oil	Between days 50 and 130 of pregnancy	Food with oregano, orange or eucalyptus essential oils	Day 45 after birth	Feeding preference test	Preference for food with oregano essential oil	Simitzis, Deligeorgis, Bizelis, & Fegeros, 2008
	Citral	Females fed diet containing citral	Last 2 weeks of pregnancy	Citral vs. AF	Day 1 after birth	Head turning preference test	No preference	Schaal, Orgeur, & Arnould, 1995

exposure to anise who showed aversion or neutral responses (Schaal, Marlier, & Soussignan, 2000). With variations in methods of exposure or behavioral testing, comparable findings have been reported after introducing novel flavors into the diet of pregnant dogs (Wells & Hepper, 2006), rabbits (Bilkó, Altabäcker, & Hudson, 1994; Coureaud, Schaal, Hudson, Orgeur, & Coudert, 2002; Semke, Distel, & Hudson, 1995), sheep (Simitzis, Deligeorgis, Bizelis, & Fegeros, 2008), pigs (Langendijk, Bolhuis, & Laurenssen, 2007), and humans (Mennella, Jagnow, & Beauchamp, 2001).

Methods of indirectly exposing fetuses to novel flavors by manipulating maternal diet have been effective in demonstrating fetal exposure learning, but they do not constitute proof that AF plays a role in fetal learning. It is plausible that fetuses gain chemosensory access to flavor additives introduced into the maternal diet via AF; human observers have reported detecting garlic odor in AF samples after maternal ingestion of garlic in both sheep (Nolte, Provenza, Callan, & Panter, 1992) and humans (Mennella, Johnson, & Beauchamp, 1995). However, olfactory sensation can be evoked by intravascular odorants in adult rats (Maruniak, Silver, & Moulton, 1983), and learning can be supported by exposure to blood-borne odorants alone (Maruniak, Mason, & Kostelc, 1983). The possibility that fetuses also may detect intravascular odorants suggests an alternative mode of prenatal exposure to constituents of maternal diet: olfactory compounds could cross the placenta to pass from maternal to fetal circulation, then diffuse directly into capillary beds within the olfactory epithelia or taste buds to give rise to chemosensation.

Although prenatal experience with blood-borne odorants remains possible, albeit untested, there is considerable empirical support for fetal learning of flavor compounds directly injected into AF. In one of the first experiments of its kind, Pedersen & Blass (1982) exposed near-term rat fetuses to citral, a lemon scent, by injecting it directly into the AF in the vicinity of the mouth. Other offspring were exposed to the odor of citral after caesarean delivery into a warm incubator environment, and a third group was exposed to citral both *in utero* and immediately after delivery. Pups that had been exposed to citral before and after birth were attracted to nipples of an anesthetized rat that were painted with citral odor, but pups that had prenatal exposure or postnatal exposure, but not both, showed no significant preference. The experimental result led the authors to conclude that general olfactory characteristics of AF learned by the fetus may be sufficient to direct search and attachment to the nipple during the first suckling episode (Pedersen & Blass, 1982). In contrast, Mickley reported that injection of a novel taste cue (saccharin) into the AF of fetal rats on E19 was sufficient to promote enhanced licking and mouthing upon saccharin reexposure to fetuses on E21 or to neonatal rats on P3, without additional postnatal experience (Mickley, Remmers-Roeber, Crouse, Walker, & Dengler, 2000). Smotherman (1982) found even longer persistence of a prenatal chemosensory memory after injecting apple juice into the AF of fetal rats on E20. When tested as adults 60 days after birth, rats that had been

exposed to apple juice in AF consumed more water flavored with apple juice than unflavored water in a two-bottle preference test. Other studies have reached similar conclusions that a single direct injection of a novel flavor into AF is sufficient to alter behavior of fetuses reexposed to the flavor later in gestation (Smotherman & Robinson, 1988a) or infants after birth (Mickley, Remmers-Roeber, Crouse, & Peluso, 2000).

These experimental findings have obvious implications for the prenatal programming of dietary preferences that may influence feeding in the infant and adult. But another line of research has extended the relevance of prenatal chemosensory experience to a clinical domain. Most nonhuman animals, such as rats, show aversion responses to alcohol and consume small volumes of dilute alcohol solutions unless water deprived. Early experience with the sensory characteristics of alcohol, however, appears to alter hedonic reactions to ethanol odor and increase amounts of consumption. The relationship between prenatal exposure and postnatal responsiveness to alcohol was noted in correlational studies of mothers that ingested alcohol during pregnancy and their children that displayed hyperactivity disorders and a propensity for alcohol abuse (Morrison & Stewart, 1971). Although such correlations have been interpreted as evidence of a genetic risk for alcoholism, an early experiment demonstrated that the progeny of rats that were fed a diet containing alcohol throughout gestation showed behavioral hyperactivity and increased ethanol intake when tested as adults (Bond & Di Giusto, 1976). This original demonstration has led to a rich literature reporting the effects of prenatal alcohol exposure on postnatal responsiveness to alcohol, notably in the laboratories of Juan Molina (Molina, Chotro, & Domínguez, 1995), Norman Spear (Spear & Molina, 2005), and M. Gabriela Chotro (Chotro, Arias, & Laviola, 2007). This research program has consistently found evidence that prenatal exposure to ethanol results in improved palatability and higher consumption of alcohol during infancy, adolescence, and adulthood. Although the pharmacological and teratogenic effects of alcohol may contribute to some of these prenatal effects, several studies have demonstrated that fetuses learn about the chemosensory qualities of alcohol during prenatal exposure. This conclusion was most dramatically demonstrated in experiments in which pups exposed to alcohol during gestation displayed increased intake of a mixture of sucrose and quinine, which mimics the psychophysical qualities of alcohol to human tasters (Domínguez, López & Molina, 1998; López and Molina, 1999). Prenatal exposure learning therefore may contribute to the nongenetic transmission of maladaptive behavior, such as alcohol addiction, as well as to suckling, dietary preference, and other functionally important behavior.

So what is the relevance of AF for prenatal exposure learning? The most direct conclusion may be that because fetuses can learn about novel olfactory cues *in utero*, then the postnatal preference demonstrated by various newborn mammals for the odor of AF may simply be the result of familiarity with unique odor characteristics of AF learned before birth. The preference for AF may be no

different than any other learned flavor preference. But does AF play any other role in supporting or facilitating the formation of these potent, long-lasting chemosensory memories? This is a question we will return to later in this chapter.

AF and the Mother: Parturition and Pain

AF also has significance beyond the fetus and newborn to facilitate the transition of birth. Newly parturient mothers of many species are attracted to the odor of the birth membranes, placenta, and AF. Sheep, for example, are repulsed by the odor of AF except during a narrow window of a few hours around the time of birth, when it is strongly attractive (Lévy, Poindron, & Le Neindre, 1983). Newborn lambs are covered with birth fluids, and parturient ewes are likely to accept a lamb as their own if it is coated in AF (Lévy & Poindron, 1987). But if newborn lambs are washed, removing the odor of AF, primiparous ewes reject them and fail to express maternal care (Lévy, Keller, & Poindron, 2004). Not surprisingly, ewes are most attracted to their own, live lambs. But when various models were tested, combining the characteristics of warmth, movement, accompanied by bleats, or covered with AF, the least attractive model was a dummy lamb lacking AF, while the most attractive model was a white bowl containing warm AF (Vince, Lynch, Mottershead, Green, & Elwin, 1985). Similar findings that AF is attractive to new mothers and promotes acceptance of offspring have been reported in rabbits (González-Mariscal et al., 1998), dogs (Abitbol & Inglis, 1997; Dunbar, Ranson, & Buehler, 1981); and primates (Brandt & Mitchel, 1971; Lundblad & Hodgen, 1980).

Newly parturient mothers not only are attracted to the odor of AF, but actively consume the placenta and embryonic membranes, along with AF, during or immediately after the birth process. This phenomenon, termed placentophagia, is typical of nearly all non-aquatic mammalian species (Kristal, 1980). Early explanations for this distinctive behavior emphasized specific hunger of the mother (noteworthy because herbivorous species exhibit this momentary shift in dietary preference toward carnivory), general hunger induced by the exertions of labor, maintenance of sanitary conditions at the site of birth, or recycling of nutrients no longer needed by the offspring. However, research over the past two decades has revealed that ingestion of placenta or AF has more immediate consequences for the mother's physiology and behavior. Although placentophagia is not necessary for mothers to express caregiving behavior, consumption of placenta or AF accelerates the onset of maternal care (Kristal, 1991). In rats, ingestion of placenta also potentiates responsiveness to morphine, suggesting a relationship between AF and the opioid system (Kristal, Thompson, & Grishkat, 1985). For this reason, Kristal has argued that one function of placentophagia may be to enhance or modulate antinociception during and after parturition. The placenta is ingested after birth, of course, so ingestion of the placenta could not contribute to pain

reduction during parturition. But rats and many other animals engage in active licking of the vaginal area during labor, and after rupture of the amniotic sac, females could ingest substantial amounts of AF. In fact, AF is more potent in its ability to potentiate morphine effects than placenta (Kristal, Thompson, & Abbott, 1986).

AF is known to contain endogenous opioids (Houck, Kimball, Chang, Pedigo, & Yamanura, 1980; Kofinas, Kofinas, Pyrgerou, & Reyes, 1987; Petrucha, Goebelsmann, Hung, Haase, & Lobo, 1983; Valette et al., 1986). However, ingested placenta or AF do not appear to produce antinociptive effects directly in the adult rat, and only potentiate the effects of exogenously administered opioid drugs (Kristal, 1991). Therefore, the putative opioid enhancing factor (dubbed "POEF" by Kristal), is unlikely to be an opioid compound itself. The analgesic effects of AF on adult rats normally follows ingestion of AF and placenta, and intragastric intubation of AF is sufficient to produce the response, indicating that the orosensory characteristics of AF do not mediate the effects of POEF. After gastric intubation, effects on morphine-induced analgesia were evident within 5 min and persisted for at least 30 min (Doerr & Kristal, 1989). Subcutaneous or intraperitoneal injection of AF did not produce effects, however, suggesting that AF must be processed within the digestive tract to exert analgesic effects in parturient rats (Abbott et al., 1991). This was confirmed in an experiment in which gastric vagotomy blocked the effects of ingested AF (Tarapacki, Thompson, & Kristal, 1992), which was likely due to disruption of vagal afferents (Robinson, Abbott, & Kristal, 1995). Finally, POEF is believed to influence central opioid systems by selectively enhancing activity at the δ (delta) and κ (kappa) subclasses of opioid receptors, but attenuating activity at μ (mu) receptors (DiPirro & Kristal, 2004). These studies all confirm the behavioral potency of AF and implicate the endogenous opioid system in mediating some of the effects of AF, at least in the adult rat.

Mechanisms of AF Effects on Behavior

Opioid Effects on Perinatal Behavioral Development

Several lines of research suggest that the endogenous opioid system (comprising endorphins, enkephalins, dynorphins, and their associated receptors) may play a central role in mediating experience-based changes in neurobehavioral organization during prenatal and neonatal life (Kehoe, 1988; Robinson & Smotherman, 1995; Smotherman & Robinson, 1992a). *In vitro* experiments have suggested that natural opioid ligands may modulate the activity of neurotrophic chemicals, such as nerve growth factor (NGF) or brain-derived growth factor (BDGF), or may directly function as growth factors to influence processes of nerve fiber outgrowth and synapse formation (Kozlova & Kalenchuk, 1994; Perez-Navarro, Alberch,

Arenas, & Marsal, 1993; Zagon & McLaughlin, 1987; Zagon, Verderame, & McLaughlin, 2002). Chronic antagonism of opioid receptors before birth has demonstrated effects on the density of dendrites and synapses in regions of the forebrain, brainstem, and spinal cord (Shepanek, Smith, Anderson, & Medici, 1995; Shepanek, Smith, Tyer, Royall, & Allen, 1989; Zagon & McLaughlin, 1984). Moreover, intermittent blockade of opioid receptors produces effects on neural development that differ from continuous blockade, suggesting that the temporal pattern and not just the presence of opioid activity may be important in structuring neural development (Hauser, McLaughlin, & Zagon, 1989). Endogenous opioids also can modulate activity in both sensory and motor elements of the CNS (Barr, 1992). Endogenous, patterned activity in the opioid system during prenatal development therefore is likely to influence activity-dependent processes that are responsible for synapse formation and elimination (Huberman, Feller, & Chapman, 2008; Sanes & Lichtman, 2001; Shatz, 1990; Thompson, 1983; Waites, Craig, and Garner, 2005).

Endogenous ligands and their mRNA precursor proteins have been identified in the CNS of fetal and neonatal rats for all three major divisions (μ, κ, ∂) of the opioid system (Leslie & Loughlin, 1993). Receptors of two divisions – μ and κ – appear to be functional and capable of modulating behavior in the rat fetus over the last few days of gestation (Attali, Saya, & Vogel, 1990; DeVries, Hogenboom, Mulder, & Schoffelmeer, 1990; McDowell & Kitchen, 1987; Petrillo, Tavani, Verotta, Robson, & Kosterlitz, 1987; Rahman, Dashwood, Fitzgerald, Aynsley-Green, & Dickenson, 1998). A third class – ∂ receptors – appears to develop during the second postnatal week, and therefore may play little or no role in perinatal behavior. Measurement of receptor binding *in vitro* has identified both μ and κ opioid receptors in rat brain (Petrillo et al., 1987; Spain, Roth, & Coscia, 1985) and spinal cord (Attali et al., 1990; Kirby, 1981; Rahman et al., 1998) at the time of birth. Although the developmental timing and abundance of different receptor types varies greatly across different CNS structures, in general, the density of μ receptors undergoes steady growth through the perinatal period, but κ receptors appear to exhibit a paradoxical bimodal distribution in density, with one peak occurring around E20 of gestation and the second 1–2 weeks after birth (Attali et al., 1990; Rahman et al., 1998). This bimodal pattern may suggest different populations of κ-opioid bearing neurons that play different functional roles during prenatal and postnatal development. Stimulation of opioid receptors by exogenous administration of selective agonist drugs has confirmed that μ and κ opioid systems are functional and capable of influencing both sensory responsiveness and motor behavior in the fetal and neonatal rat (Barr, 1992; DeVries et al., 1990; Smotherman, Simonik, Andersen, & Robinson, 1993).

The opioid system has been implicated in a wide range of effects on behavior in developing animals, including antinociception and the moderation of responses to pain, sedation or activation of motor activity, regulation of parent-offspring interactions, responsiveness to suckling stimuli, independent feeding, and learning

(Barr, 1992; Kehoe, 1988; Nelson & Panksepp, 1998; Smotherman & Robinson, 1992d). Of particular interest for our investigations into the developmental significance of AF, however, are the relationships between milk and the opioid system, and the ability of milk or exogenous opioids to support both classical and operant conditioning in perinatal animals.

Milk-Induced Opioid Activity in the Infant Rat

Milk is an especially salient biological factor for newborn animals. Infant mammals are not the passive recipients of milk from their lactating mothers, but are active participants that display a suite of coordinated behaviors with the function of searching for, locating, approaching and attaching to a nipple, and systematically stimulating the mammary gland to release milk, which is then extracted and swallowed by the infant (Blass & Teicher, 1980; Hall, 1990). The details of suckling behavior and the various sensory and neural controls over suckling vary among different species. But research has clearly demonstrated that suckling is not merely an infantile form of later feeding behavior, but rather a specialized form of ingestive behavior that is governed by different stimuli and feedback mechanisms than adult feeding (Hall & Rosenblatt, 1977; Hall & Williams, 1983). The objective of this unique behavioral control system is milk, which provides much more than nutrients, water, and electrolytes for the infant mammal. Milk also contains antibodies that can transmit resistance to disease from mother to offspring, hormones and neuroactive peptides that affect the infant's physiology and behavior, environmental toxins to which the mother has been exposed, and chemosensory cues derived from maternal diet (Hasselquist & Nilsson, 2009; Mennella & Beauchamp, 1991, 1998; Nickerson, 2006). Milk contributes to the development of cardiac regulation and organization of behavioral states (Brake, Shair, & Hofer, 1988), and as in the examples of AF discussed above, flavors in mother's milk can influence subsequent dietary preferences in offspring (Beauchamp & Mennella, 2009; Bilkó et al., 1994; Galef & Sherry, 1973; Nolte & Provenza, 1992). Indeed, several researchers have called attention to many of the striking similarities between AF and milk, suggesting a deep, developmental continuity between the two biological fluids (Marlier et al., 1998a, 1998b; Mennella & Beauchamp, 1998; Schaal, 2005; Smotherman & Robinson, 1992d).

Milk also is a potent behavioral stimulus for the infant. For example, milk has been shown to produce a variety of behavioral effects in infant rats, ranging from expression of species-typical action patterns unique to the suckling context, such as the stretch response (Lau & Henning, 1985) and sustained attachment to a nipple (Petrov, Varlinskaya, Bregman, & Smotherman, 1999), to general behavioral activation (Hall, 1979), calming (Blass, 1996) and reduction of responsiveness to noxious stimulation (Blass & Fitzgerald, 1988; Blass, Jackson, & Smotherman, 1991). Although the initial cues used by infant rats and other animals to

locate and attach to the nipple typically involve other olfactory, thermal and cutaneous stimuli, infants are exposed to literally hundreds of pairings of milk with other cues in the context of suckling, and numerous studies have shown that infant animals and humans can recognize and orient toward the odor of milk (Delaunay-El Allam, Marlier, & Schaal, 2006; Marlier & Schaal, 2005; Nolte & Provenza, 1992). Infant rats exposed to the odor of milk paired with a novel odor quickly learn the association and exhibit a conditioned preference for the paired odor (Brake, 1981; Johanson & Hall, 1982; Johanson & Teicher, 1980; Johanson & Terry, 1988), general behavioral activation (Terry & Johanson, 1987), specific consummatory responses to the conditioned odor (Johanson, Hall, & Polefrone, 1984), and sustained attachment to a non-nutritive artificial nipple (Cheslock, Varlinskaya, Petrov, & Spear, 2000). Infusion of milk into the mouth also can serve as a positive reinforcer to support operant conditioning in the infant rat. Rat pups 1–5 days old can learn to press their head or forelimbs against a paddle or touch-sensor to deliver a small infusion of milk reinforcement (Arias, Spear, Molina, & Molina, 2007; Domínguez, Bocco, Chotro, Spear, & Molina, 1993; Johanson & Hall, 1979), and can learn to discriminate between a paddle that provides milk and one that does not (Johanson & Terry, 1988).

Although it would oversimplify the results of so many different studies to attempt to find a single shared cause, convergent evidence suggests that endogenous opioid activity may play a role in mediating many of the behavioral effects of milk (Blass, 1996; Smotherman & Robinson, 1992d). Opioid involvement initially was identified in the antinociception evoked by oral infusion of milk, which could be reversed by administration of a non-selective opioid antagonist, such as naloxone or naltrexone. Blockade of opioid receptors with naloxone, for instance, was effective in reducing the latency for 10-day old pups to withdraw a paw from a noxious thermal stimulus (Blass & Fitzgerald, 1988). The same result was found for rat pups tested within a few hours after caesarean delivery (Blass et al., 1991), which suggested that postnatal suckling experience was not necessary for milk to exert its antinociceptive effects.

Milk-Induced Opioid Activity in the Rat Fetus

The conclusion that behavioral effects of milk need not be learned during suckling was extended by a series of studies in which milk was experimentally presented to rat fetuses tested *in vivo*, which precluded any influence of postnatal suckling or maternal behavior. In many of these experiments, fetal sensory responsiveness was assessed with a simple behavioral bioassay: facial wiping behavior. On the last two days of gestation (E20-21), fetal rats reliably express a facial wiping response to various forms of stimulation directed to the perioral area. A novel chemosensory fluid, such as lemon odor extract, when infused into the mouth of the fetus, elicits a flurry of activity that involves repeated forelimb strokes in which the forepaws pass

over the face, from ear to nose (Smotherman & Robinson, 1987a). A single, unilateral paw-face stroke also can be elicited by applying a punctate tactile stimulus, such as a stiff bristle, to the lateral vibrissal area of the fetus (Smotherman & Robinson, 1990, 1992a). The facial wiping response is developmentally continuous with face-washing behavior expressed during grooming and aversion responses in postnatal animals (Berridge & Fentress, 1986; Johanson & Shapiro, 1986). We have found that facial wiping evoked either by a perioral cutaneous stimulus or an intraoral infusion of lemon provides a robust measure of fetal sensory responsiveness, which is sensitive to stimulus parameters, such as concentration, volume, and rate of infusion (Brumley & Robinson, 2004), and to the behavioral and pharmacological status of the perinatal subject (Smotherman & Robinson, 1989, 1992a).

The effect of milk on the fetal wiping responses was initially assessed by infusing a small volume of milk into the mouth of the E20 rat fetus. This simple sensory exposure nearly eliminated wiping responses to either a tactile stimulus or a test infusion of lemon (Robinson & Smotherman, 1994). Other chemosensory fluids, including saline, sucrose, lactose, or corn oil (Smotherman & Robinson, 1992b), or other stimuli associated with postnatal suckling behavior, such as exposure to an artificial nipple that is effective in eliciting oral grasping and sucking responses (Robinson, Arnold, Spear, & Smotherman, 1993), were ineffective in reducing the fetal facial wiping response. This suggests that there is some specific characteristic or constitutent in milk that alters fetal sensory thresholds or its ability to generate a coordinated motor response. Previous work with both rats and human infants suggested that the key element was the ability of milk to trigger activity in the endogenous opioid system.

Pharmacological manipulation of the opioid and other neurochemical systems of the fetus can exert a marked impact on the expression of facial wiping behavior (Korthank & Robinson, 1998; Smotherman & Robinson, 1992a). For example, administration of a non-selective opioid agonist, such as morphine, or selective agonists of μ or κ opioid receptors (DAMGO and U50,488, respectively), sharply reduce the incidence of facial wiping responses to a perioral tactile stimulus (Robinson, Moody, Spear, & Smotherman, 1993; Smotherman, Moody, Spear, & Robinson, 1993). As mentioned above, the same effect of reducing responsiveness in a facial wiping test can be achieved by brief, intraoral infusion of milk. This behavioral effect of milk is reversed, however, if subjects are pretreated with the opioid antagonist naloxone. Blockade of κ-opioid receptors with the selective antagonist nor-binaltorphimine (BNI) also suppresses the effects of milk and restores high levels of facial wiping. But blockade of μ receptors with the selective antagonist CTOP or ∂ receptors with naltrindole is not effective (Korthank & Robinson, 1998; Smotherman & Robinson, 1992a; Smotherman et al., 1994). Most recently, we have replicated these findings after birth by presenting milk to neonatal rats on P1 (Méndez-Gallardo & Robinson, in press). These findings imply that milk can selectively engage the κ-opioid system of the fetus and neonate to alter responsiveness to sensory stimulation.

AF-Induced Opioid Activity in the Rat Fetus

Why should the fetus be responsive to milk, a fluid to which it would never be exposed during normal intrauterine development? Evidence from our laboratory indicates that AF also can engage the κ-opioid system of the fetus, resulting in diminished responses to chemosensory and tactile stimuli (Korthank & Robinson, 1998). In a standard testing protocol, E20 rat fetuses were prepared by removal from the uterus and embryonic membranes (to prevent contact with their own AF) and were suspended within a temperature-regulated saline bath for behavioral testing (Smotherman & Robinson, 1991). After preparation for behavioral testing, individual fetal subjects were pretreated by intraperitoneal injection of an opioid agonist or antagonist, and 5-min later were exposed to an intraoral infusion of isotonic saline (Sal) or AF. A test infusion of lemon then was delivered 60 s after the exposure stimulus to evoke a facial wiping response. As summarized in Figure 9.1 (left), control subjects, which were injected with saline and exposed to Sal before the lemon test, consistently showed multiple facial wiping strokes to the test infusion of lemon. Subjects injected with the saline vehicle and exposed to AF, however, performed fewer wiping strokes. The effect of AF to reduce wiping responses was

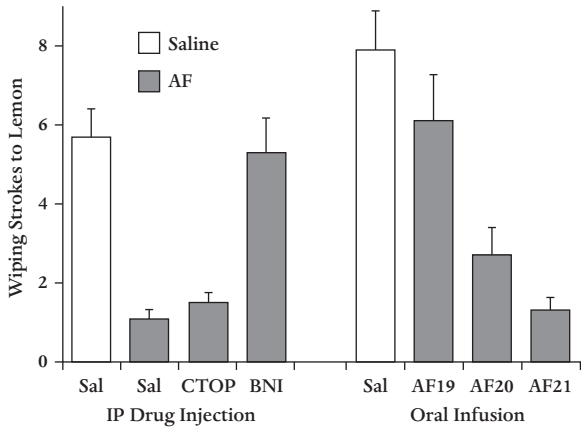

Figure 9.1. Facial wiping responses (mean number of wiping strokes ± SEM) elicited by a test infusion of lemon in various pretreatment and exposure conditions. E20 rat fetuses received an intraoral infusion of isotonic saline (white bars) or AF (black bars) and 60 s later received a second oral infusion of lemon to evoke a facial wiping response. (Left) Five min before the initial exposure infusion, fetal subjects were pretreated by IP injection of isotonic saline (Sal), the μ-opioid antagonist CTOP, or the κ-opioid antagonist BNI. The κ antagonist was effective in blocking the effect of AF and reinstating high levels of facial wiping in the lemon test. (Right) E20 fetal subjects received an exposure infusion of saline or AF collected at one of three gestational ages: E19, E20, or E21. Note that AF20 and AF21 reduced fetal wiping responses, but AF19 did not differ from saline.

not affected by pretreatment with the μ antagonist CTOP, but was completely reversed with the κ antagonist BNI. Further, administration of a range of doses of the κ agonist U50,488 produced a dose response effect on facial wiping that mimicked the effects of AF. These data strongly indicate that AF engages the κ opioid system of the fetus to alter behavioral responses to the chemosensory stimulus.

AF Effects on Spatiotemporal Motor Organization

During the late prenatal period, fetal rats exhibit spontaneous motor activity that is characterized by synchrony of movement, sequential organization, and temporal patterning. Movements tend to be brief and can be treated as discrete events for temporal analysis. Although the fetal rat expresses motor activity continuously from E16 through term (E22), the number of movements fluctuates with time and involves different regions of the body. The scoring procedures employed in our laboratory record every instance of fetal movement and the time of occurrence ($\pm$ 0.1 s) of each event, creating a continuous time series of movement data. An example of the application of these techniques can be seen with fetal subjects prepared for observation on E20 of gestation. Individual fetuses were videotaped in a 30-min session, and tapes were replayed for scoring individual movements of each limb. Spectral analysis of overall motor activity (the sum of movement events of all limbs) revealed that movements varied in a cyclical pattern, with an average frequency of 0.5 to 2.0 cycles per minute (cpm). Log-survivor analysis also indicated an over-abundance of brief intervals ($<$ 5 s) between successive movements, confirming a strong tendency for movements to occur in clusters or bouts of activity. Cyclicity and bout structure have been reported in previous studies of embryonic, fetal and neonatal motor behavior (Robertson, 1987; Robinson, Blumberg, Lane, & Kreber, 2000; Smotherman, Robinson, & Robertson, 1988), and appear to be a general characteristic of the spontaneous movements expressed during early motor development.

However, analysis of motor set organization has identified a more subtle form of spatiotemporal patterning during spontaneous fetal movement that is ignored by conventional time series techniques (Robinson & Smotherman, 1992a). Events in a time series occur in a specific sequence; by examining the events in a short sequence (e.g., a window of 10 successive events), one can obtain a coarse estimate of the relative abundance or probability of any particular event, such as hindlimb movement (abbreviated P_r). The 10-event window then is advanced iteratively through the time series, creating a second time series of the change in P_r. By identifying a criterion threshold to parse the time series into continuous epochs of high P_r activity (above threshold) and low P_r activity (below threshold), an estimate can be obtained of average epoch duration, a measure of motor set organization (Figure 9.2a). To determine whether the epoch durations observed in a particular time series reflect real organization, the time series of movement events is

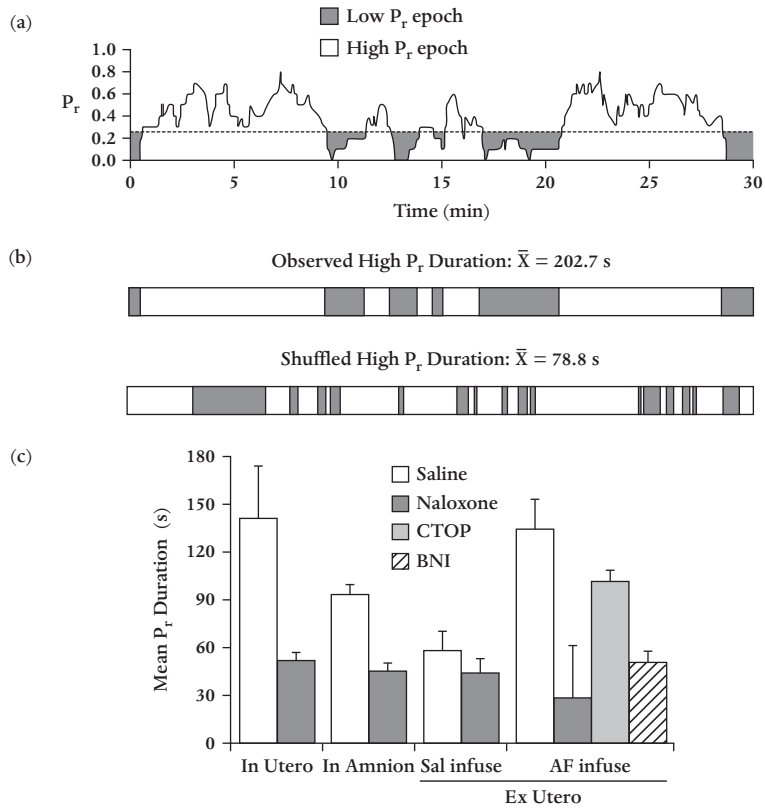

Figure 9.2. Motor set organization influenced by AF-induced opioid activity in the E20 rat fetus. (A) Representative time series of the probability of hindlimb activity (P_r) calculated within a moving 10-event window during a 30-min observation period. White areas depict fluctuating estimates of P_r during ongoing motor activity. Segments when P_r is above a criterion value of 0.3 (shown as a horizontal dashed line) represent periods of relatively abundant hindlimb activity; other segments (shaded gray) depict periods when P_r is below criterion. (B) The time series is parsed into epochs above the criterion threshold (High P_r, shown as white areas) and epochs below threshold (Low P_r, shown as gray areas). In this example, the mean duration of High P_r epochs is 202.7 s. By shuffling the sequence of movement events and inter-event intervals in the observed time series, a randomized time series provides an estimate of P_r if hindlimb movements are randomly distributed (mean duration = 78.8 s). (C) Mean duration (± SEM) of high P_r motor sets fetal rats observed in utero, in amnion, or ex utero after IP injection of saline (white bars), naloxone (black bars), the μ-opioid antagonist CTOP (gray bars), or the κ-opioid antagonist BNI (hatched bars). Fetuses in utero or in amnion remained surrounded by their own AF; fetuses ex utero received a continuous infusion of saline or AF into the mouth. High P_r epochs were significantly longer in duration when fetuses were exposed to AF, and were shorter when κ receptors were antagonized with NAL or BNI.

randomly shuffled in sequence, preserving the distribution of intervals between events, and motor set epochs measured in the resulting shuffled time series (Figure 9.2b). This method has proven most useful in characterizing temporal shifts in the topography of fetal movement, revealing that forelimb and hindlimb movements are nonrandomly distributed in time (Robinson & Smotherman, 1992a). Motor set organization appears to be a general characteristic of perinatal behavior, and we have documented it in the spontaneous activity of fetal rats, myoclonic twitching during active sleep in infant rats, and EMG recordings of forelimb and hindlimb muscles in the chronically instrumented fetal sheep.

Motor set organization originally was described from the spontaneous movements of rat fetuses that had been externalized from the uterus for behavioral observation (Robinson & Smotherman, 1992a). But experiments in our laboratory suggest that features of the intrauterine environment may actively promote this form of behavioral organization. Fetuses that remain within the uterus (*in utero*), or are externalized from the uterus but remain within the intact amniotic sac during a 30-min observation period (*in amnion*), exhibit high P_r epochs of significantly longer duration than fetuses tested outside of the uterus, after removal of the membranes, in a supportive, osmotically neutral bath environment (*ex utero*). This enhancement of motor set organization *in utero* appears to be the result of fetal exposure to AF. Fetal rats that are externalized from the uterus and amniotic sac but which receive a slow, continuous infusion of AF through an intraoral cannula (400 μl/hr), exhibit epochs of high P_r activity that are similar to motor sets expressed by fetuses *in utero* or *in amnion*, and nearly three times longer than saline-infused or untreated controls subjects tested *ex utero* (Figure 9.2c). Put more succinctly, oral exposure to AF results in longer periods of elevated hindlimb activity. This effect of AF infusion on fetal motor activity also appears to be opioid dependent. Pretreatment with naloxone, or the selective κ antagonist BNI, blocks the effects of AF and reduces the duration of high P_r epochs during AF infusion (Figure 9.2c). This pattern of results strongly implies that a constituent or characteristic of AF can evoke activity in the κ-opioid system of the E20 rat fetus, which in turn produces a change in central nervous system activity and thereby alters the spatiotemporal organization of fetal motor activity.

Ontogeny of AF-Induced Opioid Responses

The foregoing experiments strongly indicate that fetal exposure to AF results in κ-opioid activity, which exerts effects on both sensory responsiveness and the organization of motor activity. It is important to keep in mind, however, that these results hold only for E20 fetuses exposed to AF collected from other pregnancies on E20 of gestation. Presentation of AF collected at different gestational ages has revealed that AF varies in its ability to elicit opioid responses in the E20 fetus

(Figure 9.1, right). Fetal exposure to AF collected at E20 or E21 is effective in reducing fetal responses to a test infusion of lemon, but AF collected on E19 does not differ from saline. This finding implies that the constituent responsible for evoking an opioid response – the κ-opioid inducing factor or KIF – is present on the last two days of gestation in the rat, but is absent in AF at earlier ages. This developmental pattern is consistent with arguments that AF and milk share a developmental continuity, and that one of the functions of AF in general, and of the opioid-inducing effects of AF in particular, is to facilitate the behavioral transition from prenatal to postnatal life.

One strategy for testing whether the opioid-inducing qualities of AF are related to the timing of birth is to examine the evolutionary continuity of AF. Through collaboration with P. W. Nathanielsz (initially at Cornell University, later at the University of Texas Health Science Center at San Antonio), we have obtained samples of AF collected from fetal sheep (*Ovis aries*) and fetal baboons (*Papio* sp.) at various ages during the last trimester of gestation: E108–143 in sheep (gestation length is about 145 days), and E130–175 in fetal baboons (gestation length is about 180 days). Unlike rats, which give birth to relatively immature altricial offspring, these two species bear offspring that are precocial, exhibiting more mature, adultlike physiology and behavior, as well as nervous system development, at the time of birth. By presenting samples of AF collected from these species to fetal rats, we hoped to understand whether the ability of AF to evoke opioid responses was a general characteristic of mammalian fetal development. Indeed, exposure to sheep AF collected at certain ages did reduce wiping responses of fetal rats to lemon (Figure 9.3). However, in contrast to the late expression of AF-induced opioid activity in rats, AF collected from fetal sheep affected fetal behavior only when collected earlier in gestation (E108-E123). Samples collected closer to term had no effect on wiping responses of fetal rats. Presentation of baboon AF to fetal rats produced a similar pattern of data: AF collected before E150 was effective in promoting opioid-mediated reductions in facial wiping responses to lemon, but AF collected at later ages was ineffective. Moreover, the behavioral effects of sheep AF collected on E113 and baboon AF collected on E145 (ages when AF was effective in evoking an opioid response) were reversed by pretreatment with naloxone (white bars) or BNI (hatched bars), but not CTOP (gray bars), confirming that sheep AF can evoke activity in the κ-opioid system of the fetal rat (Figure 9.3, right).

To address the crucial question of whether these findings from various non-human animals are relevant to human fetal development, we also have tested a series of samples of AF collected from human subjects over a range of gestational ages. These samples, which were provided through collaboration with the Cytogenetics Laboratory in the Department of Pediatrics at the University of Iowa Hospitals and Clinics, were obtained during routine amniocentesis screening exams during weeks 15–23 of gestation, and during term delivery. Exposure to human AF collected at certain ages, when experimentally presented to fetal rats, was effective in reducing wiping responses to lemon (Figure 9.3). Moreover, the pattern of expres-

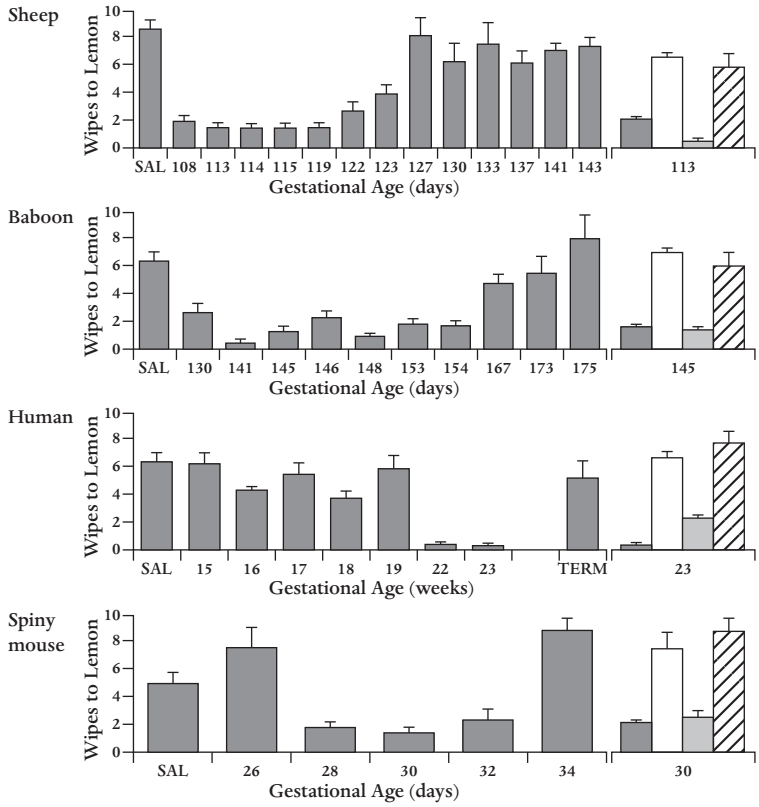

Figure 9.3. Facial wiping responses (mean number of wiping strokes ± SEM) of E20 rat fetuses elicited by a test infusion of lemon after exposure to AF collected from fetal sheep, baboons, humans or spiny mice. (Left) Effects of AF from four species collected at various gestational ages. (Right) Wiping responses of fetal rats exposed to AF of four species after pretreatment no injection (black bars), or pretreatment by IP injection of naloxone (white bars), CTOP (gray bars), or BNI (hatched bars). Samples of AF showed evidence of evoking opioid activity at some gestational ages in all four species, which was blocked with the -opioid antagonist.

sion of AF-induced opioid activity was consistent with the predicted pattern of development: samples collected earlier than week 20 were ineffective in reducing the responsiveness of fetal rats to a test infusion of lemon, as were the samples of AF collected at term (week 39). However, samples collected on weeks 22 or 23 of gestation were effective in reducing fetal responses to lemon, and the behavioral effect of AF at week 23 was completely blocked by pretreatment of rat fetuses with the opioid antagonist naloxone or the selective κ-opioid antagonist BNI (Figure 9.3, right). These findings confirm that AF collected from a range of mammalian species has the ability to evoke opioid responses in the fetus, but the qualities that engage the

opioid system are evident only at certain points in gestation. Because opioid responses were observed near term in an altricial species (rat), but at earlier points in gestation in more precocial species (sheep, baboon, human), a general developmental pattern appears to be a transient period of AF-induced opioid activity. This window of AF-induced opioid activity is not associated with the time of birth, as we previously assumed. Rather, it appears to be better correlated with the relative maturity of the central nervous system, which also is shifted to earlier points in gestation in precocial species (Clancy, Darlington, & Finlay, 2001).

To test this hypothesis, we obtained AF samples from another rodent species – the spiny mouse (*Acomys cahirinus*) – that is closely related to Norway rats, but which exhibits a precocial rather than altricial mode of reproduction (Brunjes, 1990). These samples were collected in collaboration with Jeffrey Alberts and Jill Villarreal (*nee* Menge) at Indiana University. Because spiny mice give birth to relatively mature offspring, which are capable of walking and opening eyes on the day of birth, we predicted that the period of AF-induced opioid activity would be shifted earlier in gestation, more closely resembling the pattern of sheep and baboons than their closer relative, Norway rats. Samples were collected on E26, 28, 30, 32, and 34 of the 37-day gestation. On the basis of behavioral characteristics, E20 rat fetuses most closely resemble fetal spiny mice on E28 (Robinson, 1989; Robinson & Smotherman, 1992b). Consistent with this shift in the timing of development, samples of AF collected from spiny mice on E28, E30, and E32, when experimentally presented to fetal rats, reduced facial wiping responses to lemon, but AF samples from earlier (E26) or later (E34) in gestation were ineffective. Moreover, the effects of AF from E30 spiny mice were reversed by pretreatment with naloxone or the κ-opioid antagonist BNI, but not the μ-opioid antagonist CTOP, confirming that the behavioral effect was mediated by the κ-opioid system (Figure 9.3, right). This developmental pattern corroborates the hypothesized pattern that AF can engage the endogenous opioid system only during a transient window during gestation.

Fetal Oral Exposure To AF and the Time Course of AF-Induced Opioid Activity

It is possible that the active constituent in AF is a biologically active compound that must be ingested and later absorbed to engage receptors of the κ-opioid system. However, the opioid response to AF is both rapid and short lasting, unlike what one might expect from a post-ingestive effect. The time course of opioid responses elicited by AF is shown in Figure 9.4 (left). Rat fetuses on E20 received two intraoral infusions, AF and lemon. When lemon was delivered before AF (0 s delay), normal facial wiping responses were observed. The number of wiping strokes to lemon was reduced significantly only 15 s after exposure to AF, however, and was maximally suppressed 60 and 120 s after AF infusion. At 180 s, fetuses showed some indication of recovery of responsiveness to the lemon infusion, and wiping responses were not

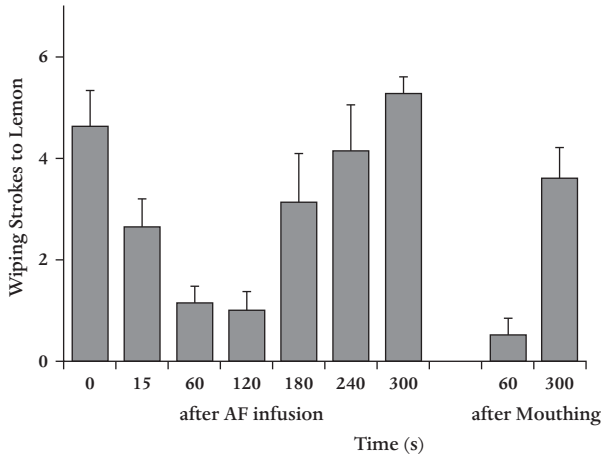

Figure 9.4. (Left) Time course of AF effects on facial wiping to a test infusion of lemon. E20 rat fetuses received the lemon infusion immediately before exposure to AF (0 s) or at one of six delays after exposure to AF. The maximal reduction in facial wiping responses was evident 60 and 120 s after AF exposure, and recovery was complete by 240 s. (Right) Wiping responses of fetuses to lemon presented at two delays after the most recent mouth movement in amnion. The suppression in facial wiping at the 60 s delay is consistent with the interpretation that fetal oral activity exposes the fetus to AF, which alters fetal responding to a chemosensory challenge.

diminished relative to controls by 240 s. This time course corresponds almost precisely to the reported time course of opioid effects after oral presentation of milk (Robinson & Smotherman, 1994). The time course implies that fetuses may experience only a brief period of opioid activity, lasting less than 5 min, after oral exposure to AF.

An obvious objection to the general pattern of AF-induced opioid activity in the fetus is that the fetus is continuously surrounded and bathed by AF in utero. Wouldn't that mean that fetuses experience continuous activation of the opioid system, or alternatively, that they eventually habituate to the active agent in AF and cease to show opioid responses to AF? In other words, if the opioid response evoked by a single pulsatile infusion of AF is short-lasting, does the near-term fetus show continuously elevated levels of opioid activity or intermittent spikes of activity? Although AF is continually present within the amniotic cavity, fetuses may be functionally exposed to AF only during periods of oral activity, which leads to swallowing and breathing of AF (Bradley & Mistretta, 1973; Marsh et al., 1963; Ross & Nijland, 1997). Before the skin becomes keratinized, water and solutes may pass directly between the fetus and the extraembryonic space through the skin. After keratinization, however, which undergoes rapid development around 20–24 weeks in the human (Hardman, Moore, Ferguson, & Byrne, 1999) and about E19–20 in the rat (Aszterbaum, Menon, Feingold, & Williams, 1992), active agents in AF must be

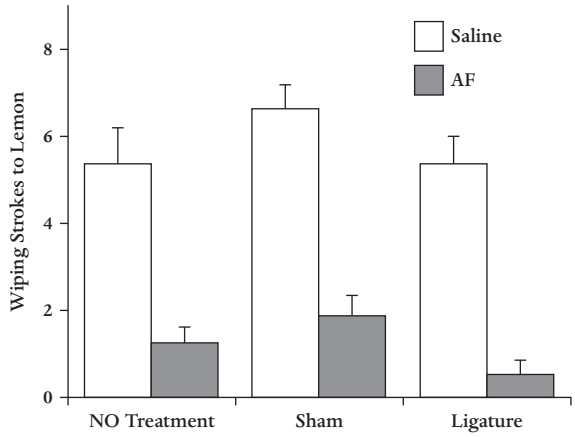

Figure 9.5. Oral mediation of the effects of AF after preparation of fetal subjects with a ligature occluding the esophagus and trachea. Control subjects received no pretreatment or a sham treatment; experimental subjects were prepared with a ligature that prevented swallowing or inspiration of fluids. Bars depict facial wiping responses (mean number of wiping strokes ± SEM) of fetal rats elicited by a test infusion of lemon after exposure to saline or AF. The ligature did not affect the ability to perform facial wiping, as indicated by high levels of wiping after exposure to saline. But both ligated and control subjects showed reduced wiping responses after exposure to AF. Surgical examination of fetal subjects after the experiment failed to reveal the presence of a dye introduced into AF in the stomach or lungs, confirming that fetuses were exposed to AF only in the oral cavity.

taken into the mouth to gain access to non-keratinized tissue in the mouth, pharynx and olfactory epithelia, the lungs, or the GI tract. To confirm the necessity of oral exposure, E20 fetal rats were prepared with a ligature occluding the esophagus and trachea to prevent swallowing and breathing (Robinson & Smotherman, 1994). As shown in Figure 9.5, oral exposure to AF after esophageal/tracheal ligature continued to suppress facial wiping responses to lemon.

Of course, if oral activity were frequent enough, fetal exposure to AF would still approach continuous exposure. But mouthing movements are expressed sporadically during spontaneous motor activity in the rat fetus (Smotherman & Robinson, 1986), and in sheep, humans, and other species (Hepper, Shannon, & Dornan, 1997; Ross & Nijland, 1997). In the rat, mouthing activity increases late in gestation, coincident with a decline in AF volume (Marsh et al., 1963; Robinson & Brumley, 2005). Moreover, fetuses that are externalized from the uterus and amniotic sac show higher levels of mouthing, and consequently briefer intervals between successive mouth movements, than fetuses in amnion. In fact, we found that periods of time occurring at least 180 s after the last mouthing movement constituted more than 17% of the time spent by fetuses in amnion. (Recall that the time course of AF effects is such that opioid responses begin to wane after 3 min; see

Figure 9.4.) These findings suggest that if AF induces opioid activity only after oral exposure, then fetuses probably experience waxing and waning periods of activity in the opioid system during normal development.

To test the hypothesis that fetal rats are exposed to the opioid-inducing qualities of their own AF only through oral activity, fetuses were prepared for observation within the intact amniotic sac and received an intraoral infusion of lemon at one of two specified delays after the most recent mouth movement. The shorter delay (60 s) was well within the time course of opioid responses evoked by AF infusion, but the longer delay (300 s) was greater than required for complete recovery from the effects of AF infusion (Figure 9.4, left). When tested within the amnion, surrounded by their own AF, fetuses showed sharply reduced facial wiping responses to lemon 60 s after a mouth movement, consistent with an expected opioid response evoked by AF exposure. But significantly more wiping strokes were evident when lemon was presented 300 s after mouthing (Figure 9.4, right), when the fetus should have recovered from a transient opioid response to AF. These findings are therefore consistent with the interpretation that fetal oral activity results in intermittent, short-lasting periods of activity in the endogenous opioid system during the last few days of gestation in the rat fetus.

Clues to the Opioid-Inducing Factor in AF

As discussed above, AF is a complex biological fluid, containing an assortment of inorganic salts, phospholipids, carbohydrates, proteins, hormones, metabolites, and cellular debris. Although numerous reports have described changes in the volume and composition of AF during gestation, only a handful of specific constituents are currently viewed as having general clinical significance, such as fetal cells in AF cultured to provide karyotype and other genetic information, alpha-fetoprotein concentration, which is an indicator of neural tube defect, and phospholipid profile, which is an indicator of pulmonary maturity (Manning, 1995). More subtle chemical indicators of fetal behavioral function and development have not yet been identified or well characterized. However, based on the available literature and previous work in our laboratory, three general classes of compounds could be present in AF that are potentially capable of inducing activity in the fetal opioid system. We will refer to the unidentified compound responsible for inducing κ-opioid activity as the putative Kappa Inducing Factor, or KIF.

First, it should be noted that two independent research programs now have identified characteristics or constituents in AF that engage the opioid system of rats. The POEF sought by Kristal and colleagues (Kristal, 1991) is believed to be present in both AF and placental tissue, and to potentiate, but not evoke, opioid activity in the newly parturient rat. KIF, as identified in research in our laboratory, also is present in AF and is responsible for eliciting activity at κ-opioid receptors in the fetal and neonatal rat (Korthank & Robinson 1998). However, there are notable

differences between AF enhancement of antinociception by POEF in adult rats and AF-induced changes in fetal responsiveness triggered by KIF. (a) The effect of ingestion of AF in adult rats is to enhance the effects of drugs or other stimulation that evokes opioid-mediated antinociception; ingestion of AF by itself does not result in antinociception. In contrast, oral exposure to AF alone is sufficient to produce changes in fetal behavior, including reduced responsiveness to sensory stimuli. Although this effect could result from enhancement of existing levels of opioid activity, administration of an opioid antagonist such as naloxone, in the absence of exposure to AF, has no effect on fetal sensory responsiveness. (b) POEF enhancement of analgesia in adult rats requires ingestion of AF: intubation of AF into the stomach produces the same effect, but mere sensory exposure to AF does not. In contrast, ingestion of AF is not necessary for KIF-induced changes in fetal responsiveness: fetuses prepared with an esophageal/tracheal ligature that prevents swallowing and breathing continue to show a strong effect of oral exposure to AF. (c) The time course of effects of AF ingestion in adult rats is approximately 5–30 minutes after intubation. In contrast, the time course of behavioral effects in the fetus is much shorter, on the order of 15–240 seconds. These marked differences in the effects of POEF in the adult rat and KIF in the perinatal rat suggest that the two phenomena may only be superficially related, and most likely depend on different underlying mechanisms.

The most obvious candidate for KIF would be an opioid compound itself, which after gaining access to the fetal CNS could directly stimulate activity at κ-opioid receptors. Opioids have been identified in assays of AF, including ß-endorphin and enkephalins (Kofinas et al., 1987; Mauri & Volpe, 1994; Petrucha et al., 1983), but these compounds act preferentially at μ and ∂ receptors, respectively (Leslie & Loughlin, 1993). Dynorphins are a naturally occurring opioid ligand that acts preferentially at κ receptors. Several forms of dynorphin have been identified in human AF, and concentrations of AF dynorphin may increase dramatically in the third trimester of gestation (Valette et al., 1986). The behavioral potency of an endogenous κ-opioid ligand was demonstrated by experimental administration of dynorphin A(1-13) directly into the brain of the fetal rat by injection into the cisterna magna (Varlinskaya, Petrov, & Smotherman, 1996). Central injection of just 10 ng of dynorphin resulted in elevated motor activity, including pronounced increases in hindlimb movements, and reduced facial wiping to a cutaneous probe. Moreover, central administration of dynorphin produced a similar time course of effects on the wiping response as AF, with reduced facial wiping evident within 30 s after injection and recovery to pre-injection baseline levels within 300 s.

However, as discussed above and depicted in Figure 9.5, for dynorphin or any alternative factor to remain a viable candidate as KIF, it must be capable of gaining access to the fetal CNS after oral exposure alone. Evidence from adult rats has suggested that chemicals within the mouth can gain direct access to the CNS without being absorbed from the gut. Injection of radiolabelled dye into the mouth of an adult rat results in significant levels of label found throughout the brain, even

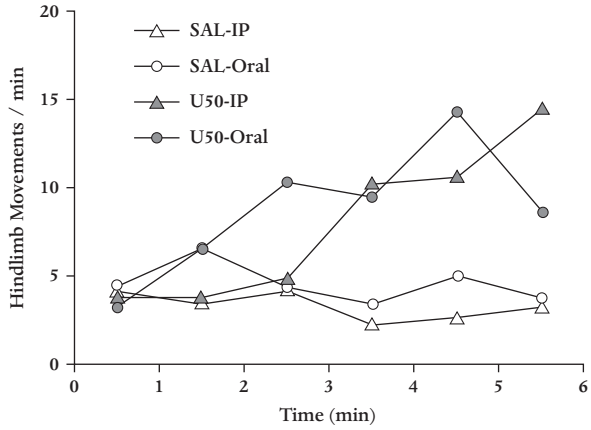

Figure 9.6. Changes in fetal hindlimb activity after administration of a κ-opioid agonist, U50,488. After a 1-min baseline period, the agonist (black symbols) or saline vehicle (white symbols) was administered to E20 fetal subjects by IP injection or by intraoral infusion, and fetal behavior was scored over the following 5 min. Note that hindlimb activity was significantly elevated in both groups treated with the κ agonist, but increased more rapidly after oral administration, within 2 min of infusion.

after an esophageal-tracheal ligature prevents ingestion of the dye (Kare, Schechter, Grossman, & Roth, 1969). Moreover, the nasopalatine duct is likely patent during the late fetal and neonatal period in rats, permitting a direct route of access from the oral cavity to the olfactory epithelia (Coppola, Budde, & Millar, 1993; Weiler & Farbman, 2003). Alternatively, AF taken into the oral cavity during fetal swallowing could gain access to the olfactory epithelia via a retronasal route (Coppola, Coltrane & Arsov, 1994; Coppola & Millar, 1994).

We have obtained supportive evidence of this model by infusing a solution of the κ-opioid agonist U50,488 directly into the mouth of the E20 fetal rat. Rat fetuses that were exposed to U50,488 by oral infusion showed a significant increase in limb activity, with a predominance of activity in the hindlimbs, over the next 5 min (Figure 9.6). The behavioral effect of orally administered U50,488 did not differ from IP injection, and was consistent with previous studies of opioid agonists administered by IP injection or intracisternal injection into the brain (Robinson, Moody, et al., 1993; Smotherman & Robinson, 1992c; Petrov, Varlinskaya, Robinson, & Smotherman, 1994). In fact, oral infusion of a selective κ agonist resulted in very similar effects as central administration of dynorphin A(1-13). Compared to intraperitoneal injection, however, behavioral effects were evident significantly more quickly after oral administration of the κ agonist, further supporting the interpretation that the κ agonist gained direct access to the fetal CNS through or around the palate via the nasopalatine duct or a retronasal route, and through the cribiform plate at the base of the skull. This experiment confirms

that opioid compounds, such as dynorphin, if they are present in AF in sufficient concentration, can produce behavioral effects when administered through an oral route.

A second candidate for KIF is dopamine, and a family of precursors and metabolites of catecholamines that have been identified in human, sheep, and rat AF (Ben-Jonathan et al., 1983; Peleg, Arbogast, Peleg, & Ben-Jonathan, 1984; Peleg, Munsick, Diker, Goldman, & Ben-Jonathan, 1986; Phillippe & Ryan, 1981). The most likely source of dopamine in AF is fetal urine. High levels of L-DOPA are produced in chromaffin tissues (organ of Zuckerkandl), some of which is converted to dopamine by dopa decarboxylase in the fetal kidney (Inoue, Kudo & Kishimoto, 1991). Both L-DOPA and dopamine then are excreted into AF during fetal micturition. Dopamine is an attractive candidate for KIF based on previous research in our laboratory, which demonstrated that administration of a dopaminergic agonist (the selective D_1 agonist SKF-38396) produces many of the same behavioral effects in the rat fetus as a κ-opioid agonist, including general behavioral activation, disproportionate increases in the movement of hindlimbs, and reduced facial wiping to a test infusion of lemon. The effects of the D_1 agonist were receptor mediated, and were effectively blocked by pretreatment with a selective D_1 antagonist (SCH-23390) (Moody, Robinson, Spear, & Smotherman, 1993). Further, the effects of the D_1 agonist were blocked by the κ-opioid antagonist BNI. But the D_1 antagonist was ineffective in blocking the effects of a selective κ-opioid agonist (U50,488) (Robinson, Moody, et al., 1993; Smotherman et al., 1993). These results indicate that the dopamine and opioid systems interact in producing effects on fetal behavior, and that the interaction between D_1-dopamine receptors and κ-opioid receptors in the rat fetus is unidirectional, with activation of the fetal dopamine system secondarily triggering a κ-opioid response (Smotherman & Robinson, 1992d). Given a DA-κ interaction, the presence of dopamine in AF could be a plausible compound that could trigger an opioid response in the fetus.

There are several difficulties with the dopamine-as-KIF hypothesis, however. First, even though catecholamines increase markedly in late gestation, peak levels of dopamine are still at very low absolute concentrations, on the order of 1–8 ng/ml (Peleg et al., 1984; Phillippe & Ryan, 1981). These concentrations are highly unlikely to be pharmacologically significant, unless they are magnified by a cascading effect on the opioid system. Second, very little free dopamine appears to be present in AF. Rather, most of the available dopamine is converted to dopamine sulfate through the action of sulfotransferase enzymes in the kidney. Sulfoconjugation is a mechanism for deactivating toxins and neurochemicals that could otherwise exert biological activity (Pacifici, 2005; Strott, 2002). Thus, the most likely means for dopamine sulfate in AF to evoke behavioral responses in the fetus would be for the sulfur to be cleaved by a sulfatase enzyme, reinstating a free form of dopamine. Interestingly, aryl sulfatase, which reverses the process and produces free dopamine, is found in many tissues, including salivary glands (Chauncey, Lionetti, Winer, & Lisanti, 1954; Vitaioli, Bondi, Menghi, & Materazzi, 1981). This fact raises the

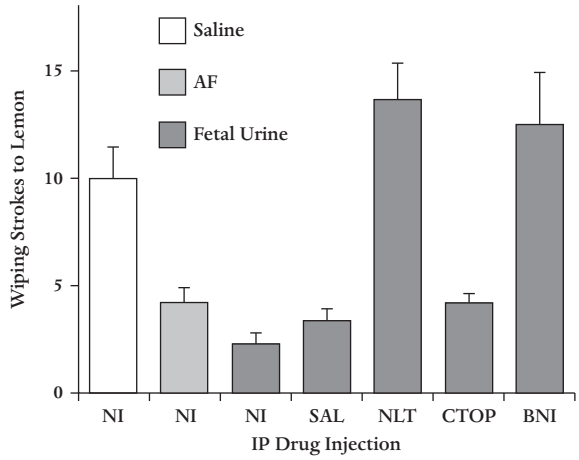

Figure 9.7. Facial wiping responses to lemon (mean number of wiping strokes ± SEM) after oral infusion of saline, AF, or urine collected from E21 fetuses. Fetal subjects were pretreated with no injection (NI) or IP injection of saline (SAL), the nonselective opioid antagonist naltrexone (NLT), the μ antagonist CTOP, or the antagonist BNI. Fetal urine was as effective as AF in reducing fetal wiping responses in the lemon test, and the effects of fetal urine were reversed with naltrexone or BNI.

intriguing possibility that dopamine sulfate, circulating in AF, is drawn into the oral cavity during fetal mouthing and swallowing, where it is activated by sulfatase in the fetal salivary glands to produce free dopamine. Dopamine then could gain direct access to the fetal CNS by the same route described above for opioid compounds, thereby triggering an opioid response.

Although this scenario may seem unlikely, preliminary evidence from our laboratory suggests that dopamine sulfate in AF is a viable candidate as KIF. The model predicts that the source of KIF is the fetus itself, which produces KIF and excretes it into AF as a constituent of urine. To test this prediction, we collected samples of urine from rat pups delivered on E21. Pups were delivered by caesarean section to prevent any postnatal influence of suckling or maternal licking. Urine samples were pooled across subjects until a sufficient amount was available for behavioral testing. Fetal subjects then were prepared for behavioral testing on E20, as in earlier experiments that tested samples of AF. Subjects were exposed via intraoral infusion of fetal urine, then 60 s later received a test infusion of lemon to evoke a facial wiping response. As can be seen in Figure 9.7, pup urine was just as effective as AF in reducing the facial wiping response to lemon. Moreover, the effect of oral exposure to fetal urine was completely blocked with naltrexone and with BNI, confirming that the behavioral effects of fetal urine are mediated by κ-opioid activity.

The final candidate factor that we have tentatively identified is dimethyl disulfide (DMDS), one of several biogenic sulfides that are produced in mouth and saliva of

the neonatal rat (Galef, Mason, Preti, & Bean, 1988; Pedersen & Blass, 1981). DMDS is not a neuroactive substance, but rather is a chemical stimulus with strong olfactory qualities. DMDS has been shown to direct the nipple search behavior and facilitate nipple attachment in newborn rats (Pedersen & Blass, 1981), and when experimentally presented to rat fetuses, evokes behavioral effects that can be blocked with a κ-opioid antagonist (Smotherman & Robinson, 1992b). Thus, in considering just these three candidate factors, it would appear to be equally likely that the opioid-inducing effects of AF could be mediated by a factor that directly engages κ-opioid receptors (e.g., dynorphin), or a factor that indirectly activates the κ-opioid system through an intermediary neurochemical response (e.g., dopamine), or a factor that lacks direct pharmacological activity but is detected by the fetus through a chemosensory modality such as olfaction (e.g., DMDS).

Blurring the Boundaries of the Organism

In the foregoing sections we have attempted to summarize the many different ways that AF may contribute to the development of behavior in mammals. In doing this, we have drawn from many sources and fields that, like the proverbial blind men describing different parts of an elephant, are seeking answers to very different questions, and in consequence have too little interchange with one another. The metaphor of the jigsaw puzzle is perhaps overused, but is particularly apt to describe the current state of our understanding of the biological significance of AF. We have tried to assemble some of the available pieces in this review, with allowance for our own idiosyncratic perspective, but there are many pieces still missing and the image that is beginning to emerge is incomplete and fragmented. Nevertheless, the picture in the puzzle is not a still life of the fetus floating serenely in an undisturbed pool, but a dynamic portrait of a life aquatic that is the evolutionary legacy and a history of each individual's experience for all placental mammals.

Although many aspects of AF appear to be regulated by the physiologies of the fetus and mother, its volume and composition, and the roles that it plays in shaping perinatal development undergo continual change during gestation. AF well deserves to be considered a true environment, both in the sense intended by the developmental psychologist that views the environment as the sum of experiences that shape the future behavior of the child, and in the sense of ecology, which views the environment as the sum of relationships between an organism and its surroundings that shape its growth and survival. And yet there is something incorrect in seeing AF as an environment physically apart from the fetus that resides in it. The picture that is emerging of AF is that it contributes in many ways to shaping the development of the fetus, but also is shaped by the fetus's physiology and behavior. It is both a key feature of the world outside the fetus and a part of the

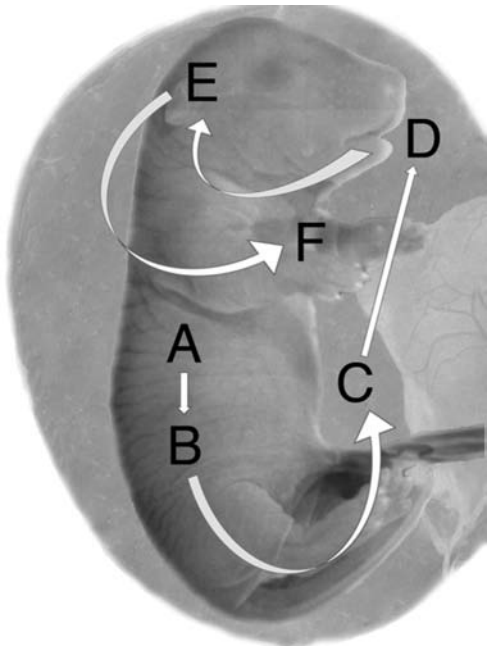

Figure 9.8. A model of the production and exposure of the rat fetus to the κ-opioid inducing factor (KIF) during the last two days of gestation. KIF or precursor chemicals (e.g., L-DOPA) are produced by fetal physiology (A) and extracted from fetal circulation in the fetal kidneys (B), perhaps undergoing further processing (e.g., synthesis of dopamine; sulfation to dopamine sulfate). KIF then is excreted into AF in urine (C), where the fetus is exposed to it via oral sampling of AF (D). After oral exposure, KIF may trigger a sensory response (e.g., the odorant DMDS) or may undergo chemical conversion in the oral cavity (e.g., by sulfatase to free dopamine). After sensory activation or direct transport to the fetal CNS (E), KIF evokes activity in the endogenous opioid system, with consequences for fetal motor behavior, sensation, and learning (F).

fetus. The picture of AF shifts as we look at it, and the image of the organism as distinct and separate from the environment is blurred.

This blurring of the boundaries of the organism during prenatal development is well illustrated by the model of AF-induced opioid activity in the rat fetus. Key pieces of evidence supporting this model may be summarized as follows (see also Figure 9.8). (a) The fetus is continually surrounded by AF during normal development in utero. (b) Behavioral experiments with fetal rats suggest that a chemical factor is present in AF that triggers activity at κ receptors of the endogenous opioid system of the fetus. (c) Although the κ-opioid Inducing Factor (KIF) has not yet been identified, KIF is present in fetal urine and therefore is likely to derive from the fetus itself. Candidates for KIF include, but are not limited to, an opioid ligand such as dynorphin, dopamine sulfate, or a sulfide compound such as dimethyl disulfide

(DMDS). (d) After development of a keratinized barrier preventing water transfer through the skin (around E19–20 in the rat), the fetus is exposed to AF primarily through its own oral activity. (e) Oral exposure to AF results in brief (3–4 min) periods of activity in the opioid system induced by KIF. (f) Because oral activity of the fetus is variable, the fetus experiences intermittent periods of activity in the κ-opioid system. (g) During periods of elevated opioid activity, the fetus exhibits altered organization of spontaneous motor activity and diminished responsiveness to sensory stimulation. (h) AF-induced opioid activity therefore is likely to influence experience-dependent and activity-dependent processes that regulate neural and behavioral development during the prenatal period.

Because oral exposure to AF may reliably induce activity in the endogenous opioid system, we also may speculate that AF can play an active role in supporting learning before birth. There is a well-established literature that documents the effects of pharmacologically-induced opioid activity on learning (Kehoe, 1988; Barr, 1992). For instance, when a novel odor cue is paired with injection of morphine, 5-day-old infant rats acquire a conditioned opioid response that is expressed upon reexposure to the odor 5 days later (Kehoe & Blass, 1986). Interestingly, administration of a selective κ-opioid agonist also resulted in conditioned odor preference when paired with an odor in 3-day-old rats, but an odor aversion in 7-day-olds (Barr, Wang, & Carden, 1994). Similar demonstrations of opioid involvement in classical conditioning have been reported in the rat fetus (Robinson, Arnold, et al., 1993). When a neutral cue is selected as a conditioned stimulus (CS), such as infusion of sucrose or oral exposure to a non-nutritive artificial nipple (Robinson et al., 1992), and the CS is paired with intraoral infusion of milk as the unconditioned stimulus (US), the rat fetus can acquire a conditioned opioid response in as few as one conditioning trial and demonstrate elevated opioid activity upon reexposure to the CS (Smotherman & Robinson, 1994). Control conditions such as explicitly unpaired presentations of the nipple CS and milk US, or exposure to the CS alone or US alone, are ineffective in producing a conditioned opioid response to the CS. Classical conditioning in this paradigm is so rapid that it implies that newborn rats may learn cues associated with milk, the nipple and the mother during the first suckling episode (Smotherman & Robinson, 1992d). This form of conditioning is dependent on the ability of milk to evoke an unconditioned response in the κ-opioid system (Smotherman & Robinson, 1994), and can be supported by central injection of dynorphin into the fetal brain serving as the US (Varlinskaya et al., 1996). Given that we also have demonstrated that AF can evoke κ-opioid activity in the fetus (Korthank & Robinson, 1998), and newborn rat (Méndez-Gallardo & Robinson, in press), it is plausible that AF also can serve as an effective unconditioned stimulus to support classical conditioning of opioid activity during development in utero and immediately after birth.

This hypothesis that AF-induced opioid activity may support associative learning in utero already has received some indirect empirical support. AF can serve as an unconditioned stimulus to support conditioning in preweanling rats (Arias &

Chotro, 2007). After 11–12-day- old rats were presented with a pairing of alcohol odor and intraoral infusion of AF collected on E20, they exhibited increased intake of alcohol and responses indicating improved palatability of alcohol. Chotro and Arias (2003) also have reported that blockade of opioid receptors by administration of naloxone to the pregnant mother prevents the preference for alcohol that typically occurs after fetal exposure to alcohol late in gestation. The authors interpret this result as evidence that alcohol pharmacologically affects the opioid system at the same time that the fetus is exposed to the orosensory qualities of alcohol, thereby supporting classical conditioning of a preference for alcohol odor. But an alternative interpretation is that naloxone blocks the opioid activity typically induced by oral exposure to AF as the fetus samples the orosensory characteristics of alcohol in AF. This suggests a more general explanation for the many demonstrations of sensory exposure learning in the fetus: any flavor compound in AF, whether derived from maternal diet or experimental injection, is detected by the fetus as it orally samples AF, and therefore is paired in a contingent fashion with the opioid-inducing effects of AF. If true, this hypothesis predicts that experimenters should be able to reduce or eliminate many forms of prenatal exposure learning by blocking opioid activity during the period of fetal exposure to the novel flavor. Moreover, the contingency created by oral exposure to AF and flavor cues in AF raises the additional possibility of operant conditioning in utero, which is known to be supported by contingent presentations of milk in newborn rats (Arias et al., 2007; Johanson & Hall, 1979).

What is clearest in this discussion is that we are only beginning to understand the many potential roles that AF may play in perinatal behavioral development. As our laboratory, along with many others, continues to pursue these investigations, it will be crucial to remember that AF is only one of many developmental resources available to the fetus. In summarizing research on AF above, we have tended to emphasize the importance of experience and learning in development to the exclusion of genes and other biological factors. But we fully expect that our research will lead to a better appreciation of the complex interplay between genes and environment during fetal development. The ability of AF to engage the opioid system and influence fetal behavior is dependent on many genes. As an illustration, let us assume, for the moment, that our speculation about dopamine sulfate as the KIF is accurate. In this eventuality, the ability of AF to induce activity in the κ-opioid system would be dependent on genes governing the synthesis of L-DOPA in chromaffin tissue, on the gene for dopa decarboxylase to convert L-DOPA to dopamine and the gene for sulfotransferase to promote the conjugation of free dopamine in the fetal kidney, on the gene for sulfatase that we speculate is produced in the fetal salivary glands to strip sulfur from dopamine sulfate, and on the various genes governing the production of dopamine and opioid ligands and receptors within the fetal CNS. Any one of these genes could represent a limiting factor in the development of fetal behavior, and a mutation, knockout or overexpression of any of them could result in dysregulation of the AF-opioid interaction

in utero, with potentially important and unpredictable consequences for the development of fetal movement and sensation, for nipple attachment and suckling after birth, and for dietary and affiliative preferences extending into adulthood.

There is no logical connection between many of these genes and such developmental consequences, but the developmental process often is nonintuitive. Who could have foreseen, for example, a rational connection between maternal licking of pups and gene expression, brain development, and adult sexual behavior (Liu, Diorio, Day, Francis, & Meaney, 2000; Moore, 1995; Weaver et al., 2004)? Certainly Gilbert Gottlieb, to whom this volume is dedicated, did not predict where his research on imprinting would lead: that auditory experience with the embryo's own vocalizations in the egg would shape auditory development and influence multisensory preferences and learning after hatching (Gottlieb, 1997). It is our hope that as further research provides more examples that blur the boundary between organism and environment, developmental science will move away from the old dichotomy of nature versus nurture to embrace a systems perspective on developmental process (Gottlieb, 1998; Johnston & Edwards, 2002; Spencer et al., 2009).

References

Abbas, T. M., & Tovey, J. E. (1960). Proteins of the liquor amnii. *British Medical Journal*, **2**, 476–479.

Abbott, P., Thompson, A. C., Ferguson, E. J., Doerr, J. C., Tarapacki, J. A., Kostyniak, P. J., et al. (1991). Placental opioid-enhancing factor (POEF): Generalizability of effects. *Physiology & Behavior*, **50**, 933–940.

Abitbol, M. L., & Inglis, S. R. (1997). Role of amniotic fluid in newborn acceptance and bonding in canines. *The Journal of Maternal-Fetal Medicine*, **6**, 49–52.

Alberts, J. R., & Cramer, C. P. (1988). Ecology and experience; sources of means and meaning of developmental change. In E. M. Blass (Ed.), *Handbook of behavioral neurobiology: Vol. 9. Behavioral ecology and developmental psychobiology* (pp. 1–39). New York: Plenum Press.

Albuquerque, C. A., Smith, K. R., Saywers, T. E., Johnson, C., Cock, M. L., & Harding, R. (2002). Relation between oligohydramnios and spinal flexion in the human fetus. *Early Human Development*, **68**, 119–126.

Arias, C., & Chotro, M. G. (2005). Increased preferences for ethanol in the infant rat after prenatal ethanol exposure, expressed on intake and taste reactivity tests. *Alcoholism: Clinical and Experimental Research*, **29**, 337–346.

Arias, C., & Chotro, M. G. (2007). Amniotic fluid can act as an appetitive unconditioned stimulus in preweanling rats. *Developmental Psychobiology*, **49**, 139–149.

Arias, C., Spear, N. E., Molina, J. C., & Molina, A. (2007). Rapid acquisition of operant conditioning in 5-day-old rat pups: A new technique articulating suckling-related motor activity and milk reinforcement. *Developmental Psychobiology*, **49**, 576–588.

Aszterbaum, M., Menon, G. K., Feingold, K. R., & Williams, M. L. (1992). Ontogeny of the epidermal barrier to water loss in the rat: Correlation of function with stratum corneum structure and lipid content. *Pediatric Research*, **31**, 308–317.

Attali, B., Saya, D., & Vogel, Z. (1990). Pre- and postnatal development of opiate receptor subtypes in rat spinal cord. *Developmental Brain Research*, **53**, 97–102.

Barr, G. A. (1992). Behavioral effects of opiates during development. In M. W. Miller (Ed.), *Development of the central nervous systems: Effects of alcohol and opiates* (pp. 221–254). New York: Wiley-Liss.

Barr, G. A., Wang, S., & Carden, S. (1994). Aversive properties of the (opioid agonist U50,488 in the week-old rat pup. *Psychopharmacology*, **113**, 422–428.

Beauchamp, G. K., & Mennella, J. A. (2009). Early flavor learning and its impact on later feeding behavior. *Journal of Pediatric Gastroenterology and Nutrition*, **48**, S25–S30.

Ben-Jonathan, N., Arbogast, L. A., Rhoades, T. A., Schillo, K. K., Pau, K.-Y. F., & Jackson, G. L. (1983). Plasma catecholamines in the chronically cannulated sheep fetus: predominance of L-dihydroxyphenylalanine. *Endocrinology*, **113**, 216–221.

Bernard, C. (1878). *Leçons sur les phénomènes de la vie communs aux animaux et aux végétaux*. Paris: J.-B. Baillière et fils.

Bernard, C. (1974). *Lectures on the phenomena common to animals and plants*. Trans. H. E. Hoff, & L. Guillemin. Springfield, IL: Charles C. Thomas. Berridge, K. C. (1990). Comparative fine structure of action: Rules of form and sequence in the grooming patterns of six rodent species. *Behaviour*, **113**, 21–56.

Berridge, K. C., & Fentress, J. C. (1986). Contextual control of trigeminal sensorimotor function. *Journal of Neuroscience*, **9**, 325–330.

Bilkó, Á., Altabäcker, V., & Hudson, R. (1994). Transmission of food preference in the rabbit: The means of information transfer. *Physiology & Behavior*, **56**, 907–912.

Blackburn, S. (2007). *Maternal, fetal, & neonatal physiology: A clinical perspective*. St Louis, MO: Saunders.

Blass, E. M. (1996). Mothers and their infants: peptide-mediated physiological, behavioral and affective changes during suckling. *Regulatory Peptides*, **66**, 109–112.

Blass, E. M., & Fitzgerald, E. (1988). Milk-induced analgesia and comforting in 10-day-old rats: Opioid mediation. *Pharmacology, Biochemistry & Behavior*, **29**, 9–13.

Blass, E. M., Jackson, A. M., & Smotherman, W. P. (1991). Milk-induced, opioid-mediated antinociception in rats at the time of cesarean delivery. *Behavioral Neuroscience*, **105**, 677–686.

Blass, E. M., & Teicher, M. H. (1980). Suckling. *Science*, **210**, 15–22.

Blumberg, M. S., Freeman, J. H., & Robinson, S. R. (Eds.). (2010). *The Oxford handbook of developmental behavioral neuroscience*. New York: Oxford University Press.

Bomsel-Helmreich, O., & Al Mufti, W. (2005). The phenomenon of monozygosity: Spontaneous zygotic splitting. In: I. Blickstein, L. G. Keith, & D. M. Keith (Eds.), *Multiple pregnancy: Epidemiology, Gestation & perinatal outcome* (pp. 94–101). Abingdon, UK: Taylor & Francis.

Bond, N. W., & Di Giusto, E. L. (1976). Effect of prenatal alcohol consumption on open-field behaviour and alcohol preference in rats. *Psychopharmacologia*, **46**, 163–165.

Bradley, R. M., & Mistretta, C. M. (1973). Swallowing in fetal sheep. *Science*, **179**, 1016–1017.

Brake, S. C. (1981). Suckling infants rats learn a preference for a novel olfactory stimulus paired with milk delivery. *Science*, **211**, 506–508.

Brake, S. C., Shair, H., & Hofer, M. A. (1988). Exploiting the nursing niche: The infant's sucking and feeding in the context of the mother-infant interaction. In E. M. Blass (Ed.), *Handbook of behavioral neurobiology: Vol. 9. Developmental psychobiology and behavioral ecology* (pp. 347–388). New York: Plenum Press.

Brandt, E. M., & Mitchel, G. (1971). Parturition in primates: Behavior related to birth. In L. Rosenblum (Ed.), *Primate behavior: Development in field and laboratory research* (pp. 177–223). New York: Academic Press.

Brown, J. R., Ye, H., Bronson, R. T., Dikkes, P., & Greenberg, M. E. (1996). A. defect in nurturing in mice lacking the immediate early gene fosB. *Cell*, **86**, 297–309.

Brumley, M. R., & Robinson, S. R. (2004). Facial wiping in the rat fetus: Variation of chemosensory stimulus parameters. *Developmental Psychobiology*, **44**, 219–229.

Brunjes, P. (1990). The precocial mouse, Acomys cahirinus. *Psychobiology*, **18**, 339–350.

Cannon, W. B. (1932). *Wisdom of the body*. New York: Norton.

Chauncey, H. H., Lionetti, F., Winer, R. A., & Lisanti, V. F. (1954). Enzymes of human saliva: I. the determination, distribution, and origin of whole saliva enzymes. *Journal of Dental Research*, **33**, 321–334.

Cheslock, S. J., Varlinskaya, E. I., Petrov, E. S., & Spear, N. E. (2000). Rapid and robust olfactory conditioning with milk before suckling experience: Promotion of nipple attachment in the newborn rat. *Behavioral Neuroscience*, **114**, 484–495.

Chotro, M. G., & Arias, C. (2003). Prenatal exposure to ethanol increases ethanol consumption: A conditioned response? *Alcohol*, **30**, 19–28.

Chotro, M. G., Arias, C., & Laviola, G. (2007). Increased ethanol intake after prenatal ethanol exposure: Studies with animals. *Neuroscience and Biobehavioral Reviews*, **31**, 181–191.

Chotro, M. G., & Molina, J. C. (1990). Acute ethanol contamination of the amniotic fluid during gestational day 21: Postnatal changes in alcohol responsiveness in rats. *Developmental Psychobiology*, **23**, 535–547.

Clancy, B., Darlington, R. B., & Finlay, B. L. (2001). Translating developmental time across mammalian species. *Neuroscience*, **105**, 7–17.

Coppola, D. M., Budde, J., & Millar, L. (1993). The vomeronasal duct has a protracted postnatal development in the mouse. *Journal of Morphology*, **218**, 59–64.

Coppola, D. M., Coltrane, J. A., & Arsov, I. (1994). Retronasal or internasal olfaction can mediate odor-guided behaviors in newborn mice. *Physiology & Behavior*, **56**, 729–736.

Coppola, D. M., & Millar, L. (1994). Stimulus access to the accessory olfactory system in the prenatal and perinatal rat. *Neuroscience*, **60**, 463–468.

Coureaud, G., Schaal, B., Hudson, R., Orgeur, P., & Coudert, P. (2002). Transnatal olfactory continuity in the rabbit: Behavioral evidence and short-term consequence of its disruption. *Developmental Psychobiology*, **40**, 372–390.

Delaunay-El Allam, M., Marlier, L., & Schaal, B. (2006). Learning at the breast: Preference formation for an artificial scent and its attraction against the odor of maternal milk. *Infant Behavior & Development*, **29**, 308–321.

DeVries, T. J., Hogenboom, F., Mulder, A. H., & Schoffelmeer, A. N. M. (1990). Ontogeny of μ-, ∂-, and κ-opioid receptors mediating inhibition of neurotransmitter release and adenylate cyclase activity in rat brain. *Developmental Brain Research*, **54**, 63–69.

DiPirro, J. M., & Kristal, M. B. (2004). Placenta ingestion by rats enhances ∂- and κ-opioid antinociception, but suppresses μ-opioid antinociception. *Brain Research*, **1014**, 22–33.

Doerr, J. C., & Kristal, M. B. (1989). Enhancement of opioid-mediated analgesia by ingestion of amniotic fluid: Onset latency and duration. *Physiology & Behavior*, **46**, 913–915.

Domínguez, H. D., Bocco, G., Chotro, M. G., Spear, N. E., & Molina, J. C. (1993). Operant responding controlled by milk or milk contaminated with alcohol as positive reinforcers in infant rats. *Pharmacology Biochemistry and Behavior*, **44**, 403–409.

Domínguez, H. D., López, M. F., & Molina, J. C. (1998). Neonatal responsiveness to alcohol odor and infant alcohol intake as a function of alcohol experience during late gestation. *Alcohol*, **16**, 109–117.

Dunbar, I., Ranson, E., & Buehler, M. (1981). Pup retrieval and maternal attraction to canine amniotic fluids. *Behavioral Processes*, **6**, 249–260.

El-Haddad, M. A., Desai, M., Gayle, D., & Ross, M. G. (2004). In utero development of fetal thirst and appetite: Potential for programming. *Journal of the Society for Gynecological Investigation*, **11**, 123–130.

Fisher, S. E. (2006). Tangled webs: Tracing the connections between genes and cognition. *Cognition*, **101**, 270–297.

Galef, B. G., Jr., Mason, R. J., Preti, G., & Bean, N. J. (1988). Carbon disulfide: A semiochemical mediating socially-induced diet choice in rats. *Physiology & Behavior*, **42**, 119–124.

Galef, B. G., Jr., & Sherry, D. F. (1973). Mother's milk: A medium for transmission of cues reflecting the flavor of mother's diet. *Journal of Comparative and Physiological Psychology*, **83**, 374–378.

Gilbert, W. M., & Brace, R. A. (1993). Amniotic fluid volume and normal flows to and from the amniotic cavity. *Seminars in Perinatology*, **17**, 150–157.

Gilbert, W. M., Newman, P. S., Eby-Wilkens, E., & Brace, R. A. (1996). Technetium Tc 99m rapidly crosses the ovine placenta and intramembranous pathway. *American Journal of Obstetrics and Gynecology*, **175**, 1557–1562.

González-Mariscal, G., Melo, A. I., Chirino, R., Jiménez, P., Beyer, C., & Rosenblatt, J. S. (1998). Importance of mother/young contact at parturition and across lactation for the expression of maternal behaviors in rabbits. *Developmental Psychobiology*, **32**, 101–111.

Gottlieb, G. (1997). *Synthesizing nature-nurture: Prenatal roots of instinctive behavior*. New Jersey: Erlbaum.

Gottlieb, G. (1998). Normally occurring environmental and behavioral influences on gene activity: From central dogma to probabilistic epigenesis. *Psychological Review*, **105**, 792–802.

Hall, W. G. (1979). The ontogeny of feeding in rats: I. Ingestive and behavioral responses to oral infusions. *Journal of Comparative Physiology Psychology*, **93**, 977–1000.

Hall, W. G. (1990). The ontogeny of ingestive behavior. In E. M. Stricker (Ed.), *Handbook of behavioral neurobiology, Vol. 10, Neurobiology of food and fluid intake* (pp. 77–123). New York: Plenum Press.

Hall, W. G., & Rosenblatt, J. S. (1977). Suckling behavior and intake control in the developing rat pup. *Journal of Comparative and Physiological Psychology*, **91**, 1232–1247.

Hall, W. G., & Williams, C. L. (1983). Suckling isn't feeding, or is it? A search for developmental continuities. In J. Rosenblatt, R. Hinde, R. Beer, & M. Busnell (Eds.), *Advances in the study of behavior* (Vol. **13.**, pp. 219–254). New York: Academic Press.

Hardman, M. J., Moore, L., Ferguson, M. W. J., & Byrne, C. (1999). Barrier formation in the human fetus patterned. *Journal of Investigative Dermatology*, **113**, 1106–1113.

Hasselquist, D., & Nilsson, J.-A. (2009). Maternal transfer of antibodies in vertebrates: trans-generational effects on offspring immunity. *Philosophical Transactions of the Royal Society B*, **364**, 51–60.

Hauser, K. F., McLaughlin, P. J., & Zagon, I. S. (1989). Endogenous opioid systems and the regulation of dendritic growth and spine formation. *Journal of Comparative Neurology*, **281**, 13–22.

Hepper, P. G. (1987). The amniotic fluid: an important priming role in kin recognition. *Animal Behavior*, **35**, 1343–1346.

Hepper, P. G. (1988). Adaptive fetal learning: Prenatal exposure to garlic affects postnatal preferences. *Animal Behaviour*, **36**, 935–936.

Hepper, P. G. (Ed.), (1991). *Kin recognition*. New York: Cambridge University Press.

Hepper, P. G. (1996). Fetal memory: Does it exist? What does it do? *Acta Paediatrica*, **85**, 16–20.

Hepper, P. G., Shannon, E. A., & Dornan, J. C. (1997). Sex differences in fetal mouth movements. *The Lancet*, **350**, 1820.

Houck, J. C., Kimball, C., Chang, C., Pedigo, N. W., & Yamanura, H. I. (1980). Placental B-endorphin-like peptides. *Science*, **207**, 78–80.

Huberman, A. D., Feller, M. B., & Chapman, B. (2008). Mechanisms underlying development of visual maps and receptive fields. *Annual Review of Neuroscience*, **31**, 479–509.

Hudson, R., & Distel, H. (1983). Nipple location by newborn rabbits: Evidence for pheromonal guidance. *Behaviour*, **82**, 260–275.

Inoue, K., Kudo, T., & Kishimoto, Y. (1991). The production mechanism of amniotic fluid dopamine in rats. *Asia Oceania Journal of Obstetrics and Gynecology*, **17**, 349–355.

Johanson, I. B., & Hall, W. G. (1979). Appetitive learning in 1-day-old rat pups. *Science*, **205**, 419–421.

Johanson, I. B., & Hall, W. G. (1982). Appetitive conditioning in neonatal rats: Conditioned orientation to an odor. *Developmental Psychobiology*, **15**, 379–397.

Johanson, I. B., Hall, W. G., & Polefrone, J. M. (1984). Appetitive conditioning in neonatal rats: Conditioned ingestive responding to stimuli paired with oral infusions of milk. *Developmental Psychology*, **17**, 357–381.

Johanson, I. B., & Shapiro, E. G. (1986). Intake and behavioral responsiveness to taste stimuli in infants rats from 1 to 15 days of age. *Developmental Psychobiology*, **19**, 593–606.

Johanson, I. B., & Teicher, M. H. (1980). Classical conditioning of an odor preference in 3-day-old rats. *Behavioral and Neural Biology*, **29**, 132–136.

Johanson, I. B., & Terry, L. M. (1988). Learning in infancy: A mechanism for behavioral change during development. In E. M. Blass (Ed.), *Handbook of behavioral neurobiology: Vol. 9. Developmental and behavioral ecology* (pp. 245–282). New York: Plenum Press.

Johnston, T. D., & Edwards, L. (2002). Genes, interactions, and the development of behavior. *Psychological Review*, **109**, 26–34.

Kare, M. R., Schechter, P. J., Grossman, S. P., & Roth, L. J. (1969). Direct pathway to the brain. *Science*, **163**, 952–953.

Kehoe, P. (1988). Opioids, behavior, and learning in mammalian development. In: Blass, E. M. (Ed.), *Handbook of behavioral neurobiology: Vol. 9. Developmental psychobiology and behavioral ecology* (pp. 309–346). New York: Plenum Press.

Kehoe, P., & Blass, E. M. (1986). Behaviorally functional opioid systems in infant rats: II. Evidence for pharmacological, physiological, and psychological mediation of pain and stress. *Behavioral Neuroscience*, **100**, 624–630.

Kirby, M. L. (1981). Development of opioid receptor binding in rat spinal cord. *Brain Research*, **205**, 400–404.

Kofinas, G. D., Kofinas, A. D., Pyrgerou, M., & Reyes, F. I. (1987). Amniotic fluid beta-endorphin levels and labor. *Obstetrics & Gynecology*, **69**, 945–947.

Korthank, A.J., & Robinson, S.R. (1998). Effects of amniotic fluid on opioid activity and fetal sensory responsiveness. *Developmental Psychobiology*, **33**, 235–248.

Kozlova, M., & Kalenchuk, V. (1994). Stimulatory effect of opioid peptides and naloxone on rat spinal cord cells in primary dissociated culture. *International Journal of Developmental Neuroscience*, **12**, 507–515.

Kristal, M. B. (1980). Placentophagia: A biobehavioral enigma (or De gustibus non disputandum est). *Neuroscience & Biobehavioral Reviews*, **4**, 141–150.

Kristal, M. B. (1991). Enhancement of opioid-mediated analgesia: A solution to the enigma of placentophagia. *Neuroscience and Biobehavioral Reviews*, **15**, 425–435.

Kristal, M. B., Thompson, A. C., & Abbott, P. (1986). Ingestion of amniotic fluid enhances opiate analgesia in rats. *Physiology & Behavior*, **38**, 809–815.

Kristal, M. B., Thompson, A. C., & Grishkat, H. L. (1985). Placenta ingestion enhances opiate analgesia in rats. *Physiology & Behavior*, **35**, 481–486.

Lai, C. S. L., Fisher, S. E., Hurst, J. A., Vargha-Khadem, F., & Monaco, A. P. (2001). A novel forkhead-domain gene is mutated in a severe speech and language disorder. *Nature*, **413**, 519–523.

Langendijk, P., Bolhuis, J. E., & Laurenssen, B. F. A. (2007). Effects of pre- and postnatal exposure to garlic and anissed flavour on pre- and postweaning feed intake in pigs. *Livestock Science*, **108**, 284–287.

Lau, C., & Henning, S. J. (1985). Investigation of the nature of the "stretch response" in suckling rats. *Physiology & Behavior*, **34**, 649–651.

Lecanuet, J. P., Fifer, W. P., Krasnegor, N. A., & Smotherman, W. P. (1995). *Fetal development: A psychobiological perspective*. Hillsdale, NJ: Erlbaum.

Leslie, F. M., & Loughlin, S. E. (1993). Ontogeny and plasticity of opioid systems. In: R. J. Hammer (Ed.), *The neurobiology of opiates* (pp. 85–123). Boca Raton, FL: CRC Press.

Lev, R., & Orlich, D. (1972). Protein absorption by the intestine of the fetal rat in utero. *Science*, **177**, 522–524.

Lévy, F., & Poindron, P. (1987). The importance of amniotic fluids for the establishment of maternal behaviour in experienced and inexperienced ewes. *Animal Behavior*, **35**, 1188–1192.

Lévy, F., Keller, M., & Poindron, P. (2004). Olfactory regulation of maternal behavior in mammals. *Hormones and Behavior*, **46**, 284–302.

Lévy, F., Poindron, P., & Le Neindre, P. (1983). Attraction and repulsion by amniotic fluids and their olfactory control in the ewe around parturition. *Physiology & Behavior*, **31**, 687–692.

Liu, D., Diorio, J., Day, J. C., Francis, D. D., & Meaney, M. J. (2000). Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nature Neuroscience*, **3**, 799–806.

López, M. F., & Molina, J. C. (1999). Chronic alcohol administration in the rat pup: Effects upon later consumption of alcohol and other palatable solutions. *Addiction Biology*, **4**, 169–179.

Lundblad, E. G., & Hodgen, G. D. (1980). Induction of maternal-infant bonding in rhesus and cynomolgus monkeys after caesarean delivery. *Laboratory Animal Science*, **30**, 913.

Manning, F. A. (1995). *Fetal medicine. Principles and practice*. East Norwalk, CT: Appleton & Lange.

Marlier, L., & Schaal, B. (2005). Human newborns prefer human milk: Conspecific milk odor is attractive without postnatal exposure. *Child Development*, **76**, 155–168.

Marlier, L., Schaal, B., & Soussignan, R. (1998a). Bottle-fed neonates prefer an odor experienced in utero to an odor experienced postnatally in the feeding context. *Developmental Psychobiology*, **33**, 133–145.

Marlier, L., Schaal, B., & Soussignan, R. (1998b). Neonatal responsiveness to the odor of amniotic and lacteal fluids: A test of perinatal chemosensory continuity. *Child Development*, **69**, 611–623.

Marsh, R. H., King, J. E., & Becker, R. F. (1963). Volume and viscosity of amniotic fluid in rat and guinea pig fetuses near term. *American Journal of Obstetrics & Gynecology*, **85**, 487–492.

Maruniak, J. A., Mason, J. R., & Kostelc, J. G. (1983). Conditioned aversions to an intravascular odorant. *Physiology & Behavior*, **30**, 617–620.

Maruniak, J. A., Silver, W. L., & Moulton, D. G. (1983). Olfactory receptors respond to blood-borne odorants. *Brain Research*, **265**, 312–316.

Mauri, A., & Volpe, A. (1994). Stress mediators in the amniotic compartment in relation to the degree of fetal distress. *Fetal Diagnosis & Therapy*, **9**, 300–305.

McDowell, J., & Kitchen, I. (1987). Development of opioid systems: Peptides, receptors, and pharmacology. *Brain Research Reviews*, **12**, 397–421.

Meisel, R. L., & Ward, I. L. (1981). Fetal female rats are masculinized by male littermates located caudally in the uterus. *Science*, **220**, 437–438.

Méndez-Gallardo, V., & Robinson, S. R. (in press). Opioid mediation of amniotic fluid effects on chemosensory responsiveness in the neonatal rat. *Developmental Psychobiology*.

Mennella, J. A., & Beauchamp, G. K. (1991). The transfer of alcohol to human milk: Effects on flavor and the infant's behavior. *New England Journal of Medicine*, **325**, 981–985.

Mennella, J. A., & Beauchamp, G. K. (1998). Early flavor experiences: Research update. *Nutrition Reviews*, **56**, 205–211.

Mennella, J. A., Jagnow, C. P., & Beauchamp, G. K. (2001). Prenatal and postnatal flavor learning by human infants. *Pediatrics*, **107**, 1–6.

Mennella, J. A., Johnson, A., & Beauchamp, G. K. (1995). Garlic ingestion by pregnant women alters the odor of amniotic fluid. *Chemical senses*, **20**, 207–209.

Mickley, G. A., Remmers-Roeber, D. R., Crouse, C., & Peluso, R. (2000). Ketamine blocks a taste recognition memory in fetal rats. *Pharmacology, Biochemistry and Behavior*, **67**, 575–581.

Mickley, G. A., Remmers-Roeber, D. R., Crouse, C., Walker, C., & Dengler, C. (2000). Detection of novelty by perinatal rats. *Physiology & Behavior*, **70**, 217–225.

Moessinger, A. C. (1983). Fetal akinesia deformation sequence: An animal model. *Pediatrics*, **72**, 857–863.

Molina, J. C., Chotro, M. G., & Domínguez, H. D. (1995). Fetal alcohol learning derived from ethanol contamination of the prenatal environment. In J. P. Lecanuet, W. P. Fifer, N. A. Krasnegor, and W. P. Smotherman (Eds.), *Fetal development: A psychological perspective* (pp. 419–438). Hillsdale, NJ: Erlbaum.

Moody, C. A., Robinson, S. R., Spear, L. P., & Smotherman, W. P. (1993). Fetal behavior and the dopamine system: Activity effects of D1 and D2 receptors manipulations. *Pharmacology Biochemistry and Behavior*, **44**, 843–850.

Moore, C. L., (1995). Maternal contributions to mammalian reproductive development and the divergence of males and females. *Advances in the Study of Behavior*, **24**, 47–118.

Morrison, J. R., & Stewart, M. A. (1971). A. family study of the hyperactive child syndrome. *Biological Psychiatry*, **3**, 189–195.

Nelson, E. E., & Panksepp, J. (1998). Brain substrates of infant-mother attachment: Contributions of opioids, oxytocin, and norepinephrine. *Neuroscience and Biobehavioral Reviews*, **22**, 437–452.

Nickerson, K. (2006). Environmental contaminants in breast milk. *Journal of Midwifery & Women's Health*, **51**, 26–33.

Nimrod, C., Varela-Gittings, F., Machin, G., Campbell, D., & Wesenberg, R. (1984). The effect of very prolonged membrane rupture on fetal development. *American Journal of Obstetrics & Gynecology*, **148**, 540–543.

Nolte, D. L. & Provenza, F. D. (1992). Food preferences in lambs after exposure to flavors in milk. *Applied Animal Behaviour Science*, **32**, 381–389.

Nolte, D. L., Provenza, F. D., Callan, R., & Panter, K. E. (1992). Garlic in the ovine fetal environment. *Physiology & Behavior*, **52**, 1091–1093.

Oyama, S., Griffiths, P. E., & Gray, R. D. (Eds.). (2001). *Cycles of contingency: Developmental systems and evolution*. Cambridge: MIT Press/Bradford Books.

Pacifici, G. M. (2005). Sulfation of drugs and hormones in mid-gestation human fetus. *Early Human Development*, **81**, 573–581.

Parfet, K. A., & Gonyou, H. W. (1991). Attraction of newborn piglets to auditory, visual, olfactory and tactile stimuli. *Journal of Animal Science*, **69**, 125–133.

Pedersen, P. E., & Blass, E. M. (1981). Olfactory control over suckling in albino rats. In R. N. Aslin, J. R. Alberts, & M. R. Petersen (Eds.), *The development of perception: Psychobiological processes* (pp. 359–381). Hillsdale, NJ: Erlbaum.

Pedersen, P. E., & Blass, E. M. (1982). Prenatal and postnatal determinants of the 1st suckling episode in albino rats. *Developmental Psychobiology*, **15**, 349–355.

Peleg, D., Arbogast, L. A., Peleg, E., & Ben-Jonathan, N. (1984). Predominance of L-dopa in fetal plasma and the amniotic fluid during late gestation in the rat. *American Journal of Obstetrics and Gynecology*, **149**, 880–883.

Peleg, D., Munsick, R. A., Diker, D., Goldman, J. A., & Ben-Jonathan, N. (1986). Distribution of catecholamines between fetal and maternal compartments during human pregnancy with emphasis on L-dopa and dopamine. *Journal of Clinical Endocrinology and Metabolism*, **62**, 911–914.

Perez-Navarro, E., Alberch, J., Arenas, E., & Marsal, J. (1993). Nerve growth factor and its receptor are differentially modified by chronic naltrexone treatment during rat brain development. *Neuroscience Letters*, **149**, 47–50.

Petrillo, P., Tavani, A., Verotta, D., Robson, L. E., & Kosterlitz, H. W. (1987). Differential postnatal development of μ-, δ- and κ-opioid binding sites in rat brain. *Developmental Brain Research*, **31**, 53–58.

Petrov, E. S., Varlinskaya, E. I., Bregman, K., & Smotherman, W. P. (1999). Sustained attachment to the nipple in the newborn rat depends on experience with the nipple, milk, and expression of oral grasping. *Behavioral Neuroscience*, **113**, 211–221.

Petrov, E. S., Varlinskaya, E. I., Robinson, S. R., & Smotherman, W. P. (1994). Kappa opioid effects on fetal behavior: Central administration of U50,488. *Physiology & Behavior*, **56**, 175–182.

Petrucha, R. A., Goebelsmann, U., Hung, T. T., Haase, H. R., & Lobo, R. A. (1983). Amniotic fluid beta-endorphin and beta-lipotropin concentrations during the second and third trimesters. *American Journal of Obstetrics and Gynecology*, **146**, 644–651.

Phillippe, M., & Ryan, K. J. (1981). Catecholamines in human amniotic fluid. *American Journal of Obstetrics and Gynecology*, **139**, 204–208.

Rahman, W., Dashwood, M. R., Fitzgerald, M., Aynsley-Green, A., & Dickenson, A. H. (1998). Postnatal development of multiple opioid receptors in the spinal cord and development of spinal morphine analgesia. *Developmental Brain Research*, **108**, 239–254.

Robertson, S. S. (1987). Human cyclic motility: Fetal-newborn continuities and newborn state differences. *Developmental Psychobiology*, **20**, 425–442.

Robinson, S. R. (1989). *A. comparative study of prenatal behavioral ontogeny in altricial and precocial murid rodents*. Unpublished doctoral dissertation, Oregon State University, Corvallis, OR.

Robinson, S. R., Arnold, H. M., Spear, N. E., & Smotherman, W. P. (1993). Experience with milk and an artificial nipple promotes conditioned opioid activity in the rat fetus. *Developmental Psychobiology*, **26**, 375–387.

Robinson, S. R., Blumberg, M. S., Lane, M. S., & Kreber, L. A. (2000). Spontaneous motor activity in fetal and infant rats is organized into discrete multilimb bouts. *Behavioral Neuroscience*, **114**, 328–336.

Robinson, S. R., & Brumley, M. R. (2005). Prenatal behavior. In I. Q. Whishaw & B. Kolb (Eds.), *The behaviour of the laboratory rat: A Handbook with tests* (pp. 257–265). Oxford: Oxford University Press.

Robinson, S. R., Hoeltzel, T. C. M., Cooke, K. M., Umphress, S. M., Murrish, D. E., & Smotherman, W. P. (1992). Oral capture and grasping of an artificial nipple by rat fetuses. *Developmental Psychobiology*, **25**, 543–555.

Robinson, S. R., Moody, C. A., Spear, L. P., & Smotherman, W. P. (1993). Effects of dopamine and kappa opioid receptors on fetal responsiveness to perioral stimuli. *Developmental Psychobiology*, **26**, 37–50.

Robinson, S. R., & Smotherman, W. P. (1991). Fetal learning: Implications for the development of kin recognition. In P. G. Hepper (Ed.), *Kin recognition* (pp. 308–333). Cambridge: Cambridge University Press.

Robinson, S. R., & Smotherman, W. P. (1992a). The emergence of behavioral regulation during fetal development. In G. Turkewitz (Ed.), *Developmental psychobiology. Annals of the New York Academy of Sciences*, **662**, 53–83.

Robinson, S. R., & Smotherman, W. P. (1992b). Motor competition in the prenatal ontogeny of species-typical behaviour. *Animal Behaviour*, **44**, 89–99.

Robinson, S. R., & Smotherman, W. P. (1994). Behavioral effects of milk in the rat fetus. *Behavioral Neuroscience*, **108**, 1139–1149.

Robinson, S. R., & Smotherman, W. P. (1995). Habituation and classical conditioning in the rat fetus: Opioid involvements. In J.-P. Lecanuet, N. A. Krasnegor, W. P. Fifer, & W. P. Smotherman (Eds.), *Fetal development: A psychobiological perspective* (pp. 295–314). New York: Erlbaum.

Robinson, T. M., Abbott, P., & Kristal, M. B. (1995). Blockade of digestion by famotidine pretreatment does not interfere with the opioid-enhancing effect of ingested amniotic fluid. *Physiology & Behavior*, **57**, 261–263.

Ross, M. G., & Brace, R. A. (2001). National Institute of Child Health and Development Conference summary: Amniotic fluid biology – basic and clinical aspects. *The Journal of Maternal-Fetal Medicine*, **10**, 2–19.

Ross, M. G., & Nijland, M. J. M. (1997). Fetal swallowing: Relation to amniotic fluid regulation. *Clinical Obstetrics and Gynecology*, **40**, 352–365.

Ryan, B. C., & Vandenbergh, J. G. (2002). Intrauterine position effects. *Neuroscience and Biobehavioral Reviews*, **26**, 665–678.

Sanes, J. R., & Lichtman, J. W. (2001). Induction, assembly, maturation and maintenance of a postsynaptic apparatus. *Nature Reviews Neuroscience*, **2**, 791–805.

Schaal, B. (2005). From amnion to colostrum to milk: Odor bridging in early developmental transitions. In B. Hopkins & S. P. Johnson (Eds.), *Prenatal development of postnatal functions* (Advances in Infancy Research series) (pp. 51–102). Westport, CT: Praeger Publishers.

Schaal, B., Coureaud, G., Langlois, D., Ginies, C., Semon, E., & Perier, G. (2003). Chemical and behavioral characterization of the mammary pheromone of the rabbit. *Nature*, **424**, 68–72.

Schaal, B., Doucet, S., Sagot, P., Hertling, E., & Soussignan, R. (2006). Human breast areolae as scent organs: Morphological data and possible involvement in maternal-neonatal coadaptation. *Developmental Psychobiology*, **48**, 100–110.

Schaal, B., Marlier, L., & Soussignan, R. (1995). Responsiveness to the odour of amniotic fluid in the human neonate. *Biology of the Neonate*, **67**, 397–406.

Schaal, B., Marlier, L., & Soussignan, R. (2000). Human foetuses learn odours from their pregnant mother's diet. *Chemical Senses*, **25**, 729–737.

Schaal, B., & Orgeur, P. (1992). Olfaction in utero: Can the rodent model be generalized? *Quarterly Journal of Experimental Psychology*, **44B**, 245–278.

Schaal, B., Orgeur, P., & Arnould, C. (1995). Olfactory preferences in newborn lambs: Possible influence of prenatal experience. *Behavior*, **132**, 351–365.

Semke, E., Distel, H., & Hudson, R. (1995). Specific enhancement of olfactory receptor sensitivity associated with foetal learning of food odors in the rabbit. *Naturwissenschaften*, **82**, 148–149.

Shatz, C. J. (1990). Impulse activity and the patterning of connections during CNS development. *Neuron*, **5**, 745–756.

Shepanek, N. A., Smith, R. F., Anderson, L. A., & Medici, C. N. (1995). Behavioral and developmental changes associated with prenatal opiate receptor blockade. *Pharmacology, Biochemistry & Behavior*, **50**, 313–319.

Shepanek, N. A., Smith, R. F., Tyer, Z. E., Royall, G. D., & Allen, K. S. (1989). Behavioral and neuroanatomical sequelae of prenatal naloxone administration in the rat. *Neurotoxicology & Teratology*, **11**, 441–446.

Simitzis, P. E., Deligeorgis, S. G., Bizelis, J. A., & Fegeros, K. (2008). Feeding preferences in lambs influenced by prenatal flavour exposure. *Physiology & Behavior*, **93**, 529–536.

Sival, D. A., Visser, G. H. A., & Prechtl, H. F. R. (1990). Does reduction of amniotic fluid affect fetal movements? *Early Human Development*, **23**, 233–246.

Smotherman, W. P. (1982). In utero chemosensory experience alters taste preferences and corticosterone responsiveness. *Behavioral and Neural Biology*, **36**, 61–68.

Smotherman, W. P., Moody, C. A., Spear, L. P., & Robinson, S. R. (1993). Fetal behavior and the endogenous opioid system: D1 Dopamine receptor interactions with the kappa opioid system. *Physiology & Behavior*, **53**, 191–197.

Smotherman, W. P., & Robinson, S.R. (1986). Environmental determinants of behaviour in the rat fetus. *Animal Behaviour*, **34**, 1859–1873.

Smotherman, W. P., & Robinson, S.R. (1987a). Prenatal expression of species-typical action patterns in the rat fetus (*Rattus norvegicus*). *Journal of Comparative Psychology*, **101**, 190–196.

Smotherman, W. P., & Robinson, S. R. (1987b). Psychobiology of fetal experience in the rat. In N. A. Krasnegor, E. M. Blass, M. A. Hofer, & W. P. Smotherman (Eds), *Perinatal Behavioral Development: A Psychobiological Perspective* (pp. 39–60). New York: Academic Press.

Smotherman, W. P., & Robinson, S. R. (1988a). Behavior of rat fetuses following chemical or tactile stimulation. *Behavioral Neuroscience*, **102**, 24–34.

Smotherman, W. P., & Robinson, S. R. (1988b). The uterus as environment: The ecology of fetal experience. In E. M. Blass (Ed.), *Handbook of behavioral neurobiology: Vol. 9. Developmental psychobiology and behavioral ecology* (pp. 149–196). New York: Plenum Press.

Smotherman, W. P., & Robinson, S. R. (1989). Cryptopsychobiology: The appearance of a species-typical action pattern during early development. *Behavioral Neuroscience*, **103**, 246–253.

Smotherman, W. P., & Robinson, S. R. (1990). Olfactory bulb transection alters fetal behavior after chemosensory but not tactile stimulation. *Developmental Brain Research*, **57**, 175–180.

Smotherman, W. P., & Robinson, S. R. (1991). Accessibility of the rat fetus for psychobiological investigation. In H. N. Shair, M. A. Hofer, & G. Barr (Eds.), *Developmental psychobiology: New methods and changing concepts* (pp. 148–164). New York: Oxford University Press.

Smotherman, W. P., & Robinson, S. R. (1992a). Kappa opioid mediation of fetal responses to milk. *Behavioral Neuroscience*, **106**, 396–407.

Smotherman, W. P., & Robinson, S. R. (1992b). Dimethyl disulfide mimics the effects of milk on fetal behavior and responsiveness to cutaneous stimuli. *Physiology and Behavior*, **52**, 761–765.

Smotherman, W. P., & Robinson, S. R. (1992c). Opioid control of the fetal stretch response: Implications for the first suckling episode. *Behavioral Neuroscience*, **106**, 866–873.

Smotherman, W. P., & Robinson, S. R. (1992d). Prenatal experience with milk: Fetal behavior and endogenous opioid systems. *Neuroscience & Biobehavioral Reviews*, **16**, 351–364.

Smotherman, W. P., & Robinson, S. R. (1994). Classical conditioning of opioid activity in the fetal rat. *Behavioral Neuroscience*, **108**, 951–961.

Smotherman, W. P., & Robinson, S. R. (1998). Prenatal ontogeny of sensory responsiveness and learning. In G. Greenberg & M. Haraway (Eds.), *Comparative psychology: A handbook* (pp. 586–601). New York: Garland Publishing.

Smotherman, W. P., Robinson, S. R., & Robertson, S. S. (1988). Cyclic motor activity in the fetal rat. *Journal of Comparative Psychology*, **102**, 78–82.

Smotherman, W. P., Robinson, S. R., Varlinskaya, E. I., Petrov, E. S., Orlowski, M., de Costa, B. R., et al. (1994). Central administration of the endopeptidase inhibitor cFP-AAF-pAB suggests dynorphin as the endogenous ligand underlying behavioral effects of milk in the fetal rat. *Pharmacology Biochemistry & Behavior*, **47**, 715–719.

Smotherman, W. P., Simonik, D. K., Andersen, S. L., & Robinson, S. R. (1993). Mu and kappa opioid systems modulate responses to cutaneous perioral stimulation in the fetal rat. *Physiology & Behavior*, **53**, 751–756.

Spain, J. W., Roth, B. L., & Coscia, C. J. (1985). Differential ontogeny of multiple opioid receptors (μ, ∂, and κ). *The Journal of Neuroscience*, **5**, 584–588.

Spear, N. E., & Molina, J. C. (2005). Fetal or infantile exposure to ethanol promotes ethanol ingestion in adolescence and adulthood: A theoretical review. *Alcoholism: Clinical and Experimental Research*, **29**, 909–929.

Spencer, J. P., Blumberg, M. S., McMurray, B., Robinson, S. R., Samuelson, L. K., & Tomblin, J. B. (2009). Short arms and talking eggs: Why we should not longer abide the nativist-empiricist debate. *Child Development Perspectives*, **3**, 79–87.

Strott, C. A. (2002). Sulfonation and molecular action. *Endocrine Reviews*, **23**, 703–732.

Tam, P. P. L., & Chan, S. T. H. (1977). Changes in the composition of maternal plasma, fetal plasma and fetal extraembryonic fluid during gestation in the rat. *Journal of Reproduction and Fertility*, **51**, 41–51.

Tang-Martinez, Z. (2001). The mechanisms of kin discrimination and the evolution of kin recognition in vertebrates: a critical re-evaluation. *Behavioral Processes*, **53**, 21–40.

Tarapacki, J. A., Thompson, A. C., & Kristal, M. B. (1992). Gastric vagotomy blocks opioid analgesia enhancement produced by placenta ingestion. *Physiology & Behavior*, **52**, 179–182.

Teicher, M. H., & Blass, E. M. (1977). First suckling response of the newborn albino rat: The roles of olfaction and amniotic fluid. *Science*, **198**, 635–636.

Terry, L. M., & Johanson, I. B. (1987). Olfactory influences on the ingestive behavior of infant rats. *Developmental Psychobiology*, **20**, 313–332.

Thompson, W. (1983). Synapse elimination in neonatal rat muscle is sensitive to pattern of muscle use. *Nature*, **302**, 614–616.

Underwood, M. A., Gilbert, W. M., & Sherman, M. P. (2005). Amniotic fluid: Not just fetal urine anymore. *Journal of Perinatology*, **25**, 341–348.

Valette, A., Desprat, R., Cros, J., Pontonnier, G., Belisle, S., & Lemaire, S. (1986). Immunoreactive dynorphine in maternal blood, umbilical vein and amniotic fluid. *Neuropeptides*, **7**, 145–151.

Varendi, H., Porter, R. H., & Winberg, J. (1996). Attractiveness of amniotic odor: Evidence for prenatal olfactory learning? *Acta Paediatrica*, **85**, 1223–1227.

Varlinskaya, E. I., Petrov, E. S., & Smotherman, W. P. (1996). Classical conditioning in the fetal rat: Reinforcing properties of dynorphin A (1-13). *Behavioral Neuroscience*, **110**, 154–167.

Vince, M. A., Lynch, J. J., Mottershead, B., Green, G., & Elwin, R. (1985). Sensory factors involved in immediately postnatal ewe/lamb bonding. *Behaviour*, **94**, 60–84.

Vince, M. A., & Ward, T. M. (1984). The responsiveness of newly born Clun Forest lambs to odour sources in the ewe. *Behaviour*, **89**, 117–127.

Vitaioli, L., Bondi, A. M., Menghi, G., & Materazzi, G. (1981). Localization of the activity of arylsulphatases A and B in the rat salivary glands. *Acta Histochemica*, **69**, 70–76.

vom Saal, F. S., & Bronson, F. H. (1980). Sexual characteristics of adult female mice are correlated with their blood testosterone levels during prenatal development. *Science*, **208**, 597–599.

Waites, C. L., Craig, A. M., & Garner, C. C. (2005). Mechanisms of vertebrate synaptogenesis. *Annual Reviews of Neuroscience*, **28**, 251–274.

Weaver, I. C., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., et al. (2004). Epigenetic programming by maternal behavior. *Nature Neuroscience*, **7**, 847–854.

Weiler, E., & Farbman, A. I. (2003). The septal organ of the rat during postnatal development. *Chemical Senses*, **28**, 581–593.

Wells, D. L., & Hepper, P. G. (2006). Prenatal olfactory learning in the domestic dog. *Animal Behaviour*, **72**, 681–686.

West, M. J., King, A. P., & Arberg, A. A. (1988). The inheritance of niches: The role of ecological legacies in ontogeny. In E. M. Blass (Ed.), *Handbook of behavioral neurobiology: Vol. 9. Developmental psychobiology and behavioral ecology* (pp. 41–62). New York: Plenum Press.

Wirtschafter, Z. T., & Williams, D. W. (1957). The dynamics of protein changes in the amniotic fluid of normal and abnormal rat embryos. *American Journal of Obstetrics and Gynecology*, **74**, 1022–1028.

Zagon, I. S., & McLaughlin, P. J. (1984). Naltrexone modulates body and brain development in rats: A role for endogenous opioid systems in growth. *Life Sciences*, **35**, 2057–2064.

Zagon, I. S., & McLaughlin, P. J. (1987). Endogenous opioid systems regulate cell proliferation in the developing rat brain. *Brain Research*, **412**, 68–72.

Zagon, I. S., Verderame, M. F., & McLaughlin, P. J. (2002). The biology of the opioid growth factor receptor (OGFr). *Brain Research Reviews*, **38**, 351–376.

10

Developmental Effects of Selective Breeding for an Infant Trait

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Introduction

This chapter describes the results of our selective breeding study in terms of its relevance for understanding the development of temperament and attachment in humans. More than that, our motivation for embarking on this long-term project stemmed from our curiosity about the possible developmental consequences of repeated selection over many generations for an infant trait and, more broadly, our interest in the relationship between development and evolution. As Gilbert Gottlieb stated in his (2007) article, "Probabilistic Epigenesis": "The basic notion here is that the emergent products of development are epigenetic, not just genetic, and this continues to be the case even when we are considering the evolutionary process" (p. 9).

Specifically, we asked what would happen if we selectively bred infant rats for high and low levels of their ultrasonic vocalization responses to isolation, a behavior that we had been studying for some time. Our curiosity was further stimulated by our growing realization that there did not seem to be any reports in the literature on the effects of selective breeding for a mammalian infant trait.

We wanted to think of our project as not just an experiment, but as a microcosm of evolution, captured within the laboratory. In one scenario, we envisioned that a predator appeared on the scene and only the pups in each litter with the lowest rate of calling survived detection to produce offspring in the next generation. In the other scenario, a change in wind and weather conditions resulted in more frequent home nest disruption, and when pups became widely isolated from the nest, only those that vocalized at the highest rates were found by the mother, retrieved to the nest, and survived to reproduce in the next generation. Of course, in our

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experiments, litter size was maintained after all pups were tested briefly in isolation at 10 days of age, and the selection took place at the time of breeding, based on the infant phenotype.

Gilbert Gottlieb would have reminded us that we must also consider the "non-evident" environmental/behavioral influences on the development of vocalization that are also inherited by our infants. He would maintain that high or low calling rates generated by selective breeding are not determined either simply or entirely by different genetic alleles accumulating in the two selected lines. The essential question that we kept asking ourselves is "what are we selecting for?" We realized that we were selecting for differences in neural systems mediating the early anxiety and arousal responses of the infants to isolation, within which the vocalization response is embedded. We knew that we must also consider the environmental contributions to the development of these systems, for variation in sensitivity to developmental events prior to testing are also potential targets for selection. We remembered vividly Gottlieb's research in ducklings on the unexpected importance of *pre-hatching* experience with their own and nestmates' vocalizations, for the later postnatal appearance of their capacity to respond to the species-typical maternal call. Could selection be acting on variations in pre- or post-natal maternal care toward their (differently) selected infants?

Given these several possibilities for how repeated selection could change infant behavior in a population, and the periods of development over which it could be acting, we were also eager to look for possible long-range developmental effects in adults of our two lines. Although, as far as we could ascertain, Gilbert did not consider the possibility of selective breeding for an infant trait, he emphasized repeatedly how early developmental processes create variation for selection to act upon. He credited St. George Mivert with the first statement, in 1871, of the idea that: "changes relevant to evolution occur during ontogeny" (Gottlieb, 1992, p. 47). But perhaps the best statements he quotes came from Walter Garstang a half century later: "that ontogeny does not recapitulate phylogeny, it creates it" (ibid, p. 90); "The real phylogeny of metazoans has never been a direct succession of adult forms, but a succession of ontogenies or life cycles" (Garstang, 1922, p. 82, quoted in Gottlieb, 1992, p. 90).

The results of our studies reported below show that long-term selection for different rates of infant isolation calling produces populations with different developmental "paths," that appear to recruit a variety of physiological systems and behaviors to produce lifelong differences in temperament. It is our loss that Gilbert Gottlieb did not live to see and comment on these widespread, enduring developmental effects of selection for a single infant behavior. For selective breeding is generally supposed to produce only limited and discrete changes in the genotype (and phenotype) within a population. We like to think that Gilbert would have enjoyed our finding that when selection is applied in early development, it can indeed produce new "ontogenies or life cycles", as Garstang (and Gottlieb) would have predicted.

Selective Breeding for "Temperamental" Traits

As in much of animal research, the major reason for embarking on a selective breeding program is to provide an animal model for a human disorder. The goal of selective breeding in an animal model is quite specific, which is to model an aspect or a component of a disorder that is believed to depend upon genetic variation in the human population. Animals are bred on the basis of high and low values of a relevant trait or phenotype that is expected to show genetic variation in the animal population. The expectation is that after many generations, the high and low lines will be "enriched" by genes facilitating the high or low phenotype; in contrast, genes that do not facilitate the selected phenotype will become less frequent over generations, thus increasing differences between lines (Plomin, DeFries, & McClearn, 1991; Snustad, Simmons, & Jenkins, 1997).

The developmental research model historically used in our laboratory has been the separation of infant rats from mother and littermates, which has provided numerous insights into the biological and behavioral regulation of infant development by the mother, and cardiovascular regulation in particular (Hofer, 1984, 1994). More recently, this research has focused on the development of the vocalization response to separation in infant rats as a model of affective regulation by the mother (Hofer, 1996). In order to create such a developmental-genetic model system, we used a novel modification of the selective breeding strategy in which the selected trait was an infantile rather than an adult behavior. The idea was to manipulate age-specific gene expression to test hypotheses that behavioral temperamental differences might predispose animals to exhibit behavioral and/or autonomic nervous system dysregulation early in development (Brunelli & Hofer, 2001). Even more interesting from a developmental perspective was the possibility of uncovering epigenetic processes that underlie predispositions for constellations of affective regulation, cognition, behavior, and biological processes ("endophenotypes"; Gould & Gottesman, 2006) that are expressed as "temperament" across the lifespan. Quite specifically we were modeling what appeared to be temperamental differences in children, uncovered by Jerome Kagan and his colleagues. The selective breeding study to be described is one attempt to examine these relationships.

Temperament in Children: Developmental Stability and Inheritance

Behavioral inhibition in childhood is a pattern of responding in which the child shows anxiety, distress, or caution in response to novelty (Biederman et al., 1993; Kagan, Reznick, & Gibbons, 1989; Kagan, Snidman, Zentner, & Peterson, 1999). Behavioral inhibition has been suggested to be a *temperamental trait*, defined as

biologically based stability of affect and behavior over the course of childhood through adolescence and into adulthood (Schwartz, Snidman, & Kagan, 1999). Thus, toddlers exhibiting behavioral inhibition continue to be inhibited as children, and are far more likely to manifest anxiety disorders as adolescents (Biederman et al., 1993; Schwartz et al., 1999). Adults that had been categorized as inhibited in childhood exhibit greater functional MRI response in the amygdala to novel versus familiar faces, consistent with greater fear to novelty and anxiety, and suggesting that behavioral inhibition represents a lifelong trait with neurophysiological correlates (Rosenbaum et al., 1993; Schwartz, Wright, Shin, Kagan, & Rauch, 2003).

Behaviorally inhibited children show significant differences in heart rates in a variety of situations and across ages (Marshall & Stevenson-Hinde, 1998; Stevenson-Hinde & Marshall, 1999; Stevenson-Hinde & Shouldice, 1995). Fetuses of highly anxious women show significant heart rate increases during a maternal stressor, whereas the fetuses of non-anxious women exhibit minor heart rate decreases (Monk et al., 2000; Monk et al., 2004; Monk, Myers, Sloan, & Fifer, 2003). Infant risk for depression and anxiety based on family associations are associated with higher heart rates and lower heart rate variability in response to stress (Yeragani, 1995; Yeragani, Rao, Pohl, Jampala, & Balon, 2001). Hyperarousal attributable to greater sympathetic activation appears to be a characteristic of anxious individuals, and particularly in children and adolescents (Biederman et al., 1990; Greaves-Lord et al., 2007; Kagan et al., 1989; Kagan et al., 1999; Yeragani et al., 2001; Yeragani, Pohl, Balon, Jampala, & Jayaraman, 2002). Consistent with this hyper-aroused phenotype, the corticotrophin releasing hormone gene has recently been linked with childhood behavioral inhibition (Schmidt, Fox, Rubin, Hu, & Hamer, 2002; Calkins & Fox, 1992; Smoller et al., 2003; Smoller et al., 2005), suggesting up-regulation of the hypothalamic–pituitary–adrenal axis (HPA), responsiveness to stress. Thus, an inherited predisposition for early anxious temperament appears to produce a constellation of characteristics involving behavioral and physiological components predictive of later childhood and adult anxiety and depression disorders (Fox et al., 2005; Warren et al., 2003).

In parallel with temperamental predisposition, insecure attachment behavior is also associated with inhibition and anxiety. Infants who at 4 months were categorized as insecurely attached, Anxious/Ambivalent (Type C) are more likely to inhibited and anxious in childhood (Kochanska, 1998, 2001; Shamir-Essakow, Ungerer, & Rapee, 2005; Stevenson-Hinde & Shouldice, 1995). Infant and child attachment classifications are associated with specific parenting styles (Dallaire & Weinraub, 2005), signifying that even the earliest manifestations of individual differences in behavior must also be considered in the context of parent-child interactions. Regardless of origin, pathologically increased separation anxiety in children has been associated with earlier onset of adult social phobias and panic disorder.

A significant percentage of children of mothers diagnosed with anxiety disorders exhibit Anxious/Ambivalent attachment (Type C), behavioral inhibition, or anxiety disorders (Manassis, Bradley, Goldberg, Hood, & Swinson, 1995). Likewise

children of parents with panic or major depressive disorder exhibit higher rates of behavioral inhibition (Biederman et al., 1990) and are at greater risk for childhood separation and anxiety disorders (Warren et al., 2003; Wickramaratne, Greenwald, & Weissman, 2000; Yeragani et al., 2001). The interaction between attachment and temperament can be seen in cardiac physiology as well: infants assessed in the first year of life for behavioral inhibition were at 14 and 24 months highly likely to be classified as Anxious/Ambivalent, and exhibited lower heart rate variability that could be associated with high sympathetic tone or low vagal tone (Calkins & Fox, 1992; Stevenson-Hinde & Marshall, 1999). Separation anxiety is far more frequent in children from families with cross-generational depressive and anxiety disorders (Weissman et al., 2005; Wickramaratne, Greenwald, & Weissman, 2000). Strongly suggestive that early separation anxiety is a marker for early affective dysregulation associated with temperament, these and other data also suggest a relationship between inherited characteristics of the child and parenting style in families either predisposed to or actively suffering from affective disorders ("gene-environment correlation"; Caspi et al., 2002, Caspi et al., 2003; Hill, 2002; Manassis et al., 1995; Rutter, Moffitt, & Caspi, 2005; Shamir-Essakow et al. 2005; Stams, Juffer, & van IJzendoorn, 2002). This gene-environment association may include a wider environment consisting of psychosocial family characteristics, and socio-demographic variables (Rutter et al., 2005).

At the other end of the spectrum of childhood disorders, aggression is a trait-like phenomenon that also runs in families, with ample evidence for interactions between environmental and genetic influences (Burgess, Marshall, Rubin, & Fox, 2003; Caspi et al., 2002; Farrington, 1989; Haberstick, Schmitz, Young, & Hewitt, 2005; Hill, 2002; Rutter et al., 2005). In keeping with the notion of temperamental continuity, childhood and juvenile aggressive psychopathologies are associated with high levels of violence in adulthood (Farrington, 1989). A number of studies have shown associations between childhood aggression and attention-deficit-hyperactivity symptoms, and suggest that the two characteristics combined are more likely to produce lifelong aggressive disorders than either one alone (Biederman et al., 1996; Caspi et al., 2003; Caspi, Moffitt, Newman, & Silva, 1996; Faraone, Biederman, Keenan, & Tsuang, 1991).

As with inhibited temperament, insecure attachment behavior is associated with child aggression. Children who at early ages (12–24 months) are categorized as showing Avoidant Attachment (A) or Avoidant-Disorganized (A-D) attachment are also more likely to manifest an uninhibited temperament, to show more activity, and more aggressive and disruptive behaviors in later childhood (Belsky & Fearon, 2002; Burgess et al., 2003). Such children also appear to be more vulnerable to environments that increase the risk of child conduct disorder and adult antisocial behavior (Caspi et al., 2003; Haberstick et al., 2005).

Finally, young children showing uninhibited temperament and Avoidant Attachment are more likely to exhibit higher aggression scores and higher respiratory sinus arryhythmia, indicative of reduced parasympathetic control of

heart rate (Burgess et al., 2003). Similar relationships have been shown between autonomic activity and a history of aggression in older samples of children (Allen, Matthews, & Kenyon, 2000; Schneider, Nicolotti, & Delamater, 2002). In a study of 7-11-year-old boys, and in aggressive adolescents, heart rate variability was inversely related to hostility and aggression, again indicative of reduced parasympathetic (vagal) antagonism to increases in heart rate (Pine et al., 1996; Pine et al., 1998). It has long been known that hostile-aggressive adults exhibit the same relationship with autonomic control of heart rate and blood pressure: this mode of reduced vagal control of heart rate and blood pressure is a significant predictor of cardiovascular disease (Sloan, Shapiro, Bagiella, Myers, & Gorman, 1999). Thus, studies in children have established that early relationships between aggression and reduced autonomic control of cardiovascular functioning are highly likely to be a marker for adult cardiovascular disease.

Selective Breeding for High and Low Rates of Separation-Induced Infant Vocalizations

As with other infant mammals, infant rodents (pups) cry initially when separated from mothers (dams) and littermates; these cries peak at 45 kHz and are therefore called ultrasonic vocalizations (USV). Rat and mouse pups, like other mammalian species, also experience maternal separation as a stressful event, producing increased autonomic nervous system activity and cardiovascular changes, activation of the hypothalamic–pituitary–adrenal (HPA) axis, and a ramping up of noradrenergic and opioid system activity (Blass & Kehoe, 1987; Harvey & Hennessy, 1995; Hennessy & Weinberg, 1990; Hofer, 1984). Thus, in response to the threat posed by separation, USV appears to be a behavioral component of an overall coordinated defensive system in rodent pups (Hofer, 1984, 1994).

Over several decades, infant rat pup USV responses to maternal separation have been well characterized pharmacologically, and studies are generally in agreement that these ultrasonic cries represent an anxiety-like state (Brunelli & Hofer, 2001; Hofer, 1994). The question has always been whether USV can be used as a model system for infant separation responses, as described by Bowlby (1969) and others. Notwithstanding caveats addressed to whether or not rats experience so complex a construct as attachment, the behavioral and physiological profiles evident in infant rodents when separated from dams and littermates show a great deal of face and construct validity with other mammalian/human separation responses.

Our interest in USV as a marker for early affective regulation of attachment prompted us to embark on a selective breeding project that would test the hypothesis that rate of infant USV in response to separation was heritable (Brunelli, 2005). Behavior-genetic studies in mice had established that the propensity for high or low rates of USV is a heritable trait based on significant dominance and additive components, as well as interactions between genes (Hahn,

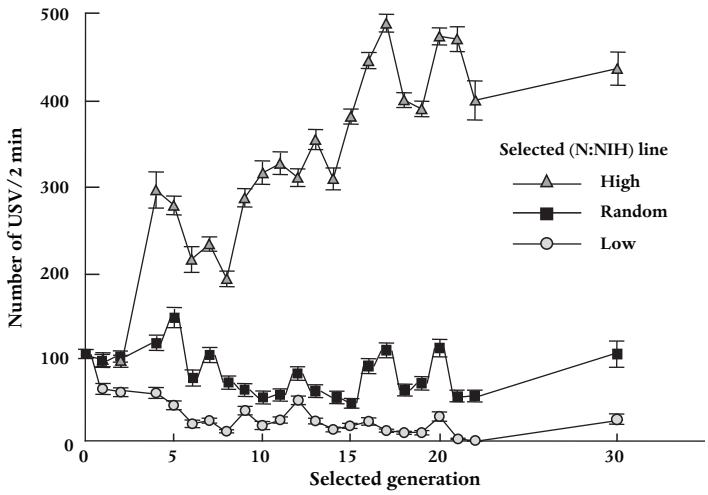

Figure 10.1. Mean (±SEM) number of USV in P10 pups for 2 min (*y*-axis) over 30 generations (*x*-axis). Low and High USV lines diverged significantly in rates of USV from the Randomly-bred control line in the S1 and S3 generations respectively. Data based on litter means.

Hewitt, Schanz, Weinreb, & Henry, 1997; Roubertoux et al., 1995). Since no previous studies had addressed whether the behavior was itself a marker for more general and permanent predispositions, a second goal of this project was to determine whether rate of infant USV in response to separation was a component of a heritable temperamental trait expressed throughout life (Brunelli, 2005).

We therefore selectively bred for two lines of rats (High and Low USV lines) for extreme differences in rates of USV in response to maternal separation in infancy. Figure 10.1 shows rates of USV in response to 2 minutes of isolation in postnatal day 10 (P10) pups over 30 generations. As shown, rates of USV in High and Low USV line pups diverged dramatically from the randomly-bred control line (Random USV line) and each other, indicative of major gene effects (Brunelli, 2005) and were maintained for 30 generations. With respect to the second goal, the following sections describe how High and Low USV lines have shown extensive and distinctly different changes in systems mediating affective regulation from infancy to adulthood, consistent with differences in early temperament.

Early Differences in Brain Monoamine Systems

A number of neurotransmitters have been implicated in the modulation of infant rat isolation-induced USV and in adult rat anxiety-related behaviors. Among these, influences of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) systems have been most systematically observed in rat pups (Brunelli & Hofer, 2001; Hofer, 1984). Because High and Low USV line rat pups show such extraordinary

extremes of USV to isolation at P10, changes in at least one of these systems seemed likely. Therefore, we examined line differences in levels and indices of activity in the three monoamine systems (Brunelli & Kehoe, 2005).

We found widespread changes in DA and 5-HT metabolism throughout cortico- and meso-limbic structures in P10 pups in both the High and Low USV lines (Figures 10.2 and 10.3). In all cases, one or both selected lines exhibited increases in

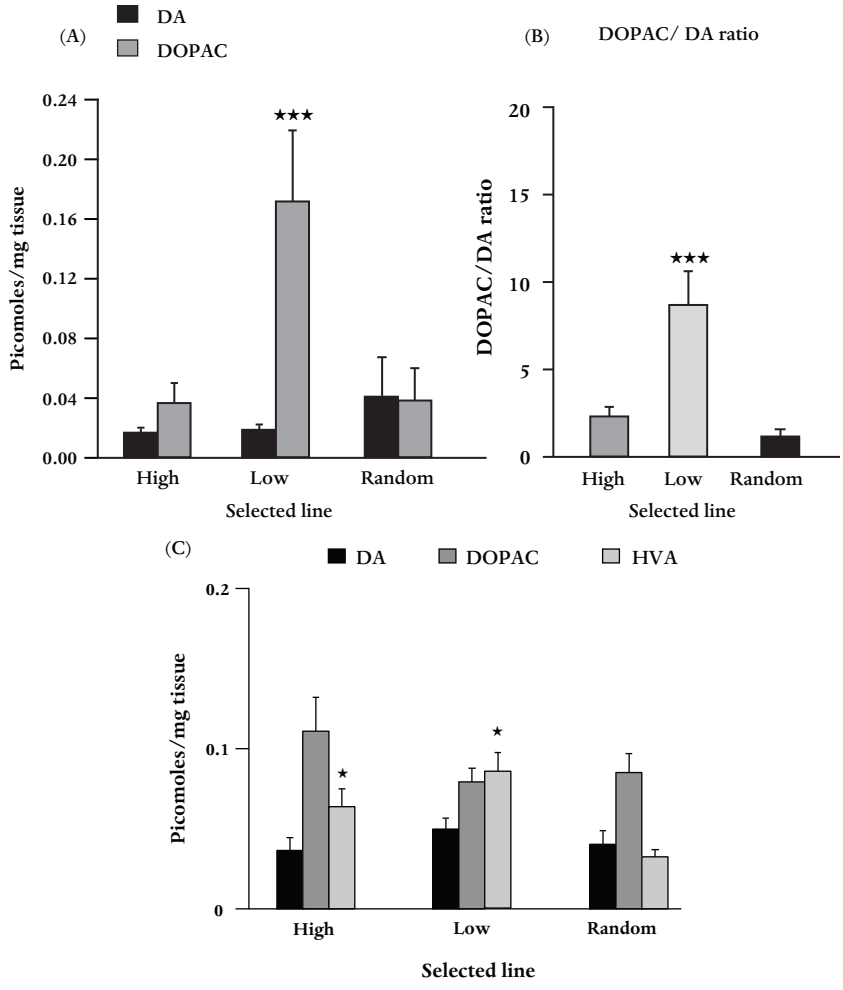

Figure 10.2. (A) Mean (±SEM) DA and DOPAC levels in anterior cingulate cortex (ACC), measured as picograms per milligram of tissue ($n = 12$ pups: All $ps < .01$ per line). Low line DOPAC significantly greater than High and Random lines). (B) DOPAC/DA ratio in ACC. Low line significantly greater than High and Random lines. (C) Mean (±SEM) DA, DOPAC and HVA levels in periaqueductal gray (PAG), measured as picograms per milligram of tissue. High and Low line HVA significantly greater than Random. (Adapted from Brunelli & Hofer, 2007).

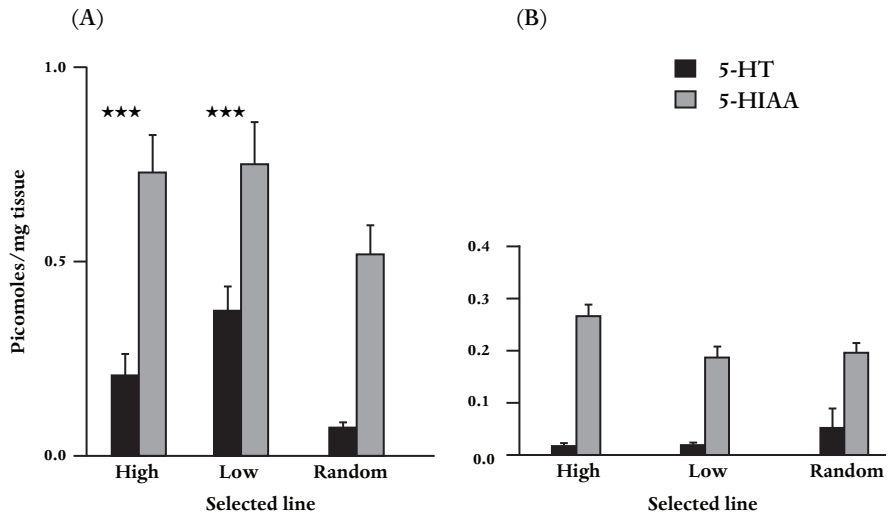

Figure 10.3. (A) Mean (±SEM) levels of 5-HT (picograms per milligram of tissue) and 5-HT turnover measured as levels of 5-HIAA (picograms per milligram of tissue) in the bed nucleus of the stria terminalis (BNST). (B) Mean (±SEM) levels of 5-HT (picograms per milligram of tissue) and 5-HT turnover in striatum (caudate ± putamen) and nucleus accumbens (NA). High line levels of 5-HIAA significantly greater than Low and Random lines. (Adapted from Brunelli & Hofer, 2007).

monoamine turnover. These differences were not state-dependent, in that levels of un-manipulated pups were not different from pups exposed to 2 minutes of isolation, suggesting permanent regulatory changes in DA and 5-HT systems, in parallel with or perhaps underlying high and low infant USV rates. No changes were found in norepinephrine levels. Overall, these global changes in monoamine function early in life pointed toward alterations in DA- and 5-HT systems that may affect current and later functioning.

In a study exploring possible differences in monoamine receptor development between the lines, Brunelli, Aviles, Gannon, Branscomb, & Shacham, 2009 examined the effects of a novel serotonin (5-HT) agonist acting exclusively at the 5HT-1A receptor (PRX-00023, EPIX Corporation), on High line rates of USV. In this study, High line infants (pups: P 11 ± 1 day) were more responsive to low doses of PRX-00023 than Random line pups. As seen in assays at P10, this difference in the degree to which PRX-00023 reduced USV rates in High versus Random line pups may involve increases in 5HT-1A receptor numbers taking place in structures such as the BNST and striatum in response to increased 5-HT activity. Thus alterations in 5HT-1A receptors in the High line appear to be one of the central mechanisms mediating infant anxiety-like behavior.

Levels of activity of these monoamines may not have the same significance in the early postnatal period as in adulthood, however, since at this time both systems

are undergoing rapid development in cortico- and mesolimbic structures (D'Amato et al., 1987; Dinopoulos, Dori, & Parnavelas, 1997; Galineau, Kodas, Guilloteau, Vilar, & Chalon, 2004; Gaspar, Cases, & Maroteaux, 2003; Perrone-Capano & Di Porzio, 2000; Tarazi, Tomasini, & Baldessarini, 1998: Zhang, 2003, and see below). Unlike adulthood, in which 5-HT generally functions as an inhibitory modulator, at this stage of life 5-HT increases neuronal activity in its role as a neurodevelopmental factor in cell growth and differentiation (Beique, Campbell, et al., 2004; Beique, Chapin-Penick, Mladenovic, & Andrade, 2004; Lemaire, Koehl, Le Moal, & Abrous, 2000; Li et al., 2004; Pellitteri, Zicca, Mancardi, Savio, & Cadoni, 2001; Zhou, Sari, & Zhang, 2000). Moreover, a variety of perinatal experiences can produce discontinuities in the development and activity of these systems so as to permanently alter monoamine functioning in target areas (Ansorge, Zhou, Lira, Hen, & Gingrich, 2004; Berger, Barros, Sarchi, Tarazi, & Antonelli, 2002; Chen, Turiak, Galler, & Volicer, 1997). Thus, without further developmental studies it is unclear whether the extensive changes in DA and 5-HT systems in High, and in Low line pups are predictive of functional alterations later in life.

Social Behavior in Juvenile High and Low USV Line Rats

In rats, play begins at about P18, shortly after eye opening and with independent feeding, and peaks during the periadolescent period between 30 and 40 days of age (Meaney & Stewart, 1981; Panksepp, 1981; Thor & Holloway, 1983; Vanderschuren, Niesink, & Van Ree, 1997). As in other mammals, play in rats is thought to have paralleled the evolution of the prefrontal cortex (De Bruin, 1990; Fagen, 1981; Pellis, Pellis, & Whishaw, 1992). Consistent with this view, rat play is comprised of complex sets of behavioral interactions that have been implicated in shaping the development of social and cognitive skills in adulthood characterized by cognitive flexibility, such as turn-taking or the ability to switch cognitive strategies (Ebner, Wotjak, Landgraf, & Engelmann, 2005; Einon & Morgan, 1977, 1978; Pellis et al., 1992).

Since selection for an early, social, affective-related behavior such as USV may also have influenced the development of other social behaviors, we investigated whether selective breeding for high and low USV rates affected common indices of juvenile play behavior, and 50 kHz ultrasonic vocalizations associated with play (Brunelli et al., 2006; Knutson, Burgdorf, & Panksepp, 1998). High, Low and Random USV line juveniles were isolated overnight and allowed to play briefly on each of three subsequent days, in same-line, same-sex sibling pairs. Interactions were observed over 10 minutes and frequencies of play behaviors, including nape contacts, pins and 50 kHz USVs were counted by pairs (Almeida & De Araujo, 2001; Almeida, Tonkiss, & Galler, 1996; Pellis, 1988; Pellis & Pellis, 1983). Definitions of these behaviors are given in detail in Brunelli et al. (2006).

Only play behaviors were reduced in the selected lines, ruling out global deficits in behavior, locomotor abilities, or differences in arousal. Compared to randomly-

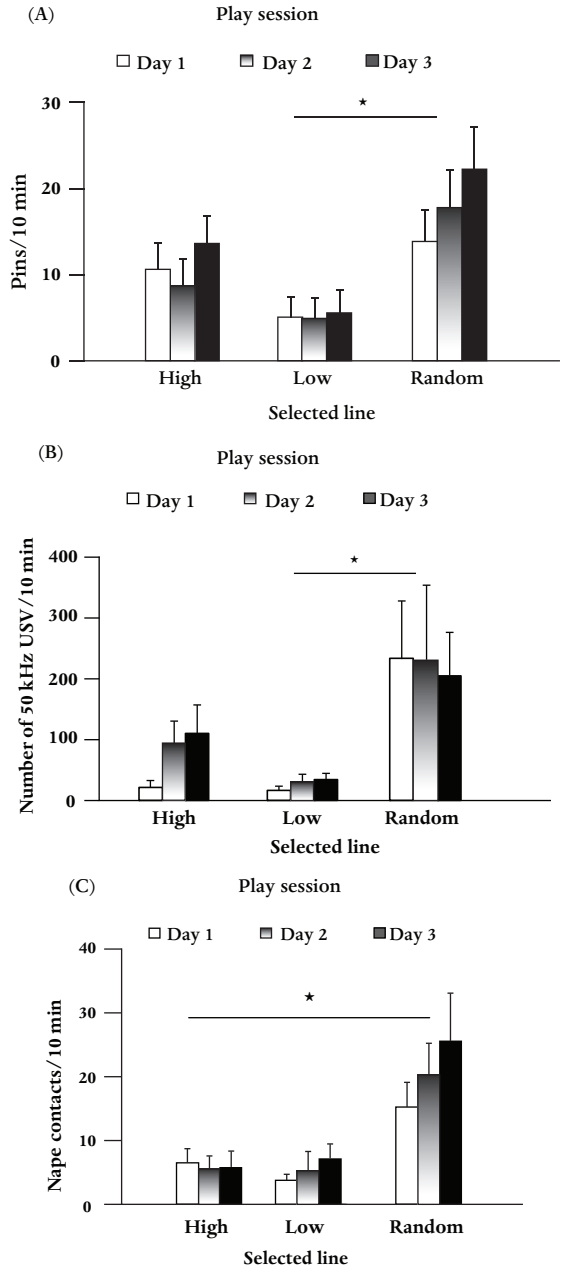

Figure 10.4. (A) Mean (±SEM) number of pins in 10 min of play during three successive play sessions (one per day over three days) in High, Low, and Random juvenile sibling pairs. Low line significantly less than Random line. Frequencies shown are for sibling pairs, which were scored as one unit. (B) Mean (±SEM) number of 50 kHz vocalizations emitted by High, Low, and Random sibling pairs in 10 min during three successive play sessions. Low line significantly less than Random line. (C) Mean (±SEM) number of nape contacts in 10 min of play during three successive play sessions (one per day over three days), in High, Low, and Random line sibling pairs. Low and High lines significantly less than Random line. (Adapted from Brunelli et al., 2006).

bred controls, High USV line juveniles had higher latencies to pin and lower levels of pinning (Figure 10.4A) and 50 kHz USVs (Figure 10.4B), but only in the *first* of the three play sessions (Play Session 1). Since 50 kHz USV is a reliable marker of positive affect during juvenile play (Knutson et al., 1998; Knutson, Burgdorf, & Panksepp, 1999), the depression of pinning and USVs in the High line on the first day could be reasonably interpreted as behavioral inhibition associated with initial conditions of exposure to a play partner. Moreover, nape contacts in High play pairs were uniformly low across all three play sessions (Figure 10.4C), consistent with the hypothesis that persistently decreased play initiation may have been associated with behavioral inhibition. In contrast, Low USV line juveniles were deficient in all play behaviors *across all three days*: in latencies to engage in play, and in frequencies of nape contact, pinning and rates of 50 kHz USV.

Alternate hypotheses for play deficits in juveniles of both lines that can be posited are, for example, that these animals lacked motivation to initiate and engage in play and therefore were affectively compromised. Juveniles may have been motivated, but unable to "read" social signals intrinsic to play, resulting in significant reductions specific to play behavior, without affecting pro-social behaviors like social investigation (Field & Pellis, 1994). This is especially true for Low line juveniles, whose play deficits were much more global and severe.

The Low line pattern is evident in juveniles subjected to social isolation, and in juveniles lacking prefrontal cortex or striatum (Einon & Morgan, 1977; Panksepp, Normansell, Cox, & Siviy, 1994; Pellis, Castaneda, McKenna, Tran-Nguyen, & Whishaw, 1993). Various forms of perinatal insults are known to affect social behavior in rats (Berger et al., 2002; Gerardin et al., 2005; Muneoka & Takigawa, 2002; Muneoka, Nakatsu, Fuji, Ogawa, & Takigawa, 1999; Muneoka, Ogawa, & Kamei, 1997; Oades et al., 2005; Sullivan, 2004; Sullivan & Brake, 2003). For example, rats that have suffered perinatal malnutrition, or have been reared without access to play partners also show similar patterns of social reactivity and inability to communicate socially (Whatson & Smart, 1978; Whatson, Smart, & Dobbing, 1975; Whatson, Smart, & Dobbing, 1976).

Pervasive deficits in social play such as these are thought to result from reductions in dopamine (DA) levels and functioning (Beatty, 1983; Field & Pellis, 1994; Thor & Holloway, 1983). Stimulation of DA activity increases play, probably through the combined action of pre- and postsynaptic dopamine D2 receptors (Vanderschuren et al., 1997).The same manipulations also produce regionally specific effects on DA levels and DA activity (measured as DA metabolite turnover) in prefrontal cortex, hippocampus, hypothalamus, and striatum (e.g., Kehoe, Mallinson, Bronzino, & McCormick, 2001; Kofman, 2002). Pharmacological reductions of DA in brain sites mediating reward also reduce 50 kHz USV in rats (Burgdorf et al., 2007). Thus, the question arises as to whether line differences in DA activity throughout the brain in the neonatal period may underlie differences in social play in the High and Low USV lines.

Developmental Continuity in Adult Affective Regulation in High and Low USV Lines

If separation-induced USV rates are an indicator of an infant anxiety-like state, does it follow that extremes of infant USV rates predict anxiety-like behavior in adulthood? Human studies examining samples of children expressing childhood inhibition and adult anxiety/depression disorders suggest that this may be the case. On the other hand, expressions of infantile states are not necessarily predictive of lifelong behavioral traits. Infantile isolation-induced USV may, for example, be an instance of a class of purely infantile behaviors or "ontogenetic adaptations" specific to the ecological niche occupied by infants in the postnatal period (West & King, 1987).

To test this question of continuity, High, Low, and Random line adults of both sexes have been examined in a variety of standardized laboratory tests measuring anxiety and depression in rodents (Zimmerberg, Brunelli, Fluty, & Frye, 2005). In a variation of the open field, High and Low line males and females were placed in a small cylinder in the open field, and the latencies at which they emerged from the cylinder were recorded. As shown in Figure 10.5, latencies for High USV line males were significantly longer than for Low USV line males. Similar significant results were noted in females (data not shown). High-line adults of both sexes also traversed fewer inner squares than Lows, another indication of higher anxiety-like state.

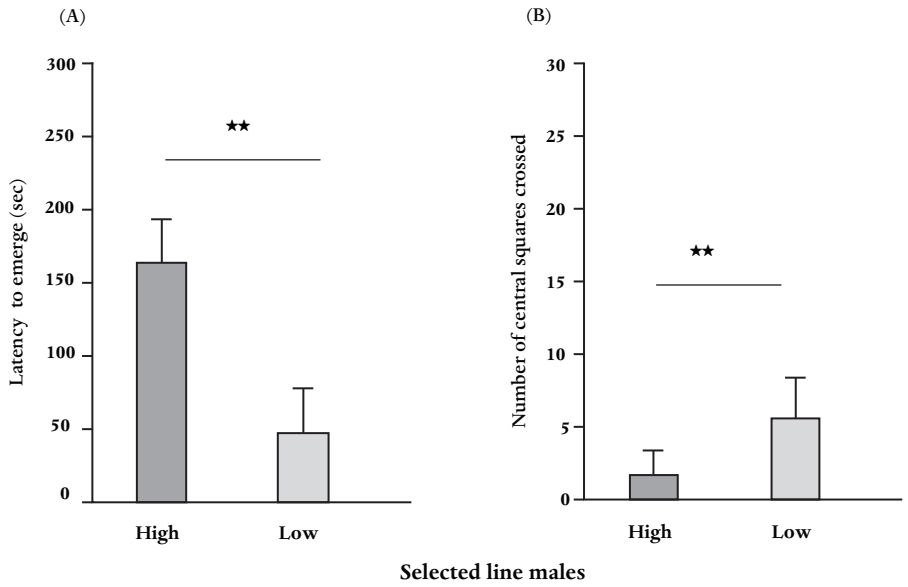

Figure 10.5. Mean (±SEM) latency (in seconds) to emerge from a cylinder into an open field by adult males in High and Low USV lines. High line significantly greater than Low line. (Adapted from Zimmerberg et al., 2005).

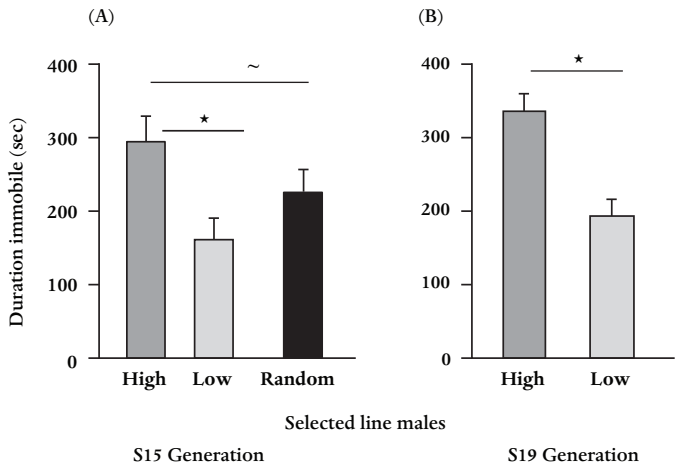

Figure 10.6. (A, left) Mean (±SEM) duration (in seconds) immobile (floating) in the Porsolt Swim by adult males in the High, Low, and Random lines in the 15th generation (S15); and (B, right) in High and Low line males in the 19th (S19) generation of selection. High line significantly greater than Low line. (Adapted from Brunelli & Hofer, 2007, and Zimmerberg et al., 2005).

Figures 10.6A and B show replications across two generations of line differences in High versus Low USV line adult males in the lengths of time spent floating, one of several behaviors measured in the Porsolt Swim. The Porsolt Swim is considered a reliable model for depression-like behavior in rodents, since behavioral measures such as floating respond primarily to antidepressant, but not anxiolytic action (Gingrich & Hen, 2001). In both generations, High USV line animals of both sexes (female data not shown) spent more time floating than Lows, indicating consistent differences between the lines (Shair, Brunelli, Velazquez, & Hofer, 2000; Zimmerberg et al, 2005).

In the same study, we measured levels of the neurosteroid, allopregnanolone (3-hydroxy-5-pregnan-20-one; 3,5-THP), a reduced metabolite of progesterone. Allopregnanolone, a positive modulator of the GABA-A receptor, reduces anxiety behavior in rats in a variety of paradigms, including rat pup isolation calls (Bitran, Hilvers, & Kellogg, 1991; Brot, Akwa, Purdy, Koob, & Britton, 1997; Carboni, Wieland, Lan, & Gee, 1996; Frye, Petralia, & Rhodes, 2000; Frye & Walf, 2002, 2004; Reddy & Kulkarni, 1997; Vivian, Barros, Maintiu, & Miczek, 1997; Zimmerberg, Brunelli & Hofer, 1994). Moreover, allopregnanolone modulates and is itself modulated by the 5-HT system and has been shown to alter depressive states via 5-HT mechanisms (Griffith & Mellon, 1999; van Broekhoven & Verkes, 2003). Because neurosteroids appear to be intimately involved in stress responses (Barbaccia et al., 1996; Barbaccia et al., 1997), we thought it possible that the adult behavioral differences observed between the Low and High USV lines might be mediated in part to selected differences in allopregnanolone-modulated receptor systems.

Allopregnanolone levels were measured in hippocampus and amygdala tissue in the same animals tested for behavior, and were significantly higher in Low USV line

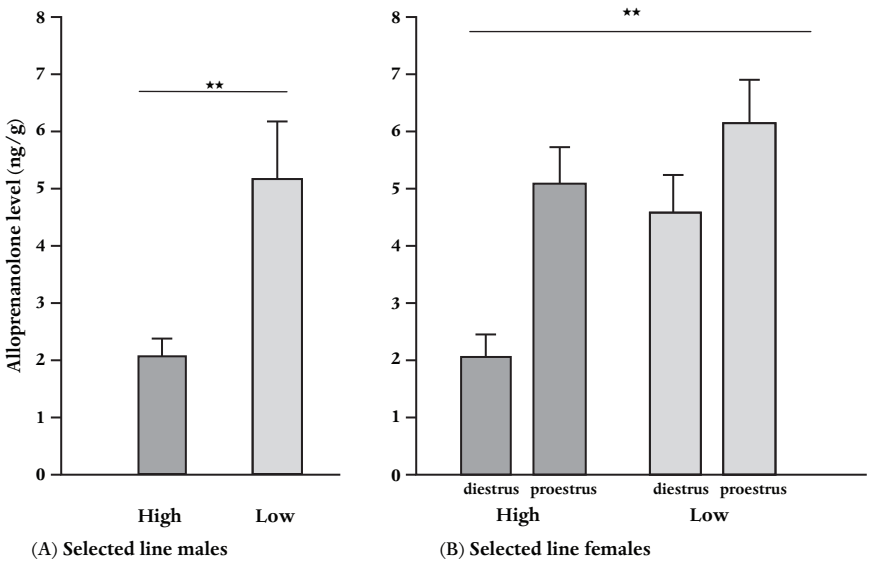

Figure 10.7. (A, left) Mean (±SEM) concentrations in ng/g tissue of allopregnanolone (3-,5-THP) in amygdala/hippocampal tissue of adult males, and (B, right) in proestrus females and diestrus females from selectively bred High and Low USV lines (significant main effects of line and hormone status, *n*'s = 13–17 subjects per bar). (Adapted from Zimmerberg et al., 2005).

male (Figure 10.7A) and female (Figure 10.7B) rats than High line rats, consistent with its acute antidepressant and anti-anxiety effects in rodents (Barbaccia et al., 1997; Bitran et al., 1991; Brot et al., 1997). In both selected lines, allopregnanolone levels fluctuated with the estrus cycles in females, so that higher allopregnanolone levels and reduced anxiety occurred during estrus when estrogen levels are highest; these were reversed during diestrus when estrogen levels are low (Figure 10.7B). It is notable that comparable changes in allopregnanolone levels have been implicated in Pre-Menstrual Syndrome disorder in women, and mechanisms underlying the relationships between fluctuations of mood with changes in ovarian hormone levels have been shown to be mediated by effects in the brain of allopregnanolone withdrawal on GABA-A subunit conformation in rodent models (Smith, 2004; Sundstrom-Poromaa, 2004). Lower allopregnanolone levels in High USV line adults of both sexes suggest either an inability to mount adequate synthesis of allopregnanolone in brain areas mediating stress, or lower baseline endogenous allopregnanolone, or its precursor progesterone, and fewer reserves to draw from during stress. In contrast, either process (i.e. the greater ability to adequately synthesize allopregnanolone or higher baseline endogenous allopregnanolone or its precursors) could have been responsible for promoting more enhanced functioning during stress in the Low line.

Zimmerberg et al. (2009) examined whether deficits in allopregnanolone could account for neonatal and adult offspring behaviors in the selectively-bred High USV line. Pregnant High USV line dams were injected twice a day with allopregnanolone or with vehicle, or handled as controls, and were tested on the elevated plus maze just before parturition. Administration of allopregnanolone during the last week of gestation was able to moderate the behavioral phenotype of anxiety/depression in these female High USV line rats. Moreover, High line pups displayed significantly lower USV rates after prenatal neurosteroid exposure compared to controls from that line. In adulthood, for both male and female High line rats, prenatal allopregnanolone exposure was associated with less depressed-type behavior, as seen by more time struggling and less time immobile in the forced swim test compared to controls. The duration of time spent engaged in these behaviors by the High line adults exposed to allopregnanolone prenatally was now comparable to that of the Low line from two previous studies (Brunelli, 2005; Brunelli & Hofer, 2007). In the plus maze only male offspring revealed a significant long-term effect of prenatal exposure, exhibiting decreased anxiety as defined by more time on the open arms, compared to male controls.

The role of prenatal allopregnanolone to reduce the anxious/depressed phenotype in the High USV line might be the result of a direct effect of the neurosteroid in the hippocampus to enhance intracellular calcium via its positive modulation of the GABA-A receptor (Berger et al., 2002). One of the possible consequences of enhancing these depolarizing currents is the support of neuronal proliferation and survival (Barbaccia et al., 1997). Brain-derived neurotrophic factor (BDNF) might be a mediator in this mechanism, as suggested by the finding that neurosteriods are critical in the rise in BDNF after damage to mature neurons (Shulga et al., 2008). Alternatively, perhaps the gestational administration of allopregnanolone replenished this neurosteroid in the fetal brain to a more optimal level at a critical time when it was needed as a developmental factor.

Autonomic Nervous System (ANS) Regulation of Heart Rates in the USV Lines

Selection for infant USV has also produced distinct modes of cardiac responsiveness to stress. Thus, compared to the Random line, both the High and Low USV line juveniles at P18 exhibited enhanced cardiac reactivity to stress (Brunelli, Myers, Asekoff, & Hofer, 2002). Figure 10.8 shows heart rate changes at Baseline (Home cage), Isolation (Novel cage) and Recovery (Home cage), in 2-min epochs, in P18 juveniles that were taken from the home cage and placed individually into a novel environment, then returned to the home cage. Though neither selected line

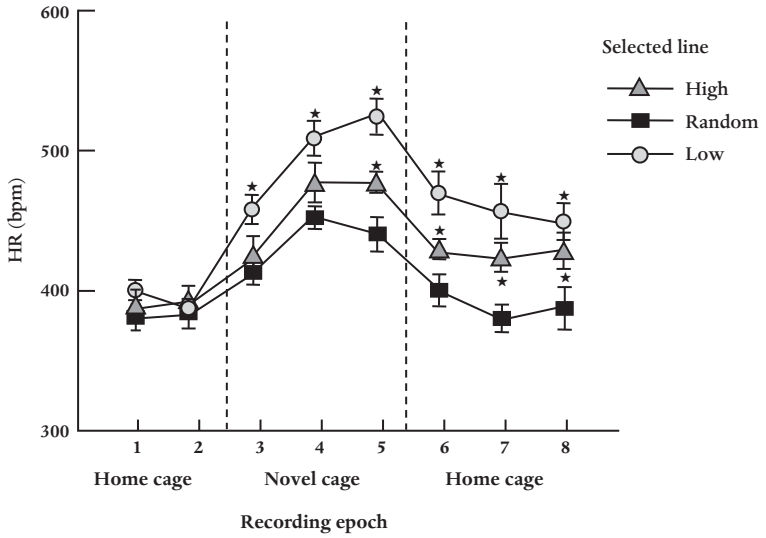

Figure 10.8. Mean (±SEM) heart rates (HRs, in beats per minute [bpm]) of Postnatal Day (P) 18 control pups in the High-USV, Low-USV, and Random-USV lines, from Baseline in the home cage (Home cage, left) through Isolation in a novel cage (Novel cage, middle), to Recovery back in the home cage (Home cage, right). (Adapted from Brunelli et al., 2002).

showed heart rate differences at baseline, High USV line heart rates were significantly higher than Random line heart rates during isolation in the novel environment, consistent with their anxiety phenotype in infancy and in adulthood. In contrast to their apparent lack of anxiety in behavioral tests, Low USV line heart rates were significantly higher than both Randoms and Highs, suggesting even greater reactivity to novelty. Compared to the drop in Random line heart rates, neither High nor Low USV line heart rates returned to baseline during recovery in the home cage, indicating continuing reactivity to the stressor in both lines.

Pharmacological blockade revealed that in High USV line juveniles, higher heart rates during isolation stress were the result of greater sympathetic *acceleratory* influence on heart rates. The even higher heart rates of Low USV line juveniles during isolation stress were clearly due to greater parasympathetic *withdrawal*, largely eliminating the only braking influence on rising heart rates; however, some sympathetic influences increasing Low USV line heart rates beyond controls could not be ruled out in this study.

Young adult males (~180 days) demonstrated continuity with juvenile heart rate responses during 30 min of restraint stress (Shair et al., 2000). As shown in Figure 10.9A, in the first 10 minutes heart rates in High and Low USV line adult males were significantly higher than Random line males; but as animals habituated to restraint, High and Random heart rates declined similarly over time. In contrast, Low USV line males maintained their high heart rates for the entire 30 minutes, consistent with more enduring reactivity to restraint.

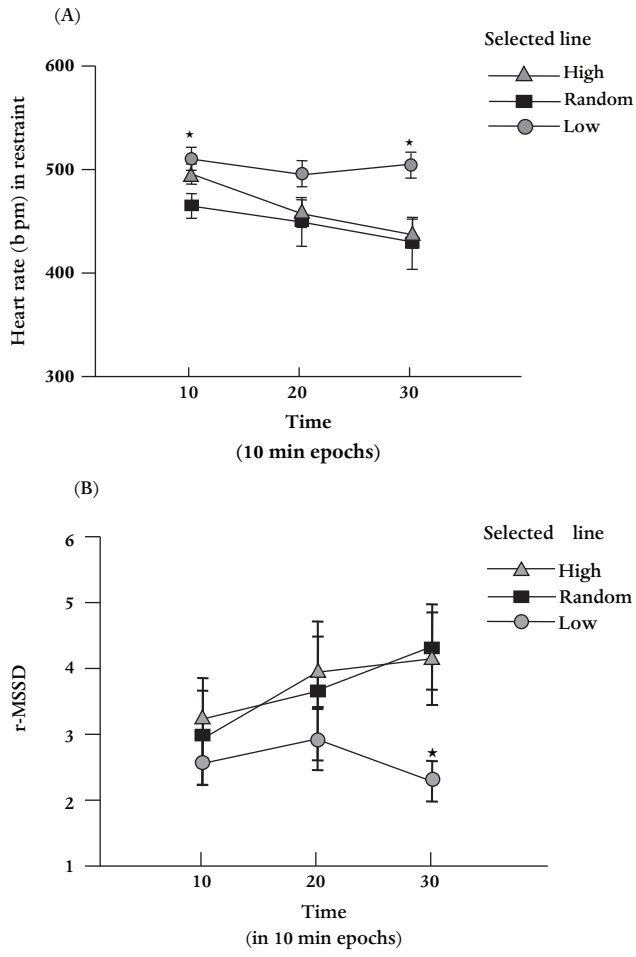

Figure 10.9. (A) Mean (±SEM) adult male heart rates over 30 min of restraint at 10 min intervals. Significant line effect ($p = .013$), which post hocs demonstrate are due to Low> Random line heart rates. Significant Line×Epoch effect, ($p = .05$) due to Low> Random/High line heart rates at the 30 min epoch. (B) Mean (±SEM) adult male r-MSSD calculated as root mean square of successive beat-to-beat variability (an index of high-frequency (vagal) variability; Murphy, Sloan, & Myers, 1991) over 30-min of restraint at 10-min intervals. Significant Line×Epoch effect ($p = .05$) due to Low< Random/High line at the 30 min epoch. (Adapted from Shair et al., 2000).

In order to determine autonomic influences on heart rate in these adult males, the square root of the mean squared differences of successive heart periods (r-MSSD) was computed. r-MSSD is a time domain measure of heart period (HP) variability that is sensitive to short-term, high-frequency (vagal) HP fluctuations. Pharmacological blockade studies have shown that the r-MSSD statistic is sensitive to vagal cardiac control with little sympathetic input (Berntson, Lozano,

& Chen, 2005; Murphy, Sloan, & Myers, 1991). Decreases in r-MSSD reflect decreases in parasympathetic tone, resulting in an increase in heart rate. r-MSSD was lower in the Low USV line males throughout the 30-minute period (Figure 10.9B), and post hoc tests demonstrated that high-frequency variability in Low line males was lower than both High and Random males, consistent with reduced parasympathetic restraint on heart rate. At the same time, blood pressure variability was greater in Low USV line adult males, a condition often associated with later elevated blood pressure (Sloan et al., 1999).

These data suggest opposite conclusions about autonomic control in High and Low USV line adult males. First, the data for High USV line males are consistent with sympathetic over-activity that characterizes High line juveniles, associated with their anxious/depressed lifetime profile. Second, heart rates in adult Low USV line males appear to be characterized primarily by parasympathetic under-activity, consistent with their juvenile control of heart rate. To generalize, findings in the High USV line are consistent with populations prone to anxiety (Calkins & Fox, 1992). Low USV line low-anxiety, high-cardiovascular reactivity profile also appears to parallel human populations exhibiting lifelong aggression/hostility (Raine, 2002; Sloan et al., 1994; Sloan et al., 1999). Moreover, Low line cardiovascular responses to stress resemble those shown by animal models of prenatal malnutrition (Tonkiss, Trzcinska, Galler, Ruiz-Opazo, & Herrera, 1998).

Low USV Line Male–Male Aggression

Based on their reduced behavioral anxiety and depression, we thought it likely that Low line males would also exhibit greater conspecific aggression. Low line adult males were paired with novel Low line adult male partners in a novel, but neutral environment (Social Interaction test: File & Seth, 2003), and compared with Random line male pairs. Since rats are not generally aggressive in this context, in order to increase aggression rates, males in both lines were previously socially isolated in home cages for 10 days, a treatment that increases reactivity in rats (e.g., increased anxiety), and aggression in a small percentage of rats (e.g., Wongwitdecha & Marsden, 1996). A significantly higher proportion of socially isolated Low line pairs engaged in bouts of fighting than Random line pairs (*Chi square* = 6.866, df = 1, $p = 0.009$). The ratio of fighting pairs in Random line rats was within the range observed in other outbred rat strains, i.e., about a third of rats responded to this treatment with increases in aggression (Barbaccia et al., 1996; Barbaccia et al., 1997).

Once it was established that higher than normal aggression in male-male encounters after social isolation was indeed highly characteristic of the Low line, we next tested the hypothesis that ethanol would increase rates of aggression in Low line pairs. Another sample of socially-isolated males was tested under the baseline conditions for aggression (Day 1) in novel Low-Low and Random-

Random pairs. Twenty-four hours later (Day 2) *one member of each pair* was injected with 1g/kg of 10% ethanol (a moderate - high dose: Varlinskaya and Spear, 2006) 10 min prior to exposure to its previous day's partner in the neutral environment. On both days pairs engaged in social interaction for 10 min, during which behaviors were videotaped for later scoring and analysis. Data for Day 1 and Day 2 were subjected separately to principal components analysis (PCA) to determine whether behaviors clustered into meaningful groupings.

Based on aggressive behaviors observed (Blanchard & Blanchard, 1977; Blanchard, et al., 1975), PCA yielded three orthogonal components for each day, comprising 69% of the variance in the data, which remained stable across the two days. They were titled: 1) *"Fighting"*, consisting of boxing, biting, clinches, pinning, and teeth chattering, accounting for an average of 27% of the variance in the data set; 2) *"Social Investigation"*, consisting of sniffing, allo-grooming, following, crossing-over/under, with freezing loading negatively onto the component, accounting for 22% of the variance in the data set; and 3) *"Aggressive Signaling"*, consisting of freezing, lateral posturing and subordinate position, with 22 kHz USV loading positively, with 55 kHz USV loading negatively on the component, and accounting for 20% of the variance in the data set. Factor scores were calculated and differences in factor scores between Random and Low lines were tested by bonferroni-adjusted *t* tests for each of the six orthogonal components, three for each day. In addition, the incidence of pairs engaging in the behaviors that constituted the principal components was tested by Chi^2 analyses. Since results of the two analyses were nearly identical, the results of only the Chi^2 analyses are presented.

Looking at baseline levels of interactions without ethanol, there were no differences between lines in the percent of animals engaging in Fighting (10.10.A, Day 1), in part because fewer Low line males in this sample fought and more Random line males fought than in the previous sample. Social Investigation (10.10.B, Day 1) and Aggressive Signaling (10.10.C, Day 1) did not differ either. On Day 2, however, 10 minutes after 1g/kg of ethanol, significantly more Low line males engaged in fighting than Random males (10.10.A, Day 2). In this case, the number of Low line pairs fighting increased sharply, whereas the opposite effect occurred in Random pairs. Significantly fewer Low line males than Random line males engaged in Social Investigation after ethanol injection (10.10.B, Day 2). Significantly more Low line pairs engaged in Aggressive Signaling after ethanol (10.10.C, Day 2), reversing their pattern of this behavior almost completely from the previous day. In contrast, ethanol decreased Fighting and Aggressive Signaling in Random pairs, while increasing Social Investigation, perhaps in response to alterations in ethanol-treated partner's behavior. Therefore, Low line males appear to be highly susceptible to the induction of aggressive behavior by social isolation, enhanced by a moderate dose of ethanol, whereas Random line males exhibit normative changes in behavior in response to isolation and ethanol in this test.

One limitation of this study is that levels of Day 2 aggression in the Low and Random line males occurred in the context of interacting with a familiar partner, since pairs were not changed from day to day. Because there were no saline-injected

controls tested daily we do not know the extent to which familiarity across days contributed to levels of aggression independently of the action of ethanol. Clearly, Low and Random line partners responded differently to the totality of the experience, but the effect of repeated exposure to these interactions is unknown.

Social isolation similar to that used in our experiment is known to *decrease* allopregnanolone levels by 60–70% in cerebral cortex, hippocampus, and blood plasma (Serra et al., 2000). Decreased frontal cortex allopregnanolone appears to be the result of reductions in 5-alpha-reductase-I, the enzyme involved in the rate-limiting step in its biosynthesis from progesterone (Mellon, 2004). This isolation-induced down-regulation can be reversed by intracerebral injections of low concentrations of allopregnanolone (Barbaccia et al., 1996; Barbaccia et al., 1997). When subsequently exposed to an acute stressor like footshock or an unknown male, such animals show significant *increases* over baseline in brain and plasma concentrations of allopregnanolone, well over the levels of socially-housed controls (Barbaccia, et al., 1996; Barbaccia et al., 1997). Given that brain allopregnanolone levels rise higher in Low USV line animals in response to a stressor (Zimmerberg et al, 2005), one possible hypothesis for the brain mechanism underlying higher aggression in socially isolated Low USV line males is that their brain allopregnanolone levels are reduced by social isolation, but then in response to subsequent interaction with strange conspecifics, and on Day 2, acting in concert with ethanol, their brain allopregnanolone may increase well over levels occurring in similarly treated Random line males, disinhibiting aggression via GABA-A receptor mechanisms (Barbaccia et al., 1997).

A Possible Role for Epigenetic Effects

It appears then, that selective breeding for high and low rates of vocalization in response to maternal separation has produced two distinct temperamental clusters or styles that include alterations in behavioral, physiological, and neurochemical characteristics. The integrity of these temperamental styles are continuous across the life span, and express themselves as adult phenotypes comparable to those seen in human populations.

The genetic and/or epigenetic mechanisms underlying these long-term changes in the USV lines are as yet unknown. However, our data do suggest the possibility of epigenetic effects as well. For instance, an unexpected effect of selection was that Low line pup birth weights have been significantly lower than both High and Random line weights since the 14th (S14) generation of breeding, suggesting prenatal nutritional deficits (Figure 10.11A). Line differences in mean litter weights have remained stable and were still significantly lower through Generation 28 (Figure 10.11B). As shown, Low USV line mean litter weights at postnatal day (P)10 have also been significantly lower than Random and High line since

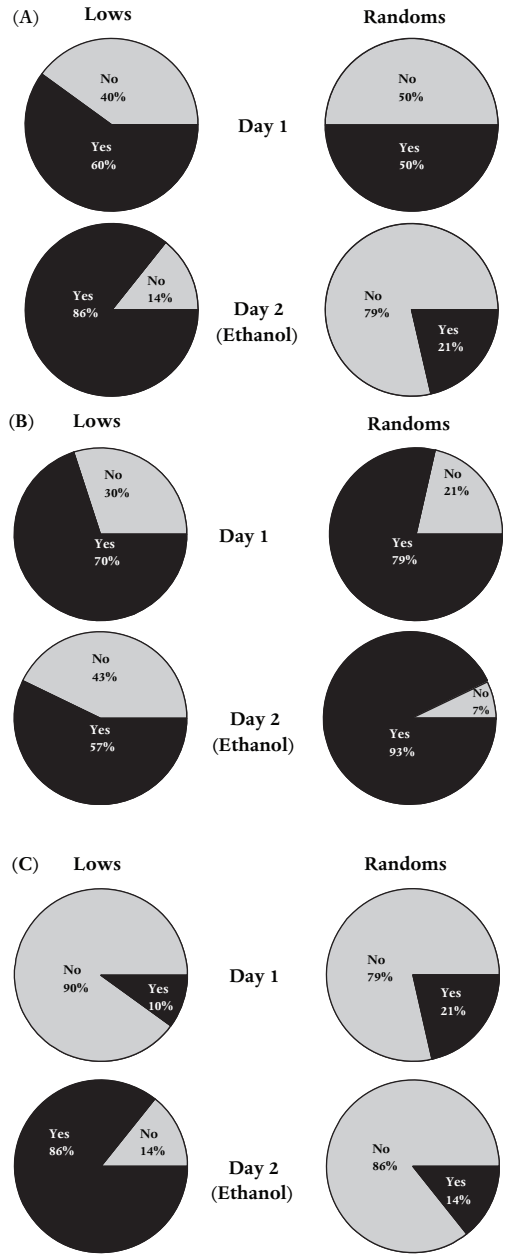

Figure 10.10. (A) Percent of animals engaged in Fighting: Significant effect of Line (*Chi* Square = 7.875, df=1, $p = 0.005$); (B) Percent of animals engaged Social Investigation: Significant effect of Line (*Chi* Square = 3.860, df=1, $p = 0.049$; (C) Percent of animals engaged in Aggressive Signaling: Significant effect of Line (*Chi* Square = 10.096, df=1, $p = 0.005$), calculated for Day 1, pre-alcohol (top of each figure) and Day 2, post-alcohol (bottom).

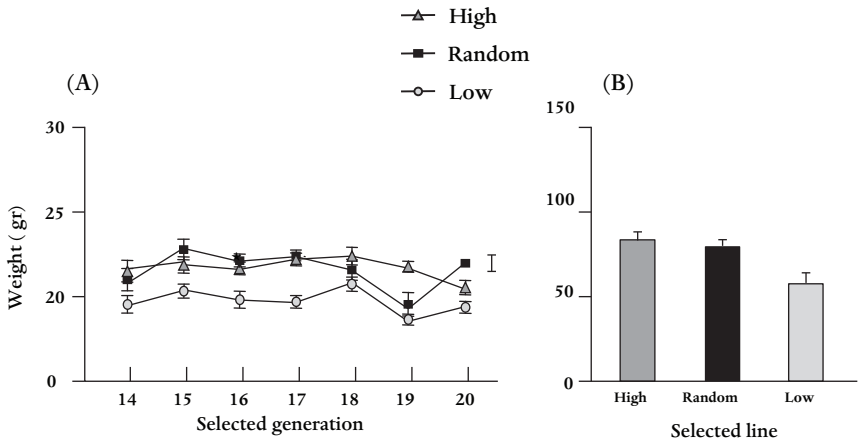

Figure 10.11. (A) Mean (±SEM) birth weights (grams) High, Low, and Random lines, across the 14th through 21st generations: Line $F(2,1196) = 44.634$, $p = 0.000$; Generation: $F(6, 1196) = 6.277$, $p = 0.000$; Generation×Line: $F(12,1196) = 2.146$, $p = 0.012$). Data based on litter means. (B) Mean (±SEM) P10 weights averaged over 22nd–28th selected generations, showing stable differences between Low and High/Random litters Line ($F(2,49) = 7.175$, $p = 0.002$). Data based on litter means (Adapted from Brunelli & Hofer, 2007).

Generation 10 (Figure 10.11B), indicating that either maternal nutritional insufficiency continues postnatally, or that litters have not caught up with High and Random line litters by the day of testing for USV (see Galler & Tonkiss, 1991). However, by weaning, Low USV line weights are not different from the High and Random lines for either sex, into adulthood (Brunelli, 2005). Thus, Low USV line fetuses may be genetically programmed for smaller size, or the Low line maternal uterine environment may be somehow unfavorable for growth and development of Low line fetuses. As noted earlier, prenatal malnutrition is associated with higher levels of aggression in both humans and animals (Raine, 2002).

We had previously examined the possibility of postnatal maternal effects in the generational transmission of isolation-induced USV and behavior at P10 days. Pups in the S13 generation were cross-fostered within 48 h of birth between dams of the two lines, that is, Low-pups to High-dams and High-pups to Low-dams (Brunelli, Hofer, & Weller, 2001). To control for fostering effects, other groups of pups were fostered to dams within their own lines (in-fostered). Comparisons with screening data obtained from unaltered litters in the entire 13th generation of the selectively bred lines showed that fostering itself significantly reduced vocalization in both the High and Low line pups, regardless of whether they were cross-fostered across or in-fostered within lines. On the other hand, P10 USV responses of cross-fostered pups were virtually the same as rates of in-fostered pups within each line, indicating that fostering of High pups to Low mothers and Low pups to High mothers revealed no postnatal maternal effects on USV rates.

Critically, however, P10 High USV line pups cross-fostered to Low USV line dams weighed significantly less than High USV line pups fostered within line, consistent with previous findings that offspring of Low line mothers weigh significant less. Fostering Low line pups to High line dams had no effect on P10 weight, suggesting that genetic or prenatal influences also contributed to their P10 weight phenotype. Thus, while there was no evidence for a postnatal maternal contribution specifically to the P10 USV phenotype, nonetheless, this study demonstrated a clear influence of Low USV line dams on weight gain in the first 7–10 days of life.

Direct comparisons of maternal behavior between High and Low USV line mothers have revealed differences in maternal pup-licking and nursing behavior: Low USV line dams lick and groom their pups significantly more than High USV line dams in the first week of life (Figure 10.12A). Low USV line dams also engage in significantly more active (high-arched) and less passive (low-arched) nursing than High USV line dams (Figure 10.12B). Does this maternal phenotype contribute to the adult Low/High USV line phenotypes? There is an obvious parallel between High/Low USV adult phenotypes and those found for offspring of low nursing/licking/grooming (LG) dams versus high LG dams, respectively, by Champagne, (2008) and by Meaney and colleagues (e.g., Meaney & Szyf, 2005). Thus adult offspring of high LG dams that naturally exhibit greater pup licking-grooming are behaviorally and physiologically less fearful than those of low-LG mothers, and this profile is consistent both with Low line dam behavior and Low line adult behavior. In a striking parallel to play results in the Low USV line, Parent and Meaney (2008), showed that juvenile male offspring of high LG dams demonstrated a significantly lower frequency of pouncing, pinning, and aggressive social grooming than male offspring of low-LG dams; high-LG males also showed lower frequencies of playful behavior than both high-LG and low-LG females. It seems possible, therefore that the same end-point may be reached through different developmental processes, which, in turn, suggests the possibility that the postnatal, as well as prenatal maternal environment might be a mediator of long-term developmental effects of selective breeding. Wichers et al. (2002) have provided evidence for similar gene–prenatal malnutrition interactions on aggressive psychopathology in children.

Perturbation of the early social environment has also been shown to alter infant and adult behavioral phenotypes in the High/Low USV lines (Martinez, Brunelli, & Zimmerberg, 2009). In a longitudinal study, High and Low USV line male and female offspring were raised in Communal Nesting (CN) groups, in which dams were housed together during pregnancy and lactation, or in standard housing (SH). As a consequence, at P7, all CN-reared pups vocalized less in isolation than SH-reared subjects. While High USV line pups continued to vocalize more than Lows in isolation tests, they showed significantly greater reductions in this response than Lows as a result

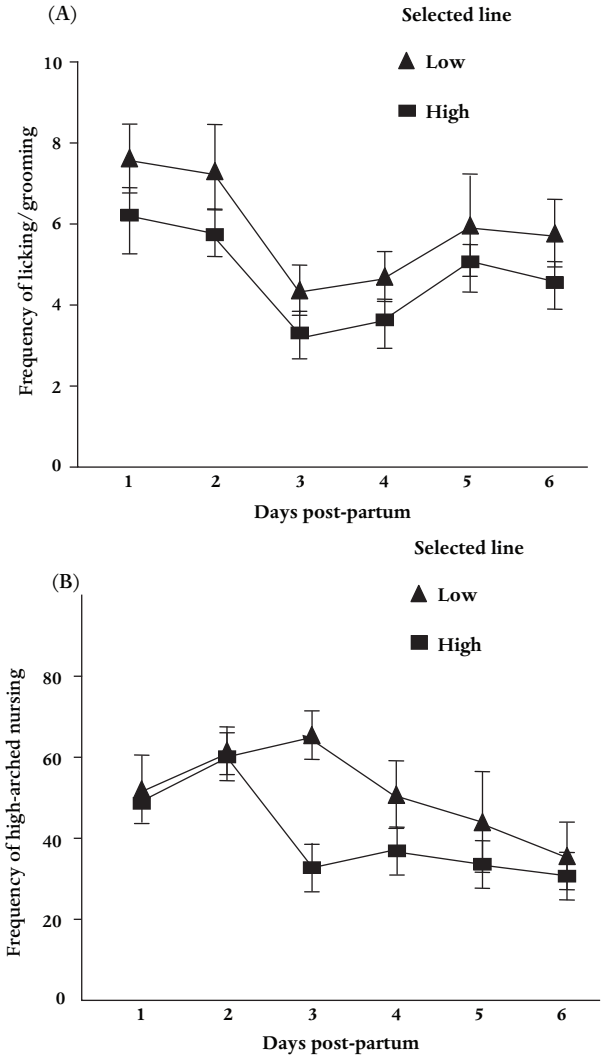

Figure 10.12. Post-partum maternal behavior in High and Low USV line dams. (A). Frequencies of licking and grooming. Significant effect of Line: Low dams significantly greater than High over the first 6 days post-partum. (B). Frequency of high-arched nursing. Significant effect of Line: Low significantly greater than High over the first 6 days post-partum. (Curley, Champaigne and Brunelli, unpublished data).

of CN, indicating a gene x environment interaction which was confined to High line pups (Figure 10.13).

So where do the genetic effects of selective breeding leave off and epigenetic influences come in to produce widespread phenotypic alterations in the USV selected lines? Such effects in selective breeding have been noted before (e.g., Rutherford &

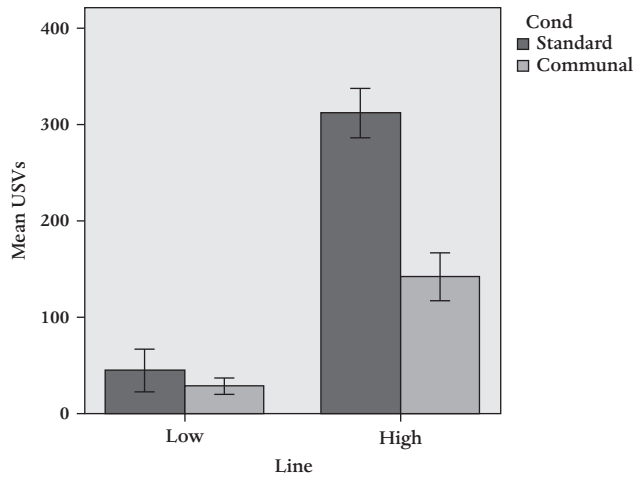

Figure 10.13. USV rates in High and Low USV line communally-reared and control litters at PN 7. Significant effect of Line (High > Low) and significant Line X Group interaction (Control High > Communal High). (From Martinez, Brunelli, & Zimmerberg, 2009).

Lindquist, 1998; Waddington, 1959) and are consistent with the notion of "genetic assimilation" as described by Waddington (1959, p. 1635), as a consequence of developmental processes, in which: "Mutations, not necessarily random, will be modified by the types of instability, and (sic) built into epigenetic mechanisms by selection for responses to environmental stresses. This is the process of `genetic assimilation´ of an acquired character."

Zuckerkandl and Cavalli (2007, p. 239) enlarge on genetic assimilation processes as occurring in the following way:

> Selectable inheritable combinations of epigenetic changes in the transcription rate of individual genes can presumably be maintained in cells or organisms over a certain window of numbers of generations. That window can be of sufficient width to permit much rarer specific mutations with equivalent effects to occur and to be selected. These specific mutations would be of high penetrance and stability. A new functional system could continue to be built in the process, with new epigenetic transmutations drawn into a growing novel gene interaction pattern based on preexisting genes or their slightly diverged duplicates.

As Hofer and colleagues have demonstrated, some of the most fundamental of these biological-behavioral interactions occur in the early postnatal period, and studies of prenatal stress show that interactions prior to birth are no less fundamental in building patterns of development. Such perinatal interactions have been conceptualized as "programming" of the young organism for optimal fitness in a given set of environmental circumstances (Meaney, Szyf & Seckl, 2007).

Rutter et al. (2005) have noted that gene-environment interactions can be parsed into different types of correlations that will influence environmental risk, but all exert their effects in response to specific signals in parent-infant and infant-parent interactions. Rutter has conceptualized such interactions as gene-environment "correlations," in which genetic variations regulate organisms' exposure to certain environments, and these can be categorized as "passive" and "active" gene-environment correlations (originally coined by Plomin, DeFries, & Loehlin, 1977). For example, "passive" correlations will be genetic influences on individual development that are outside of the individual's control but are mediated through the parent, such as prenatal exposure to uterine environments that confer risk, as may be the case of Low USV line animals. "Active" correlations are those genetic effects on offspring development that are mediated by the offspring itself. Altered USV rates or other behaviors in High and Low USV line pups may thus provide feedback to alter maternal nurturing behavior. Such effects have been observed in control rat dams in response to cross-fostered pups from prenatally malnourished litters (Galler & Tonkiss, 1991). However, as Gottlieb repeatedly emphasized in his work, even conceptualizations like "gene-environment correlations" become meaningless because of the inherent dichotomy contained therein. The discovery of active regulation of gene expression by environmental influences means that mechanisms ranging from behavioral to molecular provide constant and continuous alterations during the course of development.

Conclusions

When we asked at the outset of this research: "What would happen if we selectively bred infant rats for high and low levels of their ultrasonic vocalization response to isolation," we now wish we had asked Gilbert Gottlieb's opinion. Gilbert was interested in selective breeding (for adult traits) because he thought the results were generally being misinterpreted. He emphasized that it was not just the distribution of certain genetic variants in the laboratory population that was being altered by repeated selection: "but developmental means [also] are necessarily transmitted between generations, which may include genes, cellular machinery, and the maternal reproductive system, parental care, or interactions with conspecifics. . . that contribute to expression of the phenotype" (Gottlieb 1992, pp. 149–150). He went on to state that: "in my opinion. . . we are not dealing with absolutely different genotypes, more likely differed genetic expression in much the same genotype." And, in considering the gene frequency changes resulting in selected populations, he emphasized the greatly under appreciated role of "the vast. . . genetic store for phenotypic variation. . . almost always hidden because it normally goes unexpressed" (Gottlieb, 1992, pp. 150–152).

Acknowledgements

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References

Allen, M. T., Matthews, K. A., & Kenyon, K. L. (2000). The relationships of resting baroreflex sensitivity, heart rate variability and measures of impulse control in children and adolescents. *International Journal of Psychophysiology*, **37**, 185–94.

Almeida, S. S., & De Araujo, M. (2001). Postnatal protein malnutrition affects play behavior and other social interactions in juvenile rats. *Physiology & Behavior*, **74**, 45–51.

Almeida, S. S., Tonkiss, J., & Galler, J. R. (1996). Prenatal malnutrition affects the social interactions of juvenile rats. *Physiology & Behavior*, **60**, 197–201.

Ansorge, M. S., Zhou, M., Lira, A., Hen, R., & Gingrich, J. A. (2004). Early-life blockade of the 5-HT transporter alters emotional behavior in adult mice. *Science*, **306**, 792.

Barbaccia, M. L., Roscetti, G., Trabucchi, M., Mostallino, M. C., Concas, A., & Purdy, R. H., et al. (1996). Time-dependent changes in rat brain neuroactive steroid concentrations and GABA-A receptor function after acute stress. *Neuroendocrinology*, **63**, 166–172.

Barbaccia, M. L., Roscetti, G., Trabucchi, M., Purdy, R. H., Mostallino, M. C., & Concas, A., et al. (1997). The effects of inhibitors of GAB-A-ergic transmission and stress on brain and plasma allopregnanolone concentrations. *British Journal of Pharmacology*, **120**(8), 1582–1588.

Beatty, W. W. (1983). Hormonal organization of sex differences in play fighting and spatial behavior. In G. J. De Vries (Ed.), *Progress in Brain Research*, **61**, 315–330.

Beique, J. C., Campbell, B., Perring, P., Hamblin, M. W., Walker, P., & Mladenovic, L., et al. (2004). Serotonergic regulation of membrane potential in developing rat prefrontal cortex: Coordinated expression of 5-hydroxytryptamine 5-HT1A, 5-HT2A, and 5-HT7 receptors. *Journal of Neuroscience*, **24**, 4807–4817.

Beique, J. C., Chapin-Penick, E. M., Mladenovic, L., & Andrade, R. (2004). Serotonergic facilitation of synaptic activity in the developing rat prefrontal cortex. *Journal of Physiology*, **556**(3), 739–754.

Belsky, J., & Fearon, R. M. (2002). Infant-mother attachment security, contextual risk, and early development: A moderational analysis. *Developmental Psychopathology*, **14**(2), 239–310.

Berger, M. A., Barros, V. G., Sarchi, M. I., Tarazi, F. I., & Antonelli, M. C. (2002). Long-term effects of prenatal stress on dopamine and glutamate receptors in adult rat brain. *Neurochemical Research*, **27**(11), 1525–1533.

Berntson, G. G., Lozano, D. L., & Chen, Y. -J. (2005). Filter properties of root mean square successive difference (RMSSD) for heart rate. *Psychophysiology*, **42**, 246–252.

Biederman, J., Faraone, S. V., Milberger, S., Jetton, J. G., Chen, L., & Mick, E., et al. (1996). Is childhood oppositional defiant disorder a precursor to adolescent conduct disorder? Findings from a 4-year follow-up study of children with ADHD. *Journal of the American Academy of Child & Adolescent Psychiatry*, **35**(9), 1193–1204.

Biederman, J., Rosenbaum, J. F., Bolduc-Murphy, E. A., Faraone, S. V., Chaloff, J., & Hirshfeld, D. R., et al. (1993). A 3-year follow-up of children with and without behavioral inhibition. *Journal of the American Academy of Child and Adolescent Psychiatry*, **32**, 814–821.

Biederman, J., Rosenbaum, J. F., Hirshfeld, M. A., Faraone, S. V., Bolduc, E. A., & Gersten, M. (1990). Psychiatric correlates of behavioral inhibition in young children of parents with and without psychiatric disorders. *Archives of General Psychiatry*, **47**, 21–26.

Bitran, D., Hilvers, R. J., & Kellogg, C. K. (1991). Anxiolytic effects of 3-alphahydroxy-5-alpha[beta]-pregnan-20-one: Endogenous metabolites of progesterone that are active at the GABA-A receptor. *British Research*, **561**, 157–161.

Blanchard, R. J., & Blanchard, D. C. (1977). Aggressive behavior in the rat. *Behavioral Biology*, **21**(2), 197–224.

Blanchard, R. J., Fukunaga, K., Blanchard, D. C., & Kelly, M. J. (1975). Concpecific aggression in the laboratory rat. *Journal of Comparative and Physiological Psychology*, **89** (10),1204–1208.

Blass, E. M., & Kehoe, P. (1987). Behavioral characteristics of emerging opioid systems in newborn rats. In A. Krasnegor, E.M. Blass, M. A. Hofer, & W.P. Smotherman (Eds.), *Perinatal Development: A Psychobiological Perspective*. (pp. 61–82). New York: Academic Press.

Bowlby, J. (1969). *Attachment: Attachment and loss. Vol. 1*, New York: Basic Books.

Brot, M. D., Akwa, Y., Purdy, R. H., Koob, G. F., & Britton, K. T. (1997). The anxiolytic-like effects of the neurosteroid allopregnanolone: Interactions with GABA(A) receptors. *European Journal of Pharmacology*, **325**, 1–7.

Brunelli, S. A. (2005). Selective breeding for an infant phenotype: Rat pup ultrasonic vocalization (USV). *Behavior Genetics*, **35**(1), 53–65.

Brunelli, S. A., Aviles, J. A., Gannon, K. S., Branscomb, A., & Shacham, S. (2009). PRX-00023, a selective serotonin 1A receptor agonist, reduces ultrasonic vocalizations in infant rats bred for high infantile anxiety. *Pharmacology, Biochemistry and Behavior*, **1**, 8–15.

Brunelli, S. A., & Hofer, M. A. (2001). Selective breeding for an infantile phenotype (isolation calling): A window on developmental processes. In E. M. Blass, (Ed.), *Handbook of behavioral neurobiology: Developmental psychobiology*, Vol. *13* (pp. 433–482). New York: Kluwer Academic/Plenum Press.

Brunelli, S. A., & Hofer, M. A. (2007). Selective breeding for infant rat separation-induced ultrasonic vocalizations: Developmental precursors of passive and active coping styles. *Behavioral Brain Research*, **182**(2), 193–207.

Brunelli, S. A., Hofer, M. A., & Weller, A. (2001). Selective breeding for infant vocal response: A role for postnatal maternal effects? *Developmental Psychobiology*, **38**, 221–228.

Brunelli, S. A., & Kehoe, P. (2005). Differences in catecholamine utilization at postnatal day 10 in rats selected for ultrasonic vocalization (USV) responses to maternal separation. *Abstracts, Developmental Psychobiology*, **47**(4), 421.

Brunelli, S. A., Myers, M. M., Asekoff, S. L., & Hofer, M. A. (2002). Effects of selective breeding for infant rat ultrasonic vocalization on cardiac responses to isolation. *Behavioral Neuroscience*, **116**, 612–623.

Brunelli, S. A., Nie, R., Whipple, C., Winiger, V., Hofer, M. A., & Zimmerberg, B. (2006). The effects of selective breeding for infant ultrasonic vocalizations on play behavior in juvenile rats. *Physiology & Behavior*, **87**, 527–536.

Burgdorf, J., Wood, P. L., Kroes, R. A., Moskal, J. R., & Panksepp, J. (2007). Neurobiology of 50-kHz ultrasonic vocalizations in rats: Electrode mapping, lesion, and pharmacology studies. *Behavioral Brain Research*, **182**, 274–283.

Burgess, K. B., Marshall, P. J., Rubin, K. H., & Fox, N. A. (2003). Infant attachment and temperament as predictors of subsequent externalizing problems and cardiac physiology. *Journal of Child Psychology & Psychiatry*, **44**(6), 819–832.

Calkins, S. D., & Fox, N. A. (1992). The relations among infant temperament, security of attachment, and behavioral inhibition at twenty-four months. *Child Development*, **63**(6),1456–1472.

Carboni, E., Wieland, S., Lan, N. C., & Gee, K. W. (1996). Anxiolytic properties of endogenously occurring pregnanediols in two rodent models of anxiety. *Psychopharmacology*, **126**, 173–178.

Caspi, A., Harrington, H., Milne, B., Amell, J. W., Theodore, R. F., & Moffitt, T. E. (2003). Children's behavioral styles at age three are linked to their adult personality traits at age 26. *Journal of Personality*, **71**(4), 495–513.

Caspi, A., McLay, J., Moffitt, T. E., Mill, J., Marin, J., & Craig, I. W., et al. (2002). Role of genotype in the cycle of violence in maltreated children. *Science*, **297**(5582), 851–854.

Caspi, A., Moffitt, T. E., Newman, D. L., & Silva, P. A. (1996). Behavioral observations at age 3 years predict adult psychiatric disorders. Longitudinal evidence from a birth cohort. Archives of General Psychiatry, **53**(11), 1033–1039.

Champagne, F. A. (2008). Epigenetic mechanisms and the transgenerational effects of maternal care. *Frontiers in Neuroendocrinology*, **29**(3), 386–97.

Chen, J. C., Turiak, G., Galler, J. R., & Volicer, L. (1997). Postnatal changes of brain monoamine levels in prenatally malnourished and control rats. *International Journal of Developmental Neuroscience*, **15**(2), 257–263.

Dallaire, D. H., & Weinraub, M. (2005). Predicting children's separation anxiety at age 6: The contributions of infant-mother attachment security, maternal sensitivity, and maternal separation anxiety. *Attachment & Human Development*, **7**(4), 393–408.

D'Amato, R. J., Blue, M. E., Largent, B. L., Lynch, D. R., Ledbetter, D. J., & Molliver, M. E., et al. (1987). Ontogeny of the serotonergic projection to rat neocortex: Transient expression of a dense innervation to primary sensory areas. *Proceedings of the National Academy of Science, USA*, **84**(12), 4322–4326.

De Bruin, J. P. (1990). Social behaviour and the prefrontal cortex. *Progress in Brain Research*, **85**, 485–496.

Dinopoulos, A., Dori, I., & Parnavelas, J. G. (1997). The serotonin innervation of basal forebrain shows a transient phase during development. *Developmental Brain Research*, **99**, 38–52.

Ebner, K., Wotjak, C. T., Landgraf, R., & Engelmann, M. (2005). Neuroendocrine and behavioral response to social confrontation: Residents versus intruders, active versus passive coping styles. *Hormones & Behavior*, **47**(1), 14–21.

Einon, D. F., & Morgan, M. J. (1977). A critical period for social isolation in the rat. *Developmental Psychobiology*, **10**, 123–132.

Einon, D. F., & Morgan, M. J. (1978). Brief periods of socialization and later behavior in the rat. *Developmental Psychobiology*, **11**, 213–225.

Fagen, R. M. (1981). *Animal play behaviour*. New York: Oxford University Press.

Faraone, S. V., Biederman, J., Keenan, K., & Tsuang, M. T. (1991). Separation of DSM-III attention deficit disorder and conduct disorder: Evidence from a family-genetic study of American child psychiatric patients. *Psychological Medicine*, **21**(1), 109–121.

Farrington, D. P. (1989). Early predictors of adolescent aggression and adult violence. *Violence Victims*, **4**(2), 79–100.

Field, E. F., & Pellis, S. M. (1994). Differential effects of amphetamine on attack and defense components of play fighting in rats. *Physiology & Behavior*, **56**(2), 325–330.

File, S. E., & Seth, P. (2003). A review of 25 years of the social interaction test. *European Journal of Pharmacology*, **463**(1–3), 35–53.

Fox, N. A., Nichols, K. E., Henderson, H. A., Rubin, K., Schmidt, L., & Hamer, D., et al. (2005). Evidence for a gene-environment interaction in predicting behavioral inhibition in middle childhood. *Psychological Science*, **16**(12), 921–926.

Frye, C. A., Petralia, S. M., & Rhodes, M. E. (2000). Estrous cycle and sex differences in performance on anxiety tasks coincide with increases in hippocampal progesterone and 3alpha, 5alpha-THP. *Pharmacology, Biochemistry, & Behavior*, **67**(3), 587–596.

Frye, C. A., & Walf, A. A. (2002). Changes in progesterone metabolites in the hippocampus can modulate open field and forced swim test behavior of proestrous rats. *Hormones & Behavior*, **41**, 306–315.

Frye, C. A., & Walf, A. A. (2004). Estrogen and/or progesterone administered systemically or to the amygdala can have anxiety-reducing, fear-reducing, and pain-reducing effects in ovariectomized rats. *Behavioral Neuroscience*, **118**, 306–313.

Galineau, L., Kodas, E., Guilloteau, D., Vilar, M. P., & Chalon, S. (2004). Ontogeny of the dopamine and serotonin transporters in the rat brain: An autoradiographic study. *Neuroscience Letters*, **17**, 266–271.

Galler, J. R., & Tonkiss, J. (1991). Prenatal protein malnutrition and maternal behavior in Sprague-Dawley rats. *Journal of Nutrition*, **121**(5), 762–769.

Garstang, W. (1922). The theory of recapitulation: A critical re-statement of the biogenetic law. *Journal of the Linnean Society of London Zoology*, **35**, 81–101.

Gaspar, P., Cases, O., & Maroteaux, L. (2003). The developmental role of serotonin: News from mouse molecular genetics. *Nature Reviews Neuroscience*, **4**, 1002–1012.

Gerardin, D. C., Pereira, O. C., Kempinas, W. G., Florio, J. C., Moreira, E. G., & Bernardi, M. M. (2005). Sexual behavior, neuroendocrine, and neurochemical aspects in male rats exposed prenatally to stress. *Physiology & Behavior*, **84**(1), 97–104.

Gingrich, J. A., & Hen, R. (2001). Dissecting the role of the serotonin system in neuropsychiatric disorders using knockout mice. *Psychopharmacology (Berlin)*, **155**(1), 1–10.

Gottlieb, G. (1992). *Individual development and evolution*. New York: Oxford University Press.

Gottlieb, G. (2007). Probabilistic epigenesis. *Developmental Science*, **10**(1), 1–11.

Gould, T. D., & Gottesman, I. I. (2006). Psychiatric endophenotypes and the development of valid animal models. *Genes, Brain & Behavior*, **5**(2), 13–19.

Greaves-Lord, K., Ferdinand, R. F., Sondeijker, F. E., Dietrich, A., Oldehinkel, A. J., & Rosmalen, J. G., et al. (2007). Testing the tripartite model in young adolescents: Is hyperarousal specific for anxiety and not depression? *Journal of Affective Disorders*, **102**(1–3), 55–63.

Griffith, L. D., & Mellon, S. H. (1999). Selective serotonin reuptake inhibitors directly alter activity of neurosteroidogenic enzymes. *Proceedings of the National Academy of Sciences, USA*, **96**, 13512–13517.

Haberstick, B. C., Schmitz, S., Young, S. E., & Hewitt, J. K. (2005). Contributions of genes and environments to stability and change in externalizing and internalizing problems during elementary and middle school. *Behavior Genetics*, **35**(4), 381–386.

Hahn, M. E., Hewitt, J. K., Schanz, N., Weinreb, L., & Henry, A. (1997). Genetic and developmental influences on infant mouse ultrasonic calling. I. A diallel analysis of the calls of 3-day olds. *Behavior Genetics*, **27**(2), 133–143.

Harvey, A. T., & Hennessy, M. M. (1995). Corticotropin-releasing factor modulation of the ultrasonic vocalization rate of isolated rat pups. *Brain Research: Developmental Brain Research*, **87**(2), 125–134.

Hennessy, M. M., & Weinberg, J. (1990). Adrenocortical activity during conditions of brief social separation in preweaning rats. *Behavioral Neural Biology*, **54**(1), 42–55.

Hill, J. (2002). Biological, psychological and social processes in the conduct disorders. *Journal of Child Psychology & Psychiatry*, **43**(1), 133–164.

Hofer, M. A. (1984). Early stages in the organization of cardiovascular control. *Proceedings of the Society for Experimental Biology and Medicine*, **175**, 147–157.

Hofer, M. A. (1994). Early relationships as regulators of infant physiology and behavior. *Acta Paediatrica, Supplement*, **397**, 735–738.

Hofer, M. A. (1996). Multiple regulators of ultrasonic vocalization in the infant rat. *Psychoneuroendocrinology*, **21**(2), 203–217.

Kagan, J., Reznick, J. S., & Gibbons, J. (1989). Inhibited and uninhibited types of children. *Child Development*, **60**, 838–945.

Kagan, J., Snidman, N., Zentner, M., & Peterson, E. (1999). Infant temperament and anxious symptoms in school age children. *Developmental Psychopathology*, **11**(2), 209–224.

Kehoe, P., Mallinson, K., Bronzino, J., & McCormick, C. M. (2001). Effects of prenatal protein malnutrition and neonatal stress on CNS responsiveness. *Brain Research: Developmental Brain Research*, **132**(1), 23–31.

Knutson, B., Burgdorf, J., & Panksepp, J. (1998). Anticipation of play elicits high-frequency ultrasonic vocalizations in young rats. *Journal of Comparative Psychology*, **112**(1), 65–, 73.

Knutson, B., Burgdorf, J., & Panksepp, J. (1999). High-frequency ultrasonic vocalizations index conditioned pharmacological reward in rats. *Physiology & Behavior*, **66**(4), 639–, 643.

Kochanska, G. (1998). Mother-child relationship, child fearfulness, and emerging attachment: A short-term longitudinal study. *Developmental Psychology*, **34**(3), 480–490.

Kochanska, G. (2001). Emotional development in children with different attachment histories: The first three years. *Child Development*, **72**(2), 474–90.

Kofman, O. (2002). The role of prenatal stress in the etiology of developmental behavioral disorders. *Neuroscience & Biobehavioral Revues*, **26**, 457–470.

Lemaire, V., Koehl, M., Le Moal, M., & Abrous, D. N. (2000). Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. *Proceedings of the National Academy of Science, USA*, **97**(20), 11032–11037.

Li, Q. H., Nakadate, K., Tanaka-Nakadate, S., Nakatsuka, D., Cui, Y., & Watanabe, Y. (2004). Unique expression patterns of 5-HT2A and 5-HT2C receptors in the rat brain during postnatal development: Western blot and immunohistochemical analyses. *Journal of Comparative Neurology*, **469**(1), 128–140.

Manassis, K., Bradley, S., Goldberg, S., Hood, J., & Swinson, R. P. (1995). Behavioural inhibition, attachment and anxiety in children of mothers with anxiety disorders. *Canadian Journal of Psychiatry*, **40**(2), 87–92.

Marshall, P. J., & Stevenson-Hinde, J. (1998). Behavioral inhibition, heart period, and respiratory sinus arrhythmia in young children. *Developmental Psychobiology*, **33**(3), 283–292.

Martinez, A. R., Brunelli, S. A., & Zimmerberg, B. (2009). Communal nesting alters the development of affective and social behavior in rats selectively bred for an infantile trait. *Developmental Psychobiology*, **51**, 593.

Meaney, M. J., & Stewart, J. (1981). A descriptive study of social development in the rat (*Rattus norvegicus*). *Animal Behavior*, **129**, 34–45.

Meaney, M. J., & Szyf, M. (2005). Environmental programming of stress responses through DNA methylation: Life at the interface between a dynamic environment and a fixed genome. *Dialogues in Clinical Neuroscience*, **7**(2), 103–123.

Meaney, M. J., Szyf, M., & Seckl, J. R. (2007). Epigenetic mechanisms of perinatal programming of hypothalamic-pituitary-adrenal function and health. *Trends in Molecular Medicine*, **13**(7), 269–277.

Mellon, S. H. (2004). Synthesis, enzyme localization, and regulation of neurosteroids. In S. S. Smith (Ed.), *Neurosteroid effects in the central nervous system: The role of the GABA-A receptor* (pp. 1–46). Boca Raton, FL: CRC Press.

Monk, C., Fifer, W. P., Myers, M. M., Sloan, R. P., Trien, L., & Hurtado, A., (2000). Maternal stress responses and anxiety during pregnancy: Effects of maternal heart rate. *Developmental Psychobiology*, **36**(1), 67–77.

Monk, C., Myers, M. M., Sloan, R. P., & Fifer, W. P. (2003). Effects of women's stress-elicited physiological activity and chronic anxiety on fetal heart rate. *Journal of Developmental and Behavioral Pediatrics*, **24**(1), 32–38.

Monk, C., Sloan, R. P., Myers, M. M., Ellman, L., Werner, E., & Jeon, J., et al. (2004). Fetal heart rate reactivity differs by women's psychiatric status: An early marker for developmental risk? *American Academy of Child & Adolescent Psychiatry*, **43**(3), 283–290.

Muneoka, K., Nakatsu, T., Fuji, J., Ogawa, T., & Takigawa, M. (1999). Prenatal administration of nicotine results in dopaminergic alterations in the neocortex. *Neurotoxicology & Teratology*, **1**(5), 603–609.

Muneoka, K. T., Ogawa, T., & Kamei, K. (1997). Prenatal nicotine exposure affects the development of the central serotonergic system as well as the dopaminergic system in rat offspring: Involvement of route of drug administrations. *Brain Research: Developmental Brain Research*, **102**(1), 117–126.

Muneoka, K. T., & Takigawa, M. (2002). A neuroactive steroid, pregnenolone, alters the striatal dopaminergic tone before and after puberty. *Neuroendocrinology*, **75**(5), 288–295.

Murphy, C. A., Sloan, R. P., & Myers, M. M. (1991). Pharmacological responses and spectral analyses of spontaneous fluctuations in heart rate and blood pressure in SHR rats. *Journal of the Autonomic Nervous System*, **36**(3.), 237–250.

Oades, R. D., Sadile, A. G., Sagvolden, T., Viggiano, D., Zuddas, A., & Devoto, P., et al. (2005). The control of responsiveness in ADHD by catecholamines: Evidence for dopaminergic, noradrenergic and interactive roles. *Developmental Science*, **8**(2), 122–131.

Panksepp, J. (1981). The ontogeny of play in rats. *Developmental Psychobiology*, **14**(4), 327–332.

Panksepp, J., Normansell, L., Cox, J. F., & Siviy, S. M. (1994). Effects of neonatal decortication on the social play of juvenile rats. *Physiology & Behavior*, **56**, 429–443.

Parent, C. I., & Meaney, M. J. (2008). The influence of natural variations in maternal care on play fighting in the rat. *Developmental Psychobiology*, **50**(8), 767–776.

Pellis, S. M. (1988). Agonistic versus amicable targets of attack and defense: Consequences for the origin, function, and descriptive classification of play-fighting. *Aggressive Behavior*, **14**, 85–104.

Pellis, S. M., Castaneda, E., McKenna, M. M., Tran-Nguyen, L. T. L., & Whishaw, I. Q. (1993). The role of the striatum in organizing sequences of play fighting in neonatally dopamine- depleted rats. *Neuroscience Letters*, **158**, 13–14.

Pellis, S. M., & Pellis, V. C. (1983). Locomotor-rotational movements in the ontogeny and play of the laboratory rat *Rattus norvegicus*. *Developmental Psychobiology*, **16**(4), 269–286.

Pellis, S. M., Pellis, V. C., & Whishaw, I. Q. (1992). The role of the cortex in play fighting by rats: Developmental and evolutionary implications. *Brain & Behavioral Evolution*, **39**(5), 270–284.

Pellitteri, R., Zicca, A., Mancardi, G. L., Savio, T., & Cadoni, A. (2001). Schwann cell-derived factors support serotoninergic neuron survival and promote neurite outgrowth. *European Journal of Histochemistry*, **45**(4), 367–376.

Perrone-Capano, C., & Di Porzio, U. (2000). Genetic and epigenetic control of midbrain dopaminergic neuron development. *International Journal of Developmental Biology*, **44**, 679–687.

Pine, D. S., Wasserman, G., Coplan, J.D., Fried, J., Sloan, R.P., & Myers, M. M., et al. (1996). Serotonergic and cardiac correlates of aggression in children. *Annals of the New York Academy of Sciences*, **794**, 391–393.

Pine, D. S., Wasserman, G. A., Miller, L., Coplan, J. D., Bagiella, E., & Kovelenku, P., et al. (1998). Heart period variability and psychopathology in urban boys at risk for delinquency. *Psychophysiology*, **35**(5), 521–529.

Plomin, R., DeFries, J. C., & Loehlin, J. C. (1977). Genotype–environment interaction and correlation in the analysis of human behavior. *Psychological Bulletin*, **84**, 309–322.

Plomin, R., DeFries, J. C., & McClearn, G. E. (1991). *Behavioral genetics: A primer*. New York: W. H. Freeman & Co.

Raine, A. (2002). Annotation: The role of prefrontal deficits, low autonomic arousal, and early health factors in the development of antisocial and aggressive behavior in children. *Journal of Child Psychology and Psychiatry*, **43**(4), 417–434.

Reddy, D., & Kulkarni, S. K. (1997). Differential anxiolytic effects of neurosteroids in the mirrored chamber behavior test in mice. *Brain Research*, **752**, 61–71.

Rosenbaum, J. F., Biederman, J., Bolduc-Murphy, E. A., Faraone, S. V., Chaloff, J., & Hirshfeld, D. R., et al. (1993). Behavioral inhibition in childhood: A risk factor for anxiety disorders. *Harvard Review of Psychology*, **1**(1), 2–16.

Roubertoux, P. L., Martin, B., Le Roy, I., Beau, J., Marchaland, C., & Perez-Diaz, F., et al. (1995). Vocalizations in newborn mice: Genetic analysis. *Behavior Genetics*, **26**(4), 427–437.

Rutherford, S. L., & Lindquist, S. (1998). Hsp90 as a capacitor for morphological evolution. *Nature*, **396**, 336–342.

Rutter, M., Moffitt, T. E., & Caspi, A. (2005). Gene–environment interplay and psychopathology: Multiple varieties but real effects. *Journal of Child Psychology & Psychiatry* **47**(3/4), 226–261.

Schmidt, L. A., Fox, N. A., Rubin, K. H., Hu, S., & Hamer, D. H. (2002). Molecular genetics of shyness in preschoolers. *Personality & Individual Differences*, **33**, 227–238.

Schneider, K. M., Nicolotti, L., & Delamater, A. (2002). Aggression and cardiovascular response in children. *Journal of Pediatric Psychology*, **27**(7), 565–573.

Schwartz, C. E., Snidman, N., & Kagan, J. (1999). Adolescent social anxiety as an outcome of inhibited temperament in childhood. *Journal of the American Academy of Child & Adolescent Psychiatry*, **38**(8), 1008–1015.

Schwartz, C. E., Wright, C. Z., Shin, L. M., Kagan, J., & Rauch, S. L. (2003). Inhibited and uninhibited children "grown up": Adult amygdalar response to novelty. *Science*, **300**(5627), 1952–1953.

Serra, M., Pisu, M. G., Littera, M., Papi, G., Sanna, E., & Tuveri, F., et al. (2000). Social isolation-induced decreases in both the abundance of neuroactive steroids and GABA-A receptor function in rat brain. *Journal of Neurochemistry*, **75**, 732–740.

Shair, H. N., Brunelli, S. A., Velazquez, Z., & Hofer, M. A. (2000). Adult behavioral tests of rats selectively bred for infantile ultrasonic vocalization. *Developmental Psychobiology*, **356**.

Shamir-Essakow, G., Ungerer, J. A., & Rapee, R. M. (2005). Attachment, behavioral inhibition, and anxiety in preschool children. *Journal of Abnormal Child Psychology*, **33**(2), 131–143.

Shulga, A., Thomas-Crusells, J., Sigl, T., Blaesse, A., Mestres, P., & Meyer, M., et al. (2008). Posttraumatic GABA(A)-mediated [Ca2 +] increase is essential for the induction of brain-derived neurotrophic factor-dependent survival of mature central neurons. *Journal of Neuroscience*, **28**, 6996–7005.

Sloan, R. P., Shapiro, P. A., Bagiella, E., Myers, M. M., & Gorman, J. M. (1999). Cardiac autonomic control buffers blood pressure variability responses to challenge: A psychophysiological model of coronary artery disease. *Psychosomatic Medicine*, **51**, 58– 68.

Sloan, R. P., Shapiro, P. A., Bigger, J. T., Bagiella, E., Steinman, R. C., & Gorman, J. M. (1994). Cardiac autonomic control and hostility in healthy subjects. *American Journal of Cardiology*, **74**, 298–300.

Smith, S. S. (2004). Withdrawal effects of a neuroactive steroid as a model of PMS: Synaptic physiology to behavior. In S. S. Smith (Ed.), *Neurosteroid effects in the central nervous system: The role of the GABA-A receptor* (pp. 144–172). Boca Raton, FL: CRC Press.

Smoller, J. W., Rosenbaum, J. F., Biederman, J., Kennedy, J., Dai, D., & Racette, S. R., et al. (2003). Association of a genetic marker at the cortiocotrophin-releasing hormone locus with behavioral inhibition. *Biological Psychiatry*, **54**(12), 1376–1381.

Smoller, J. W., Rosenbaum, J. F., Biederman, J., Kennedy, J., Dai, D., & Racette, S. R., et al. (2005). The corticotrophin-releasing hormone gene and behavioral inhibition in children at risk for panic disorder. *Biological Psychiatry*, **57**(12), 1485–1492.

Snustad, D. P., Simmons, M. J., & Jenkins, J. B. (1997). *Principles of genetics*. New York: Wiley.

Stams, G. J., Juffer, F., & van IJzendoorn, M. H. (2002). Maternal sensitivity, infant attachment, and temperament in early childhood predict adjustment in middle childhood: The case of adopted children and their biologically unrelated parents. *Developmental Psychology*, **38**(5), 806–821.

Stevenson-Hinde, J., & Marshall, P. J. (1999). Behavioral inhibition, heart period, and respiratory sinus arrhythmia: An attachment perspective. *Child Development*, **70**(4), 805–816.

Stevenson-Hinde, J., & Shouldice, A. (1995). 4.5–7 years: Fearful behaviour, fears and worries. *Journal of Child Psychology & Psychiatry*, **36**(6), 1027–1038.

Sullivan, R. M. (2004). Hemispheric asymmetry in stress processing in rat prefrontal cortex and the role of mesocortical dopamine. *Stress*, **7**(2), 131–143.

Sullivan, R. M., & Brake, W. G. (2003). What the rodent prefrontal cortex can teach us about attention-deficit/hyperactivity disorder: The critical role of early developmental events on prefrontal function. *Behavioral Brain Research*, **146**(1–2), 43–55.

Sundstrom-Poromaa, I. (2004). Premenstrual dysphoric disorder. In: S. S. Smith (Ed), *Neurosteroid effects in the central nervous system: The role of the GABA-A receptor* **146**(1–2) (pp. 291–304). Boca Raton, FL: CRC Press.

Tarazi, F. I., Tomasini, E. C., & Baldessarini, R. J. (1998). Postnatal development of dopamine and serotonin transporters in rat caudate-putamen and nucleus accumbens septi. *Neuroscience Letters*, **254**(1), 21–24.

Thor, D. H., & Holloway, W. R., Jr. (1983). Play-solicitation behavior in juvenile male and female rats. *Animal Learning & Behavior*, **11**(2), 173–178.

Tonkiss, J., Trzcinska, M., Galler, J. R., Ruiz-Opazo, N., & Herrera, V. L. M. (1998). Prenatal malnutrition-induced changes in blood pressure: Dissociation of stress and nonstress responses using radiotelemetry. *Hypertension*, **32**, 108–114.

van Broekhoven, F., & Verkes, R. J. (2003). Neurosteroids in depression: A review. *Psychopharmacology*, **165**, 97–110.

Vanderschuren, L. J. M. J., Niesink, R. J. M., & Van Ree, J. M. (1997). The neurobiology of social play behavior in rats. *Neuroscience & Biobehavioral Reviews*, **21**(3), 309–326.

Varlinskaya, E. I., & Spear, L. P. (2006). Ontogeny of acute tolerance to ethanol-induced social inhibition in Sprague-Dawley rats. *Alcoholism: Clinical & Experimental Research*, **30**(11), 1833–1844.

Vivian, J. A., Barros, H. M., Maintiu, A., & Miczek, K. A. (1997). Ultrasonic vocalizations in rat pups: Modulation at the gamma-aminobutyric acid-A receptor complex and the neurosteroid recognition site. *Journal of Pharmacology & Experimental Therapeutics*, **282**, 318–325.

Waddington, C. H. (1959). Evolutionary systems – animal and human. *Nature*, **183**, 1634–1638.

Warren, S. L., Gunnar, M. R., Kagan, J., Anders, T. F., Simmens, S. J., & Rones, M., et al. (2003). Maternal panic disorder: Infant temperament, neurophysiology, and parenting behaviors. *Journal of the American Academy of Child & Adolescent Psychiatry*, **42**(7), 814–825.

Weissman, M. M., Wickramaratne, P. J., Nomura, Y., Warner, V., Verdeli, H., & Pilowsky, D. J., et al. (2005). Families at high and low risk for depression: A three-generation study. *Archives of General Psychiatry*, **62**(1), 29–36.

West, M. J., & King, A. P. (1987). Settling nature and nurture into an ontogenetic niche. *Developmental Psychobiology*, **20**(5), 549–562.

Whatson, T. S., & Smart, J. L. (1978). Social behavior of rats following pre- and early postnatal undernutrition. *Physiology & Behavior*, **20**, 749–753.

Whatson, T. S., Smart, J. L., & Dobbing, J. (1975). Dominance relationships among previously undernourished and well fed male rats. *Physiology & Behavior*, **14**, 425–429.

Whatson, T. S., Smart, J. L., & Dobbing, J. (1976). Undernutrition in early life: lasting effects on activity and social behavior of male and female rats. *Developmental Psychobiology*, **9** (6), 529–538.

Wichers, M. C., Purcell, S., Danckaerts, M., Derom, C., Derom, R., & Vlietinck, R., et al. (2002). Prenatal life and post-natal psychopathology: Evidence for negative gene-birth weight interaction. *Psychological Medicine*, **32**, 1165–1174.

Wickramaratne, P. J., Greenwald, S., & Weissman, M. M. (2000). Psychiatric disorders in the relatives of probands with prepubertal-onset or adolescent-onset major depression. *Journal of the American Academy of Child and Adolescent Psychiatry*, **39** (11), 1396–1405.

Wongwitdecha, N., & Marsden, C. A. (1996). Social isolation increases aggressive behaviour and alters the effects of diazepam in the rat social interaction test. *Behavioral Brain Research*, **75**(1/2), 27–32.

Yeragani, V. K. (1995). Heart rate and blood pressure variability: Implications for psychiatric research. *Neuropsychobiology*, **32**, 182–191.

Yeragani, V. K., Pohl, R., Balon, R., Jampala, V. C., & Jayaraman, A. (2002). Twenty-four-hour QT interval variability: Increased QT variability during sleep in patients with panic disorder. *Neuropsychobiology*, **46**(1), 1–6.

Yeragani, V. K., Rao, K. A., Pohl, R., Jampala, V. C., & Balon, R. (2001). Heart rate and QT variability in children with anxiety disorders: A preliminary report. *Depression & Anxiety*, **13**, 72–77.

Zhang, Z. W. (2003). Serotonin induces tonic firing in layer V pyramidal neurons of rat prefrontal cortex during postnatal development. *Journal of Neuroscience*, **23**(8), 3373–3384.

Zhou, F. C., Sari, Y., & Zhang, J. K. (2000). Expression of serotonin transporter protein in developing rat brain. *Brain Research: Developmental Brain Research*, **119**(1), 33–45.

Zimmerberg, B., Martinez, A. R., Skudder, C. M., Killien, E. Y., Robinson, S. A., & Brunelli, S. A. (2009). Effects of gestational allopregnanolone administration in rats bred for high affective behavior. *Physiology & Behavior*, **99**(2), 212–217.

Zimmerberg, B., Brunelli, S. A., Fluty, A. J., & Frye, C. A. (2005). Differences in affective behaviors and hippocampal allopregnanolone levels in adult rats of lines selectively bred for infantile vocalizations. *Behavioral Brain Research*, **159**(2), 301–311.

Zimmerberg, B., Brunelli, S. A., & Hofer, M. A. (1994). Reduction of rat pup ultrasonic vocalizations by the neuroactive steroid allopregnanolone. *Pharmacology Biochemistry & Behavior*, **47**, 735–738.

Zuckerkandl, E., & Cavalli, G. (2007). Combinatorial epigenetics, "junk DNA" and the evolution of complex organisms. *Gene*, **390**, 232–242.

11

Emergence and Constraint in Novel Behavioral Adaptations

Kathryn E. Hood

We live, it appears, in a world of emergent evolution: of problems whose solutions, if they are solved, beget new and deeper problems. (Karl Popper, 1974)

How do novel behavioral adaptations emerge and persist? What is the role of developmental and cognitive attainment in supporting emergent properties? Everyday life presents a range of opportunities and accompanying constraints that support the generation of both novel and conventional behavioral modes. The goal of this chapter is to consider various approaches in understanding emergent adaptations through the methods of psychology, philosophy, cognitive studies, physiology, and the study of developmental change. The contributions of constraints are also of interest, as constraints in physical settings and social directives may be structured to guide, limit, redirect, or eliminate aspects of behavioral expression. The levels of interplay between these two modes, emergence and constraint, suggest that both opportunities and limits may be usefully generative in novel settings.

The concepts of emergence and constraint have been defined in numerous ways by philosophers, psychologists, biologists, behaviorists, developmental and evolutionary thinkers. A formal definition of emergence seems premature, given the range of phenomena that support emergent outcomes. However, we can recognize emergent outcomes, which may occur as a sudden appearance of something new which was not expected. An emergent property may arise at a boundary or a border, or as a novel adaptation or a method for an individual or a social group. Emergent entities, properties, or substances arise from lower level entities, and they emerge as wholes, irreducible with respect to historical, cultural, and biological systems. They cannot be parsed into more simple forms; that is, they are not manifested at lower levels than their own. By contrast, the reality of

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constraint is omnipresent as gravity, limitation, direction, and restriction. Positive results of constraint also are tangible: sticking to a deadline despite other attractions, avoiding peril, keeping faith. It is the balance of these two factors, emergence and constraint, that offers stability and novelty in a changing world.

The range of conditions that support the emergence of novel properties point to a variety of interpretations and perspectives. Scientific and philosophical studies provide a starting point for thinking about emergent phenomena, with examples that instantiate the boundaries of emergent properties, as they are described here in relation to philosophy (Clayton, 2006), genetic and related factors (Jablonka & Lamb, 2002, 2005; Nijhout, 1990), developmental processes (Eckerman, 1993; Fischer et al., 1997; Gottlieb, 1975; Jung, 1954; Rosenblatt & Mayer, 1995; Thelen and Smith, 1998), social and family relationships (Cox & Paley, 1997; Sameroff, 1994), cultural change (Bates et al., 1998; Sapolsky, 2006), and evolution (Gottlieb, 1984, 1992; Müller & Streicher, 1989; Waddington, 1939; 1968). Not accounted for in this chapter are the interesting computational and modeling approaches that also are related to emergence of novel entities (see Greenberg, Partridge & Ablah, 2006).

As an example of an emergent form in the domain of human culture, the creation of a new form of music within the Western tradition was accomplished by Arnold Schoenberg. In 1923, he radically reordered the way in which music can sound. Followers of classical music were shocked to hear the 12-tone scale of Schoenberg, and were unprepared to experience this new music's disquieting emotional qualities. After Schoenberg's death, the 12-tone system was expanded by his passionate adherents, and there were equally passionate detractors. His once-unimaginable system of music has since been carried forward, enriching new forms of jazz and contemporary music in novel modes (Dyer, 2006).

Topics that follow in this chapter include a selective review of emergent factors across levels (genetic, epigenetic, individual, developmental, and social), as well as constraints that may limit operations in developmental settings. Contemporary theories related to evolution and emergence are presented in relation to novel adaptations in four species: bonobos, baboons, snow monkeys and mice. Within an interest in the role of constraints, a view of factors shaping behavioral development is served in two examples, the construction of novel adaptive responses through social learning among free-ranging and provisioned macaques in a constrained environment, and working within the constraints of materials in the context of human craftmaking.

In the following section, active programs of research are structured to include constraints on behaviors and to specify the conditions for relevant behaviors. For example, in the study of selectively bred mice, social behaviors and exploratory behaviors as well as patterns of epigenetic inheritance of maternal behaviors and preferences are documented. In addition, Gilbert Gottlieb's long-held interest in evolutionary change, based on the proposal that "behavior is the leading edge of evolution," supported the emergence of an evolutionary novelty arising through

intergenerational adaptations. To illuminate the concept of emergence at a different level, the section concludes with a brief literary and psychological study of the acquisition of self- and other-consciousness and conscience in C. G. Jung's interpretation of *The Book of Job*. In the conclusion, the appreciation of Gilbert Gottlieb's impact in the field is made evident; for an example, see Halpern, Hood & Lerner (2007), in the "Special Issue: The Scientific Contributions of Gilbert Gottlieb" in the *European Journal of Developmental Science*.

Historical Precedents

The valuable history of emergence and evolution by Blitz (1992) usefully documents the many efforts to characterize emergent entities. After G. H. Lewes in 1875 coined "emergent" properties as discontinuous aspects of qualitative change, the comparative psychologist and later philosopher of nature, C. Lloyd Morgan proposed in 1913 that there are three levels of phenomena: physio-chemical, relations of organic types, and cognitive types. Limitations of locale defined the proposition by philosopher R. W. Sellars in 1922 that emergence is local rather than universal. This proposition fits readily with sociologist G. H. Mead's 1932 understanding of emergent events. Mead proposed that novel properties inevitably force a rewriting of time, in particular, the past, so that "once discovered, the emergent then ceases to be emergent and follows from the past which has replaced the former past" (p. 134). The embryologist J. Needham wrote in his 1937 Spencer lecture to address "Integrative Levels: A Reevaluation of the Idea of Progress" based on dialectical theory. In his 1945 *Science* paper, A. B. Novikoff published "The Concept of Integrative Levels and Biology," and referred to physical, chemical, biological, and sociological levels (also see Needham, 1945). This work was followed by T. C. Schneirla's comparative psychological approach to levels of complexity as "overlapping and interrelated adaptive stages" (1949, 1951, 1965). Biologist E. Mayr proposed that emergent entities can produce effects at lower levels, as "the return action of higher levels on lower ones" (1982). Among philosophers, A. N. Whitehead, N. Hartman, and M. Bunge joined in considering these views. Other theorists have produced their versions of ontological levels, some heuristically concise, others ornate, such as the system of Yale biophysicist Harold Morowitz (2002) which includes 28 levels of phenomena over the course of history.

The present resurgence of interest in emergent processes points to a fresh look at the theoretical base for scientific and philosophical studies of development and novel adaptations among developmental and evolutionary thinkers (Clayton & Davies, 2006; Gottlieb, 1992; Greenberg et al., 2006; Hood, 1995; Lerner, 2002). For understanding the world as continuously "becoming" through the processes that constitute emergence, the perspective of Levins and Lewontin (1985) as the

"dialectical biologist" shows the world as continually becoming among the relationships of qualities, processes, and entities. "These are the properties of things we call dialectical: that one thing cannot exist without the other; that one acquires its properties from its relation to the other, that the properties of both evolve as a consequence of their interpenetration" (Levins & Lewontin, 1985, p. 3). Levins (1974) specifies "complexity itself as an object of study" and has stressed "interconnection, wholeness, qualitative relations, multiple causality, the unity of structure and process, and the frequently contra intuitive results of contradictory processes" (p. 124). Lewontin (1986) reminds us that "an organism is not determined by its genes and the environment in which it develops acting jointly, but by genes, environment, and random developmental factors acting at the level of single cell divisions and thermal noise" (p. 813; also see 1983). For example, the concept of "Mind" carries legitimacy while "brain" can be resisted as reductively causal, where plasticity is viewed as epigenetically determined and contingent (Rose, 1981). Within such a view, emergence lends itself to the recognition of methods and laws that are appropriate to each level, including novel organizing relationships across levels.

A Selective Review: Ontology and Emergence of Novelty

From the 17th to the 21st century, philosophers' perspectives on the conditions for emergence of novelty have brought to the fore unique domains of interrelatedness, each with their own vision. Hegel is widely regarded as the early and the great advocate of emergence theory (1812/1969). His system of dialectical tension generates new entities through the higher category of Becoming, in a continuous process of redefinition and refinement of concepts sufficient to support the Hegelian system (Clayton, 2006; Clayton & Davies, 2006; Hood, 1995). More recently, Mario Bunge (1977) proposed his version of ontology – physical, biochemical, psychobiological, and linked social-technical systems. A. N. Whitehead (1978, pp. 229–230) proposed that the experience, subjectivity, consciousness, and ultimately even the "consequent nature" of God are emergent products of evolution.

Also of interest is biologist Salthe's (1985) proposal that lower levels of organization constitute constraints in development and evolution, while entities at higher levels are related by way of creative synthetic processes. Deacon (2003) has specified that relational properties and relatedness are key factors in emergence, which is about configurations and topologies which are unlimited in scale and scope. To be clear, he stipulates that "It is the spontaneous, unprecedented production of new relational properties that constitutes emergence, not the production of new kinds of substance or physical law" (p. 276). An additional quality arises from "recurrent or circular redundancy of the influence of constraints or biases exhibiting the same form across levels of scale" with forms to be "re-entrantly compounded and fed-forward" (p. 284). "Third order emergence

includes developmental and evolutionary aspects with recursive causality and amplification of complexity. . . . It will be sufficient to operate in third order, because third order emergence includes an unbounded capacity to evolve new forms of emergence itself, including top-down holistic control of the super-organism of symbolic culture." In this model, process-oriented thinkers can understand language development as an emergent process by which individual humans represented at some point a veritable flood of new forms of verbal expression and self-representation. The near-universal process of elaborated language development fits with Deacon's levels of organization, "an amplification dynamic that can spontaneously develop in. . . interacting elements by. . . circulation of interaction constraints and biases. . . Circular connectivity enables constituents to reinforce one another iteratively." In this three-tiered account, first-order emergence entails properties such as laminar flow or viscosity, through relational-physical features [meaningful sounds, idiosyncratic baby talk]. Second order emergence is related to self-organizing systems, such as a snow crystal or a self-correcting, autocatalytic system [correctly linking the meanings of words]. Third-order emergence is related to development and evolution as systems with memory and redundancy, with historical traces that are re-entered into the system [as representation of complex ideas] (Deacon, 2003; 2006, p. 124). In his contemporary volume, Clayton puts forward the irreducibility and unpredictability of emergent properties that assure higher level, more complex entities can affect lower-level entities. "Strong emergence – that is, emergence with downward causation – has the merit of preserving commonsense intuitions and corresponding to our everyday experience as agents in the world" (Clayton, 2006; Kauffman & Clayton, 2006).

Philosopher David Chalmers (2006) offers one of the most intriguing interpretations of emergence, as he lays claim to the irreducibility of individual experience, the inaccessibility of "raw feels" and the experience of others, proposing that "there is exactly one clear case of a strongly emergent phenomenon, and that is the phenomenon of consciousness. . .. It is a key fact about nature that it contains conscious systems; I am one such" (pp. 300–306; also see Bitbol, 2007).

In sources represented by the philosopher Evan Thompson (2008), an assessment is delivered in which emergence of a self mutually entails the emergence of a world. "The environment (Umwelt, or lived phenomenal world) emerges from the world through the actualization of the being of the organism. . . which can exist only if it succeeds in finding in the world an adequate environment" (Thompson, 2008). It is the activation of the organism-world entity that engages a capacity for agency, making sense of selected interactions. It seems fitting to include here the observation that organisms also inherit a developmental matrix, which may change over time, a somatic ecology of the organism consisting of generations of conspecifics, parasites, beneficial bacteria, and simple organisms colonizing the organism, as well as prey and predator species. (For details, see below: Jablonka, 2001; Jablonka & Lamb, 2002, 2005; West and King, 1987).

Developmental Biologists: Coordination and Constraints

The historical perspectives of biological systems thinkers include nonreductionist agendas, such as C. Lloyd Morgan's early view (1925) that "whole and parts cooperate to constitute one natural cluster." Within an evolutionary framework, C. H. Waddington (1942) represented a version of dialectical materialism in which entities modify one another so that "the genotype of evolving organisms can respond to the environment in a more co-ordinated fashion." How his theory of canalizing constraints could guide the direction of changes in evolution was a continuing issue, illustrated in his image of the "epigenetic landscape." This landscape, with valleys that guide and hills that constrain the developmental process, "points to alternative sources of influence in constructing the phenotype, beyond the limited roles of DNA" (1942, p. 79). In 1968, Waddington remarked that a phenotype "takes its place between the gentoype and the environment. It is the phenotype which acts on the environment. . . and it is on phenotypes that the environment exerts its natural selective forces."

In an important elaboration of emergence theory, with an emphasis on consciousness and self-consciousness, Nobel laureate and neuroscientist Roger Sperry specified that higher level entities can deliver "downward causation" in lower levels of organization, altering the structure and function of lower levels to accommodate influences from higher levels. As an example, he specified that "the conscious subjective properties. . . have causal potency in regulating the course of brain events; the mental forces or properties exert a regulative control influence in brain physiology" (Sperry, 1969, 1980, p. 165). Considering origins of constraint in model systems, developmental neurobiologists Sporns and Edelman (1993) used a modeling approach to applied nonlinear systems theory in the context of self-organizing systems. The self-organizing component was realized through the integration of sensory and motor systems to produce, for example, in cortical regions, dynamically coupled sets of neuronal groups acting to guide or constrain behavioral outcomes (also see Kauffman, 1983 on developmental constraints). Processes of constraint will be revisited below in the section on experimental constraints.

Contemporary Views of Evolutionary Change: Emergence and Epigenesis

It is increasingly clear that development includes epigenetic processes that abundantly reside in fully inhabited domains with a panoply of resources for effective adaptations. Many of these epigenetic factors are reversible. Waddington explored epigenetic relationships as early as 1968, proposing that for each

phenotype, what is required is a process of "passing through a space of 'epigenetic operators' which is not wholly constituted by the active genes, but in which environmental influences may act as programme modifers. . . in connexion with the much deeper ecological and population genetical knowledge which we possess today" (1968, p. 527), Gilbert Gottlieb in 1970 clarified the difference between older views of predetermined or fixed developmental outcomes, in contrast to his more current views of probabilistic, open developmental systems which support the emergence of progressive features during the developmental process.

The concept of epigenesis stands in for a variety of factors that may impact the multilevel heterogeneous developmental or ecological process, from the simplest version of DNA copies to complex maternal and social environments for offspring. Epigenetic descriptors may function as, for example, variant states in cell cycles which may be induced by the environment. In recent investigations of epigenetic factors, maternal care and environmental patterns have been inherited through several generations by way of genetic elements (DNA) and/or epigenetic elements such as social relationships, environmental conditions (home sites, food availability, predator density), and molecular processes that alter genes indirectly.

In the broad view of Nijhout (1990, pp. 443–444),

> the genes whose products are necessary during development are activated by stimuli that arise from the cellular and chemical processes of development. . . Genes are passive sources of materials upon which a cell can draw. . . by providing the means of synthesizing, importing or structuring the substances required for metabolism, growth and differentiation. The function of regulatory genes is. . . that they simply provide efficient ways of ensuring that the required materials are supplied at the right time and place.

In addition, it is worth mentioning that regulatory genes can be co-opted for novel uses, such as HOX genes: homeotic genes that build a structure or a unit. Modularity in genetic combinations impacts the complex, interdependent, and hierarchical systems that also function as evolutionary factors shaping developmental modules (Raff, 1996). Other thinkers, such as Stearns (1982) have considered the organism as an important unit of selection, along with consideration for the role of mutual constraint that arises as developmental plasticity "frees the gene pool from the immediate impact of selection, which acts on phenotypes. . . while plasticity carries with it the cost of putting the phenotype at the mercy of the environment. . . Canalization also makes the phenotype independent of the environment" (p. 252).

Regulatory genes respond to stimuli external to the genome, such as hormones, heat, light, nutritional status, developmental factors, and neurotransmitters. Regulatory genes operate at the molecular level to influence expression, inhibition, or modification of particular sections of DNA in tissue-specific regions. DNA

wraps around a chromatin core made of histones, which can be methylated (or phosphorylated, acetylated, or ubiquitinated) in processes that may serve to deactivate, activate, or repair DNA regions. These elements are not gene-specific. However, there are preferred methylation sites at particular loci in different cell locations, especially in neural cells, which result in relatively stable patterns of methylation. These mythelation patterns may be instantiated in early life, may be reversible, and can be maintained throughout the life span by DNA methyltransferase. In general, one-third of the epigenome can be silenced by methylation processes. Most interesting is the proposal that epigenetic variations, such as methylation patterns, may be related to individual differences in human social adaptations (McGowan, et al., 2009).

In an important test case for assessing the genetic contributions to behavior, Gilbert and Jorgensen (1998, p. 261) concluded that although we know the complete DNA sequence of the genome for *C. elegans*, "the predicted behavior does not emerge from this knowledge. We also know the complete neural connectivity of the worm, but the behaviors of the animal cannot be read from the patterns of the neural connections." Part of what is missing may be the epigenetic operators imputed by Waddington.

Intergenerational features are of interest to Jablonka and Lamb (2002), including "processes of spontaneous self-organization that depend on the physical and chemical properties of the internal and external environments, as well as evolved gene-dependent mechanisms." These processes include life-span epigenetic adaptations of "immune system, cell memory mechanisms involving heritable changes in chromatin and DNA methylation, and the self-propagating properties of some protein conformations,. . . RNA gene silencing, enzyme – mediated DNA rearrangements and repair. Much of this work stems from [Barbara] McClintock's work" (pp. 88–89). Accounts of epigenesis in Jablonka (2001) describe cells that give rise to daughter cells that maintain modular holistic inheritance patterns from cell to cell; these may be self-organizing units, and they may be sensitive to environmental conditions. In addition, inducible RNA or chromatin can change the functional state of a cell. It can be inherited horizontally as a clone, and it can be copied by an enzyme (e.g., methyltransferase), so that parental patterns of methylation (for example) can be reconstituted in offspring, or induced by the environment. Prenatal exposure to high levels of hormones (corticosterone, testosterone) represents an epigenetic effect, by which offspring may be altered in patterns that could be reconstituted over generations. Active forms of niche construction by animals may represent a form of epigenetic inheritance related to material sites and structures which may be inherited. Birds and whales can acquire and transmit dialects of their songs, which offspring continue to acquire over generations (Jablonka, 2001). In the end, it may be that many levels of organization and self-organization are available to produce reliable and robust outcomes over the course of development, reproduction, and changing social/environmental circumstances.

In theories of artificial life and automata, models of developmental progressions have been represented by a program enacted by agents that show "fully emergent, spontaneous self-organization. . . Self-organization may require that we rethink all of evolutionary theory, for the order seen in evolution may not be the sole result of natural selection but of some new marriage of contingency, selection, and self-organization. New biological laws may hide in this union" (Clayton, 2006; Kauffman, 2008, pp. 60ff; Kauffman & Clayton, 2006) Two relevant and critical aspects of individual and group agency include boundaries for individuals, and the deliberate construction of constraints within the life space of autonomous agents and groups.

Behavioral Novelty and Emergent Structures

To realize the possibilities for study of emergent outcomes, several ongoing programs of research that exemplify these interests are described in their variety of settings, species, and measures. In particular, domains related to novel adaptations are explored with reference to emergent evolution (Smuts 2006), cultural novelty (Sapolsky, 2006), and Michael Meaney's recent studies on maternal behavior and epigenetic components (McGowan et al., 2009; Szyf, Weaver, & Meaney, 2007). Of particular interest to behavioral scientists are the material, maternal, and social factors influencing developmental adaptations. These adaptations may persist over generations of animals as uniquely effective forms of non-genetic inheritance.

Evolutionary Emergence of a Unique Social Organization in a Primate Species

Among the great apes, chimpanzees, gorillas, bonobos, and humans are phylogenetically related. However, it was only in the 1930s that primatologists first noted that the primates residing south of the Zaire River aren't just small chimpanzees, but that they are a distinct and different species. Later, in the 1950s, differences in bonobo social organization were characterized with females showing concealed estrus and unusually prosocial relations among females as well as males Smuts (2006) in her chapter on "Emergence in social evolution" proposes that the evolutionary divergence of chimpanzees and bonobos occurred about 2.5 million years ago, with chimpanzees most similar to the common ancestor. Primatologists had already established that chimpanzee society is structured with male dominance, that males are larger than females, and that multi-male alliances are formed in part to support deliberate raids on other chimp groups. Male chimps readily threaten and attack females, while females are known to forage in groups away from male competition. (It is worth noting that in captivity, groups

of female chimps can stand against males successfully, in an apparent emergent response to the constraints of enclosure in zoos and sanctuaries.)

The species-typical social forms for bonobos are quite different, distinctive and species-typical. Females are both dominant and peaceful, even though males are larger than females. In an unusual reversal, adolescent female bonobos (but not males) typically transfer to new troops, where females may choose to mate with particular males during their much more frequent estrus and copulation (compared to chimps), and with concealed ovulation (chimps display signs of ovulation). Compared to chimpanzee males, male bonobos have lower levels of testosterone, and show lower levels of intermale competition along with higher levels of conflict resolution and reconciliation. Female bonobos bring up young animals without help from males, and sons inherit their mother's rank. Also unlike chimpanzees, female bonobos readily enter into all-female alliances, which allow access to the best forage. Females typically and successfully ignore aggressive charges by males against females. Female bonobos uniquely reinforce their social bonds by frequent mutual genital stimulation (by rubbing) between females. Many of these observed behavioral adaptations are unique to this species, especially the very high rates of sexual interactions among females, and between females and males, and very much higher rates of conflict resolution and reconciliation by males.

On a related historical note, geography and climate change enabled the success of the bonobo species. North of the Zaire River, chimps and gorillas long have lived and foraged for the same grassy turf. However, a cold and dry period eons ago eliminated the gorilla from the habitat south of the Zaire River: No gorillas are found there, but bonobos are well established. Had it gone otherwise, bonobos might have been forced to accommodate or compete with gorillas for grass turf, or worse. They might have lost their niche, with unpredictable consequences. Kauffman refers to such an historical character of unique evolutionary events, noting that "*History enters* when the space of the possible is vastly larger than the space of the actual. At these levels of complexity, the evolution of the universe is vastly nonrepeating, hence vastly nonergodic" (2008; p. 123, italics in original).

Emergence of Cultural Change in a Baboon Society

Among Robert Sapolsky's studies of social behaviors among primates, one of the most intriguing developments occurred as a result of unexpected events. His Kenyan study subjects, a typical troop of savannah baboons – aggressive, highly stratified, and male-dominant – were under observation in the wild, at a time when the local nature-tourist hotel was expanding. After changes in the management of the hotel, additional opportunities for foraging in the hotel's dump became available, with food remains and rotting left-overs for baboons to fight about. The result of these easy pickings were disastrous to the dominant males, and provided an opening for emergent cultural change among the remaining troop members.

At one of Sapolsky's study sites, savannah baboons from two different troops had converged, with one troop raiding discarded food at the new and enlarged dump site. Over the course of a year, most of the aggressive males in the troop which had been dump-site habitués had also died from exposure to tuberculosis, presumably from infection lingering in the dump. Males that never visited the dump did not contract tuberculosis. The males that remained (which never visited the dump site) already were less aggressive and more socially skilled than the males which had died, and the majority of the remaining animals were socially skilled and gregarious females. With the loss of the relatively belligerent and dominant males, an emergent quality of social life became apparent through the socialization provided by resident females for in-migrating males from nearby troops. With ample welcome, social grooming and copulations, resident females supported prosocial adaptations among males and females in that troop.

Fast-forward 20 years. The unique social environment established by female troop members remained intact through two decades of in-migration by young males. Active socialization of young males through the welcoming behaviors of resident females, with high levels of socially-inclusive sexual solicitation and four-fold increases in patterns of grooming, distinguish this troop as qualitatively distinct from other troops. Sapolsky's (2006) interpretation of two decades' process is that:

> this troop's special culture. . . simply emerges, facilitated by. . . resident members. . . The savanna baboon [previously had become], literally, a textbook example of an aggressive, highly stratified, male-dominated society. Yet within a few years, members of the species demonstrated enough behavioral plasticity to transform a society of theirs into. . . baboon utopia.

Emergence of Intergenerational Patterns of Behavior and Physiological Adaptations

Ongoing studies of epigenetic effects in Michael Meaney's program of research demonstrate the remarkably effective transmission of specific behavioral forms across generations. In a variety of studies, very young rodents which were amply licked and groomed by their dams grew to attain as adults a more active orientation in novel environments. By contrast, pups which were not licked and groomed effectively by their dams grew as adults into less active patterns in novel environments. The inheritance of maternal patterns of care among foster-reared pups was observed, and as female pups attained maturity and licked and groomed their own pups, they assumed patterns of licking and grooming that replicated the foster-dam's behavioral patterns of licking and grooming, rather than the biological dam's pattern.

At the physiological level, infant rodents which had received high levels of licking and grooming by dams in the first week of life showed substantial increases

of function in the hippocampus, which is associated with lower levels of stress hormones as a result of hypothalamic feedback. Licking and grooming by dams induced in pups a demethylation of nerve growth factor protein. Alterations of DNA methylation patterns and chromatin-histone effects were found to contribute to the programming of relevant genes in early life and through adulthood. Most interesting is the observation that "social behavior such as maternal care affects epigenetic programming of specific genes in brain of *other* subjects. . . [i.e., the dam's pups]" (Szyf et al., 2007, p. 16, emphasis added).

In a study of adult suicide victims who had experienced childhood abuse, compared to adult suicide victims without childhood abuse, differential effects were observed at multiple levels. At autopsy, among childhood abuse cases only, decreased levels of hippocampal glucocorticoid receptor expression were due to methylation effects at specific sites, which were also associated with increased hypothalamic-pituitary-adrenal system activity that persisted into adulthood. No such changes were observed in suicide victims who had not experienced childhood abuse. The possibility exists that the intergenerational inheritance of methylation patterns at a genetic level could suffice to reinstate patterns of abuse in subsequent generations (McGowan et al., 2009).

Constraints as Factors Shaping Behavioral Development

Opportunities for the emergence of new adaptations may be limited by constraints in a variety of systems at different levels, in relation to the task that is posed: mechanical, personal, historical, social, intellectual, mathematical, temporal, physiological, material, and evolutionary. Constraints within the organism and constraints in the environment may or may not coalesce, while encompassing the trends of developmental processes. These multilevel sources (organism, environment, developmental process) are independent within their discrete and fundamental domains, while potentially contributing to genetic-evolutionary outcomes.

Theorists vary in their interpretation of the action of constraints. For example, some propose that developmental constraints can define the origin and limits of morphological change. Internal constraints also can support directional change, sufficient to open up new adaptive domains for developing organisms (Alberch, 1982). Lower level constraints (e.g., lack of dietary methyl sources) may act to restrict components at higher levels (upward causation), while higher level entities (organisms' behaviors, goals, partner preferences) may constrain or influence lower level processes through downward causation (e.g., change in sleep patterns; digestive function, diffuse anxiety or fearfulness). When constraints operate in upward and downward directions simultaneously, a dialectical structure may emerge in the process of acquiring behavioral and interpersonal skills, with an excitatory approach component, potentially accompanied by a withdrawal

response (if the task is too demanding) and (perhaps later) a self-regulatory phase of recognizing constraints.

In Waddington's well-known metaphor for development, a hilly landscape represents the dual aspects of constraint and opportunity as components in the processes of evolutionary stability and change (1957). Genetic and epigenetic effects that are stable are represented as following down well-worn paths, over the course of development. Over time, genetic and epigenetic effects that are evolving may change the landscape, eroding or accumulating the hills in this landscape and its material, be it responsive sand or resistant stone. Within the landscape metaphor, discrete opportunities arise amidst the constraints of past and current challenges, and in the potential emergence of correlated genetic and epigenetic change.

T. C. Schneirla's theory of behavioral development accounts for these taxes as two fundamental processes, approach and withdrawal (1949, 1951). These two fundamental tendencies are elaborated during the process of development to become complex behavioral forms. This view is grounded in Hegel's theory of *Becoming* in a spontaneously active, novelty-producing world (1812/1969). Schneirla's biphasic approach/withdrawal theory proposes that over the course of development, young organisms tend to approach a source of low-intensity stimulation. As organisms develop, they acquire the capacity to withdraw from a source of high-intensity stimulation. The opposition of approach and withdrawal and their interactions and elaborations are sufficient to account for the appearance of complex species-typical behaviors and for the variety of individual behavioral adaptations. Schneirla wrote that "My biphasic A/W theory is grounded on the universality of this state of affairs in the relationship of every species to its characteristic habit. Selection pressure would favor a corresponding approach-withdrawal dichotomy, and would promote its stabilization in the genotype through genetic assimilation" (Schneirla, 1965, in 1972, p. 348). Approach to the source of stimulation increases the intensity of the stimulation, while the opponent process, withdrawal, serves to distance an organism from the source of stimulation, and thereby to reduce intensity (see Figure 11.2 for additional discussion).

A self-evident proposition is that constraints may arise at each level of organization within individuals and species, and within the particulars of each experimental or natural setting (Fentress, 1976, 1991, 1999; also see Staddon, 1979a; 1979b; 1980). Sporns and Edelman (1993) specify the constraints that are critical to include in any account of "becoming," including constraints related to exploratory or social behaviors. Most interesting here is the process of generating "the variability and degeneracy that are so vexing [in early efforts, which]. . . are in fact a necessary prior condition for successful selection [of motor or behavioral gestalts]. . . Movements are selected as *whole patterns*" (p. 968; italics in original).

Settings for the study of behavior may be designed to promote behavioral coherence in naturalistic environments. Within the constraints of an internal value system such as a preferred activity or social partner, and external environmental

constraints and structures suitable for activity, the scale of constraints and opportunities in such a setting may point to how learning occurs in relation to constraints. Examples from two programs of research may clarify how a developmental process shapes outcomes of development. In an empirical study assessing intergenerational change in tobacco hornworms, developmental processes were found to be organized at the level of genetic modules, with one module for body size, and another module that determines timing of maturity (Suzuki & Nijhout, 2008). Under selection pressure over six generations, the genetic module regulating size responded to constraints under selection pressure, while the module regulating timing of maturity did not respond to selection pressure. The outcome showed that correlated traits (organized as genetic modules) can be mutually constrained or alternatively, some traits can be uncoupled under strong directional selection. In this study, regulatory genes operated on ontogenetic processes and were strongly conserved, whereas structural genes were more likely to be co-opted in the service of new adaptations. Most intriguing are the homeotic genes (HOX) which are sufficient for building a functional structure (Raff, 1996).

In an evolutionary comparison, the development and evolution of a contemporary bird hindlimb and a dinosaur (sauropsid) hindlimb, Müller and Streicher (1989) found that homologous processes in early development provided a transient foundation for the emergence of a functional component of the hindlimb in both species. The result is that "a caenogenetic [transient developmental] feature triggers phenotypic novelty" as a homologous feature in the early lifespan of these two related species.

How Lower-level Processes Operate as Constraints and Novel Adaptations Emerge

In a long-running study of a troop of free-ranging, island-dwelling, and provisioned Japanese macaques (snow monkeys: Nakamichi, Kato, Kojima & Itoigawa, 1998), one of the changes in management made by scientists to reduce the incidence of aggressive encounters among macaques was related to centralized food stores. The solution was to constrain the distribution of food to small sized bits of food which were widely dispersed in various sandy locations on the beach. The sandy locale also constrained the rate of consumption, as sand was mixed in with the small bits of food. These constraints were successful in reducing aggression, and also presented an opportunity. The emergence of a novel behavioral adaptation, first observed in a prepubescent female macaque named Imo, was to carry the sandy bits of potato into the shallow surf, allowing potato bits to float and, after the sand washed off, be eaten. Soon other young females learned this novel approach, then older females and, later, other troop members acquired the capability. In a subsequent paradigm, wheat grains were scattered in the beach environment, providing the same animal, Imo, the challenge of first carrying the wheat "puffs" into the surf and then, after the sand

washed off, skimming the wheat from the water and consuming it. More recently, members of the troop adopted an additional application of the washing process, pulling grasses up from the soil, then carrying and washing a naturally occurring food, grass roots, in a nearby stream. Some troop members were observed to beat the grasses against a flat rock near a river to dislodge dirt in the grass roots, a process which might well be viewed as emergent or incipient tool use.

External Constraints in Support of Emergence: Play, Craft, and Work

The interpretation of play as serious business includes the possibility that material constraints in play may usefully serve to resist children's egoistic interpretations. In later learning, children may construct rules for repetition, some of them very strict. These eventually may be modified to allow for increasing complexity in play. Albert Einstein's autobiographical account of 1945 includes the view that "combinatory play seems to be the essential feature in productive thought – before there is any connection with logical construction in words or other kind of signs which can be communicated to others" (Holton, 1973, pp. 368–369). Erikson (1977, p. 104) noted that during the progression to adulthood, "play is transformed into work, game into competition and cooperation, and the freedom of imagination into the duty to perform with full attention to the techniques which make imagination communicable, accountable, and applicable to defined tasks."

The place of craft, craftsmanship, and uses of materials in contemporary societies continues to be highly valued in a technological world. Sociologist Richard Sennett (2008) notes that for the skilled crafts person, it is not unusual to find resistance or constraint in a process, or in a material, or in the desired use of a tool. Making time and attention to acquire the patience to recast the resistance, to approach it in a different way, and in the end to identify with the resistance; this allows the effort to proceed. In particular, the recommendation is to identify with the smallest and most forgiving element of the process (pp. 220–222), "seeing the problem, as it were, from the problem's point of view. . . Working *with* resistance means. . . converting boundaries into borders" as sites for exchange, independent of central control (pp. 226–229, italics in original). Borders may serve as constrained opportunities for assessing novel solutions within settled structures, while also casting a larger interpretation of relationships of trade.

How Higher-level Processes Operate as Constraints and Novel Adaptations Emerge

Self-consciousness and awareness of self-consciousness in others are markers for the emergence of developmental and interpersonal richness in human lives. The growth of self-consciousness is a focus of the remarkable text by C. G. Jung, the

psychoanalyst and psychologist. His book, *Answer to Job* (1954) probes the emerging consciousness of God in relation to Job, who was an honest and thriving family man (see *The Book of Job* in the *Bible*). Yet severe losses and ill fortune fell on Job's house. In the midst of his suffering, Job had converse with God. Jung's voice serves as the interlocutor: "Job is no more than the outward occasion for an inward process of dialectic in God" (p. 43). "His (God's) consciousness seems to be not much more than a primitive 'awareness' which knows no reflection and no morality" (p. 86). "A more differentiated consciousness must, sooner or later, find it difficult to love. . . a God whom on account of his unpredictable fits of wrath, his unreliability, injustice and cruelty, it has every reason to fear. . . Yahweh's (God's) moral defeat in his dealings with Job had its hidden effects: man's unintended elevation" (pp. 116–117). In the end, "The encounter with the creature changes the creator" (p. 130). Jung's archetypes for God are compelling. "We cannot tell whether God and the unconscious are two different entities. Both are border-line concepts for transcendental contents" (p. 199). The mythic result is the process of growing self-consciousness in God, as occasioned by the counterpoint from God's servant, Job.

Constraints and Emergence in Research Investigations

Constraints figure importantly in the study of behavioral development. In several settings and studies, Cairns and Cairns (1994) have highlighted in their book, *Lifelines and Risks*, the structure of opportunities and constraints in the neighborhoods, schools, and families of children at risk for aggression. In this set of studies, interpersonal relations are the core phenomenon of interest, such that "The behavior of one person in an interchange provides constraints on the freedom of action of the other person, and vice versa. . . selection and socialization are mutually supportive" (Cairns & Cairns, 1994; p. 117). What follows from this perspective are the social dilemmas of youth and adolescents as they approach the adult world. Aggressive behavior had been determined to be a major instigating factor in the formation of maladaptive peer groups, while the lack of aggressive behavior was remarkable among children in leadership roles. Race and social class also served as definitive constraints within the social lives of adolescent groups. The expression by girls of indirect aggression, exclusion, and ostracism was a continuing constraint for unaligned girls (Cairns, Cairns, Neckerman, Ferguson, & Gariépy, 1989; Feshback & Sones, 1971; Xie, Swift, Cairns, & Cairns, 2003). Buffers against delinquency include additional constraints arising from parental preferences and teachers' values; however, social isolation also may serve as a buffer against delinquency. Cairns & Cairns (1994) noted the historical process within social contexts, as "Individuals are changed by their associations, and they carry to the next set of relationships

the behavioral residue of the recent past" (p. 129). Aggressive behavior problems pointed to broader issues of socialization through adaptation or constraint, with peer judgments weilding a heavy hand.

However, novelties do arise: for example, girls with abusive parents were able to find unrelated women who supported their development in adolescence, as they became increasingly emancipated. Skill development in sports or band gave access to different peer groups, providing for girls and for boys some novel opportunities for change. While Cairns and Cairns (1994) emphasized the reciprocal process of the individual/environment interplay, "the actions and interactions of other people constitute major external sources of behavioral organization" (Cairns & Cairns, 1994, p. 243). More formally,

> Patterns of psychological functioning develop like dynamic systems, in that they are extremely sensitive to the conditions under which they are formed. . . Conservation in development is supported by constraints from without and from within, and by the correlated action of internal and external forces. The upshot is that social and cognitive organization in development tends to be continuous and conservative, despite continuous change. (pp. 245–246)

We also have pursued a series of studies using animal models. Among the selectively bred mice we have studied over 25 years, these include a line of mice bred for high aggression, a line of mice bred for low aggression, and a control line. In our observations of these animals, we (Robert Cairns, Jean-Louis Gariépy, and I) have studied social and aggressive behaviors, exploratory behaviors, group housing and isolation housing effects on aggressive behavior, open field, enriched environments, novel environments, the light-dark box, the plus maze, cytokine responses, maternal behaviors, and defensive maternal aggression in the home cage (with pups removed). Bob Cairns initiated the study of heterochrony in aggressive expression, characterizing the changes in developmental timing of the onset of aggressive behavior over 30 generations of selective breeding (Cairns, 1979, 1986; Cairns, MacCombie, & Hood, 1983; Cairns, Gariépy, & Hood, 1990). Louis Gariépy carried out complex studies of links among neurobiology, behavior, and evolution within the selectively bred lines. In my lab at Penn State, female mice from the selectively bred lines have been favorite subjects.

Among these studies, important aspects of constraint in experimental design include the institution of isolation housing, which has proved to be essential in producing aggressive behavior. Only after a two- or three-week period of isolation housing will mice reliably fight other mice. This constraint (restriction of social contacts) reliably shifts the isolated animal into a more aggressive mode. By contrast, the socially-housed male test partner is less likely to enter into an aggressive mode. Because of the restriction of social contacts in isolation housing, the expression of aggression becomes more available to investigation. Figure 11.1 (panels A, B, and C) show the continued effect of isolation housing in augmenting

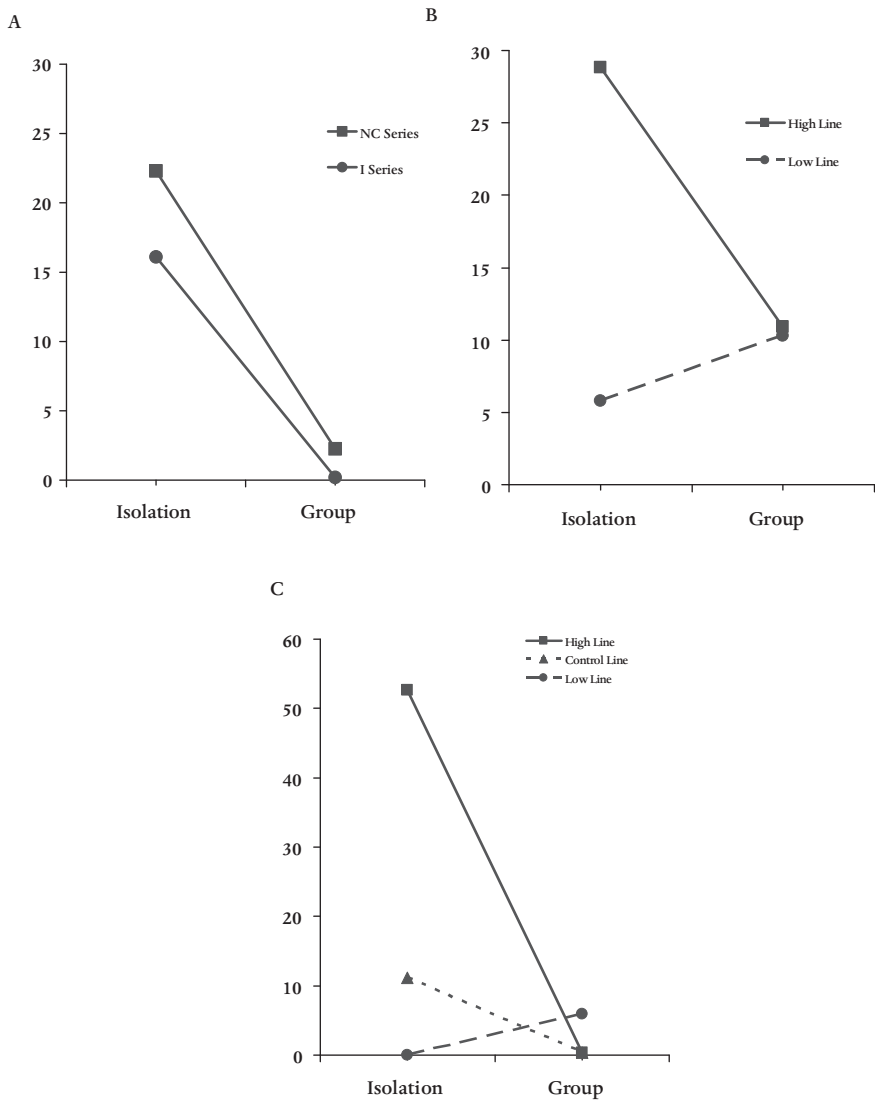

Figure 11.1. Aggressive behavior after rearing in isolation housing or group housing. A. Foundational generation, B. Generation S_6; C. Generation S_{39}.

aggressive behavior in males over generations. Females also show increased aggression after isolation housing (Hood & Cairns, 1989).

A theoretical model of aggressive interactions is portrayed in Figure 11.2, based on the approach-withdrawal theory of T. C. Schneirla (1951, 1965), with postulated moment-by-moment changes in behavioral tendencies. Efficient uses of approach and withdrawal tendencies are at the core of adaptive responses in young animals. In Figure 11.2 (Hood, 1995), there are two modalities represented, an approach tendency

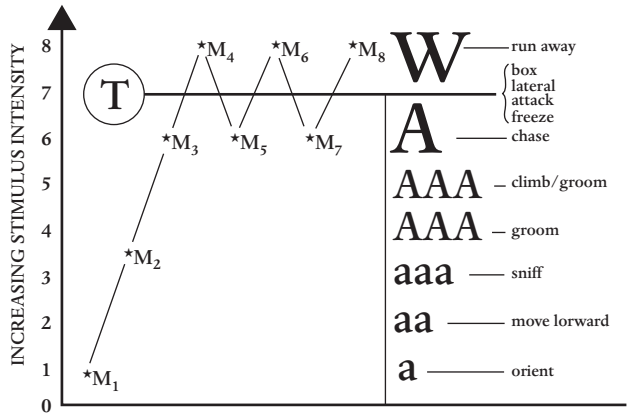

Figure 11.2. From moment to moment (M_1 to M_2 to M_3, etc.), perceived stimulus intensity (left vertical axis) produces *A*, approach behavior or W, withdrawal behavior (if the stimulus intensity exceeds level "7" in the hypothetical schema). Increasing stimulus intensity produces increasingly strong and directed approach behaviors, but beyond the threshold (circled "T"), too-intense stimulation produces withdrawal, an all-or-none response. As the withdrawal response increases distance from the source of stimulation and reduces stimulus intensity, approach behaviors may reappear. By this model, organisms will seek the maximum level of stimulation below threshold, titrating stimulation by approach and withdrawal behaviors. The sequence M_4-M_5-M_6-M_7-M_8 illustrates how the threshold may capture behavior. The switching of *A* and W at threshold opens the possibility of new behavioral forms arising (here: highly stereotypic forms of mice: box, lateral display, attack, freeze). *Source*: Hood 1995.

which is dimensional (a graded response; fast, medium or slow to approach in relation to the level of stimulation) and a withdrawal response to intense stimulation which is not graded but serves as an all-or-nothing response. A transformational process occurs at the threshold of stimulation (the circled "T" indicates the threshold in Figure 11.2), where approach behaviors are transformed into withdrawal behaviors, in response to high-intensity stimulation. As the animal moves away from the stimulus, intensity is reduced and returns to the range evoking strong approach behaviors. The threshold may capture the oscillation of approach and withdrawal around a set point, and it is at this point that the potential for emergence of novel behaviors, such as aggressive behavior (boxing, lateral display, attack) or freezing (prolonged immobility) is realized. Cycling between these two modes, approach and withdrawal, sets the conditions for behavioral expression in young animals. However, social-behavioral expression may become more complex as animals mature, and as learning shifts the threshold between approach and withdrawal responses, including processes of habituation, sensitization, and selective learning.

Ethel Tobach and T. C. Schneirla's discussion of approach-withdrawal theory includes an analysis of aggressive behavior. "Attack on the biosocial level is an

intense approach response; on the psychosocial levels it is an integration of both approach and withdrawal. The withdrawal in this instance is complexly elaborated on the psychosocial levels as it derives from anticipation of future events based on past experience, which must be defined as fear." Schneirla notes that individual animals may be biased toward approach or withdrawal behaviors: in the selectively bred lines of high-aggressive or low-aggressive mice, the outcome is expectable. The "apparently paradoxical character of aggression as both approach and withdrawal" implies that aggression is a uniquely complex form (Hood, 1995; Tobach & Schneirla, 1968). By placing two behavioral forms, aggression and exploration (of novel environments) into relation within a more general behavioral system of response to stimulation, the interplay of inhibition, exploration, and stimulus-seeking might well be drawn as approach-withdrawal gradients. This perspective offers a fresh look at the foundations of behavioral expression, suggesting that "both approach and withdrawal responses can be aroused simultaneously" (Hood, 1995; Rosenblatt & Mayer, 1995).

Approach and Withdrawal in Social and Exploratory Behaviors

An important component in the study of exploratory behavior is the opportunity for animals to actively explore novel environments. By placing the home cage containing one male mouse into the large novel arena, and opening a hole in the home cage, animals could exit or enter at will. The resulting structure of exploratory behavior by young high-aggressive line males in a large novel arena consisted of long latency to emerge from the home cage, suggesting a reluctance to enter and explore novel settings (Hood & Quigley, 2008). However, after entering the novel environment, high-aggressive line males had shorter latencies to approach and make first contact with a novel object (a plastic pipe, a wire climbing structure). Significant differences in latency scores for male mice from the three lines distinguish the relative strength of the approach component of exploratory behavior, supporting the proposition that high-aggressive line males are slower to explore (and perhaps are more anxious), compared to males from the low-aggressive and control lines (Table 11.1). In the second component of the exploratory setting, contacting a novel object, shorter latency may be related to impulsivity or low inhibitory control in high-aggressive line males. Also telling is the related pattern of high-aggressive line males to repeatedly return to the security of the home cage, and to reenter the home cage more often, compared to low-aggressive and control line males.

These aspects of exploratory behavior can be represented in a dialectical analysis with an emphasis on higher level processes that unite seemingly contradictory outcomes. An interpretation of opposed tendencies (approach, withdrawal) under high reactivity to novelty suggests that in the novel arena, the opposed tendencies are expressed in turn, as these tendencies are related to each other in a dialectical-emergent behavioral structure. High levels of stimulation from the novel arena first

Table 11.1. Exploratory behavior in a novel enriched environment. Male mice show line effects: Generations S_{24}, S_{28} and S_{29}. (mean and standard error)

	Age		*Line*		
	45 days	*120 days*	*100*	*500*	*900*
Contact home cage (frequency)	NS	NS	26.68 (1.81)	31.71 (3.70)	39.66* (3.61)
Enter home cage (frequency)	NS	NS	3.81 (0.56)	3.21 (0.80)	7.28* (1.21)
N	33	21	22	14	18

Note: * = $p < .05$.

engender withdrawal responses, which delay the departure from the home cage. When withdrawal tendencies subside (associated with long latencies to enter the novel environment), then approach tendencies to novel objects may be more readily manifested (quickly contacting novel objects: for a discussion, see Hood, 1995).

In a complementary set of studies, males from the S_{32} generation (the 32nd generation of selective breeding) were given access to a dark compartment in the light-dark box. Young mice, age 42–56 days, were placed in the dark compartment, and could freely enter the lighted box at any time during the 10 min trial. The high-aggressive line males were slowest to enter the light box, spent less time in the light box, and had fewer transitions than low-aggressive and control line males (Table 11.2).

In a related assessment providing access to a novel and preferred food (diluted sweetened milk) for 30 min on four consecutive days under dim light, a novel environment was instituted on the fifth (test) day, with bright light instead of dim light, and with the novel addition of an half an inch of warm water in the bottom of the cage. High-aggressive line males reduced their consumption of the sweetened milk by half on the test day when drinking under the novel circumstances, while low-aggressive line males did not alter their consumption under the novel conditions. The conclusion from these various modes of assessment is that high-aggressive line males are more sensitive to alterations in the environment, and are more reactive to novelty, compared to male mice from the low-aggressive and control lines (Hood, 2005; Hood & Quigley, 2008).

Recent studies at Penn State have assessed alcohol preference and tolerance in female and male mice, followed by studies of maternal behavior by high-drinking and low-drinking dams (Colby & Hood, in progress). The most promising observational series involves epigenetic maternal effects among female mice. A significant minority of female mice prefer to drink alcohol, given a continuous choice of water and saccharin, and also a continuous choice for alcohol, water and

Table 11.2. Exploratory behavior in the light-dark box: Male mice (mean and standard error)

	Line		
	100	*500*	*900*
Exploratory Behaviors			
Latency to enter light box (*sec*)	18.33[a]	19.41[a]	37.50[b]
	(4.13)	(3.05)	(8.88)
Time in the light box (*sec*)	193.16[a]	161.02[b]	141.87[b]
	(11.20)	(11.26)	(7.86)
Transitions between light and dark compartments (*n*)	30.40[a]	31.11[a]	22.38[b]
	(3.41)	(2.99)	(1.42)
N	15	17	16

Note. For each row, superscripts indicate significant differences ($p < .05$).
Young adult male mice from the S_{32} generation of selective breeding are subjects.

saccharin, over a 6 day period. This access to alcohol occurred months before females became pregnant. Briefly, cross-fostered pups were switched at birth and were reared by S_{49} foster dams, within each line (high-aggressive, low-aggressive and control lines). These foster-reared pups acquired the alcohol preference that was characteristic of the foster dam (when that dam had access to alcohol at age 30 days). Preference for alcohol in foster-reared S_{50} pups at age 30 days was more strongly correlated with S_{49} foster-dams' own alcohol preference at age 30 days ($r\ (36) = .32$, $p = .06$), rather than with the S_{49} biological dam's preference (after cross-fostering, $r\ (36) = -.12$, $p = .46$). If this finding is replicated, we will extend our examination of emerging relationships among cross-fostering dams and acquired effects among foster-pup outcomes in our selectively bred lines.

Gilbert Gottlieb: Radical Scientific Philosopher and Innovative Experimentalist (1929–2006)

The gift of generative investigators and original thinkers consists of sharing with colleagues in the field fresh perspectives on old and new ways of understanding development. For more than two decades, it was a great gift to learn from Gilbert Gottlieb's understanding of the human and natural elements of the world. His talents were impressively varied, including his early experimental work with Zing Yang Kuo, and the film he and Kuo produced on "The Development of Behavior in the Duck Embryo" with Gottlieb's voice-over (available at Penn State Media Services), the many empirical studies of prenatal behavior and non-obvious

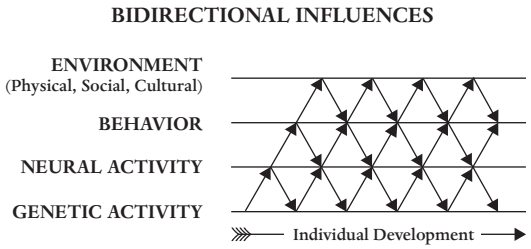

Figure 11.3. Bidirectional influences. *Source*: Gottlieb (1992, p. 186).

self-stimulation, recognition of the maternal assembly call, and peer social relations among ducklings, consistently revealing their hidden capabilities. For example, using a technique that he perfected, Gottlieb temporarily immobilized the syrinx of neonatal ducklings by painting it with Collodion to prevent them from hearing their own vocalizations: this account represents an incisive use of constraint in an experimental design (Gottlieb, 1975).

Equally impressive are Gottlieb's widely admired and insightfully clear theoretical treatises on development, evolution, and the appropriate understanding of the role of genetics as part of a complex web of interactions across levels, while not jumping over more than one level at a time (Gottlieb, 1984). His theoretical figure captures the relationships among levels of interaction in the developmental process, the fully bidirectional model (Figure 11.3; Gottlieb, 1992, 2007: also see the review chapters since 1983 from Gottlieb for the *Handbook of Child Psychology*).

For this focus on emergence theory, Gottlieb's 2002 paper "Developmental–behavioral initiation of evolutionary change" is most appropriate (also see Gottlieb, 1994). His proposal in his Bolder Speculation that "behavior is the leading edge of evolution" fueled his precision and his passion for the study of incipient speciation processes. To his mind, individual animals that carried out exploratory behaviors in novel environments were the pioneers of their species, opening the possibilities of fresh behavioral adaptations and possibly, after substantial exposure to the novel environment, evolutionary change. In *Rhagoletis pomonella*, the apple maggot fly, Gottlieb found an opportunity. This fly, an American native, accommodated to the importation of apple trees in the mid-1800s. Compared to the original host, the hawthorn fruit, apples matured earlier in the season, and so did the newly adapted flies. The anticipation of realizing the theoretical pathways that Gottlieb had spelled out for the emergence of a novel endophenotype aroused considerable excitement: his theory was to be realized in this newly evolving fly. Faithful to his stage theory of the behavioral neophenotype, Gottlieb expected to find these markers of evolutionary novelty: changes in behavior (ovipositing within the odors of apple trees, rather than hawthorn); changes in morphology (novel uses of the apple habitat leading to latent changes in the timing of reproduction by apple maggot flies) and changes in genes sufficient to proclaim the potential emergence of a

new species (some genetic changes have occurred in the apple maggot fly, but the two variants still can crossbreed). This last step is still in process. Gottlieb's papers set the stage for an application of these principles, in flies or in other changing species. He stipulates that "because the genes do not make behavior, it is the genes-in-the-recurring-developmental-system that make for the stability of the behavioral changes across generations" (2002, p. 211).

The hope is that others will arise to carry forward Gottlieb's Bolder Speculation: If "behavior is the leading edge of evolution," then novel behaviors may be sufficient to instantiate persistent new adaptations in changing environments, and these behavioral changes may prove sufficient to support the emergence of durable evolutionary novelties through selective reproduction and through recurring developmental scenarios. To carry further the vigor with which Gottlieb has emphasized these principles, it may be sufficient to investigate the scope and depth of these epigenetic outcomes, be they long lasting and durable intergenerational outcomes or transient adaptations to situational settings. In the midst of transformative thinking about the place of epigenetic factors, Gottlieb was at the fore as a champion for integrative understanding. "New variations and adaptations are a consequence of changes in individual development mediated by transgenerationally persistent changes. In this view, natural selection is not the cause of the new adaptations but acts only as a filter through which the new adaptations must pass. Changes in behavior create the new variants on which natural selection works" (Gottlieb, 2002, p. 217; see Partridge & Greenberg (this volume) for a comprehensive account of Gottlieb's work).

Conclusion

The theoretical and empirical scholarship of Gilbert Gottlieb, his intellectual leadership, and his congenial relations with colleagues were abundantly shared and widely appreciated in supporting progressive domains of interest. Especially, the biweekly meetings on theories of developmental emergence and constraints that he and I organized at the Center for Developmental Science during my sabbatical in 1999–2000 included colleagues, post-doctoral students, and graduate students. From these and other precedents, and his friendly conversations with scientists which were intermittently shared over a span of more than 30 years, it remains to further embed Gilbert's way of thinking into the orientation of developmental science, writ large. By freely sharing his capacious understanding, he created an enduring legacy within the fields of developmental psychobiology and evolutionary theory.

One perspective presented by Whitehead, the philosopher, proposed an unusual prescription for maintaining such shared understandings:

> This actual entity is the original percipient of that nexus [which consists of an entity containing data and feelings]. But any other actual entity which includes in its own actual world that original percipient also includes that previous nexus as a portion of its own actual world. Thus each actual world is a nexus which in this sense is independent of its original percipient. It enjoys an objective immortality in the future beyond itself... Both [God and the World] are in the grip of the ultimate metaphysical ground, the creative advance into novelty. Either of them, God and the World, is the instrument of novelty for the other. (Whitehead, 1978, p. 349)

This enlarged view of the persistence of shared meanings reinforces the value of the community of scholars and explorers who continue to be enriched by Gottlieb's voice.

References

Alberch, P. (1982). Developmental constraints in evolutionary processes. In J. T. Bonner (Ed.), *Evolution and development*. New York: Springer-Verlag.

Bates, E., Elman, J., Johnson, M. H., Karmiloff-Smith, A., Parisi, D., & Plunkett, K. (1998). Innateness and emergentism. In W. Bechtel & G. Graham (Eds.), *A companion to cognitive science*. Oxford: Blackwell.

Bitbol, M. (2007). Ontology, matter and emergence. *Phenomenology and Cognitive Science*, **6**, 293–307.

Blitz, D. (1992). *Emergent evolution: Qualitative novelty and the levels of reality*. Boston: Kluwer Academic Publishers.

Bunge, M. (1977). The GST challenge to the classical philosophies of science. *International Journal of General Systems*, **4**, 29–37.

Cairns, R. B. (1979). *Social development: The origins and plasticity of interchanges*. San Francisco: Freeman.

Cairns, R. B. (1986). An evolutionary and developmental perspective on aggressive patterns. In C. Zahn-Waxler, E.M. Cummings, & R. Iannotti (Eds.), *Altruism and aggression: Biological and social origins*. New York: Cambridge University Press.

Cairns, R. B., & Cairns, B. D. (1994). *Lifelines and risks: Pathways of youth in our time*. New York: Cambridge University Press.

Cairns, R. B., Cairns, B. D., Neckerman, H. J., Ferguson, L. L., & Gariépy, J.-L, (1989). Growth and aggression: I. Childhood to early adolescence. *Developmental Psychology*, **25**, 320–330.

Cairns, R. B., Gariépy, J.-L., & Hood, K. E. (1990). Development, microevolution, and social behavior. *Psychological Review*, **97**, 49–65.

Cairns, R. B., MacCombie, D. J., & Hood, K. E. (1983). A developmental-genetic analysis of aggressive behavior in mice. *Journal of Comparative Psychology*, **97**, 69–89.

Chalmers, D. J. (2006). Strong and weak emergence. In P. Clayton & P. Davies (Eds.), *The re-emergence of emergence. The emergentist hypothesis from science to religion*. New York: Oxford University Press.

Clayton, P. (2006). Conceptual foundations of emergence theory. In P. Clayton & P. Davies (Eds.), *The re-emergence of emergence. The emergentist hypothesis from science to religion*. New York: Oxford University Press.

Clayton, P., & Davies, P. (2006). *The re-emergence of emergence: The emergentist hypothesis from science to religion*. New York: Oxford University Press.

Cox, M. J., & Paley, B. (1997). Families as systems. *Annual Review of Psychology*, **48**, 243–267.

Deacon, T. W. (2003). The hierarchic logic of emergence: Untangling the interdependence of evolution and self-organization. In B. H. Weber & D. J. Depew (Eds.), *Evolution and learning: The Baldwin effect revisited*. Cambridge, MA: The MIT Press.

Deacon, T. W. (2006). Emergence: The hole at the wheel's hub. In P. Clayton & P. Davies (Eds.), *The re-emergence of emergence: The emergentist hypothesis from science to religion*. New York: Oxford University Press.

Dyer, R. (2006). Love him or loathe him, he transformed music. The Boston Globe February 12.

Eckerman, C. (1993). Toddlers' achievement of coordinated action with conspecifics: A dynamic systems perspective. In L. B. Smith & E. Thelen (Eds.), *A dynamic systems approach to development*. Cambridge, MA: MIT.

Erikson, E. H. (1977). *Toys and reasons: Stages in the ritualization of experience*. New York: W. W. Norton & Company.

Fentress, J. C. (1976). Dynamic boundaries of patterned behaviour: Interaction and self-organization. In P. P. G. Bateson & R.A. Hinde (Eds.), *Growing points in ethology*. New York: Cambridge University Press.

Fentress, J. C. (1991). Analytical ethology and synthetic neuroscience. In P. P. G. Bateson (Ed.), *The development and integration of behaviour*. New York: Cambridge University Press.

Fentress, J. C. (1999). The organization of behavior revisited. *Canadian Journal of Experimental Psychology*, **53**, 8–19.

Feshback, N. D., & Sones, G. (1971). Sex differences in adolescent reactions to newcomers. *Developmental Psychology*, **4**, 381–386.

Fischer, K. W., Ayoub, C., Singh, I., Noam, G., Maraganore, A., & Raya, P. (1997). Psychopathology as adaptive development along distinctive pathways. *Development and Psychopathology*, **9**, 749–779.

Gilbert, S. F., & Jorgensen, E. M. (1998). Wormwholes: A commentary on K. F. Schaffner's "Genes, behavior and developmental emergentism.". *Philosophy of Science*, **65**, 259–266.

Gottlieb, G. (1975). Conceptions of prenatal development: Behavioral embryology. *Psychological Review*, **83**, 215–234.

Gottlieb, G. (1984). Evolutionary trends and evolutionary origins: Relevance to theory in comparative psychology. *Psychological Review*, **91**, 448–456.

Gottlieb, G. (1992). *Individual development and evolution*. New York: Oxford University Press.

Gottlieb, G. (1994). Review of David Blitz, *Emergent evolution: Qualitative novelty and the levels of reality*. *Journal of the History of the Behavioral Sciences*, **30**, 234–237.

Gottlieb, G. (2002). Developmental–behavioral initiation of evolutionary change. *Psychological Review*, **109**, 211–218.

Gottlieb, G. (2007). Probabilistic epigenesis. *Developmental Science*, **10**, 1–11.

Greenberg, G., Partridge, T., & Ablah, E. (2006). The significance of the concept of emergence for comparative psychology. In D. Washburn (Ed.), *Primate perspectives on behavior and cognition*. Washington, DC: American Psychological Association.

Halpern, C. T., Hood, K. E., & Lerner, R. M. (Eds.) (2007). Special issue: The scientific contributions of Gottlieb Gottlieb. *European Journal of Developmental Science*, **1**, 1–7.

Hegel, G. W. F. (1812/1969). *Science of logic*. (A. V. Miller, Trans.) London: George Allen & Unwin.

Holton, G. (1973). *Thematic origins of scientific thought*. Cambridge, MA: Harvard University.

Hood, K. E. (1995). Dialectical and dynamical systems of approach and withdrawal: Is fighting a fractal form? In K. Hood, G. Greenberg, & E. Tobach (Eds.), *Behavioral development: Concepts of approach-withdrawal and integrative levels. The T. C. Schneirla Conference Series, Vol. 5* (ch. 2, pp. 19–76). New York: Garland Press.

Hood, K. E. (2005). Toward an integrative account of the development of aggressive behavior. In D. M. Stoff & E. J. Susman (Eds.), *Developmental psychobiology of aggression* (pp. 225–251) New York: Cambridge University Press.

Hood, K. E., & Cairns, R. B. (1989). A developmental-genetic analysis of aggressive behavior in mice: IV. Genotype-environment interaction. *Aggressive Behavior*, **15**, 361–380.

Hood, K. E., & Quigley, K. (2008). Exploratory behavior in mice selectively bred for developmental differences in aggressive behavior. *Developmental Psychobiology*, **50**, 32–47.

Jablonka, E. (2001). The systems of inheritance. In S. Oyama, P.E. Griffiths, & R. D. Grey (Eds.), *Cycles of contingency: Developmental systems and evolution*. Cambridge, MA: MIT Press.

Jablonka, E., & Lamb, M. J. (2002). The changing concept of epigenetics. *Annals of the New York Academy of Sciences*, **981**, 82–96.

Jablonka, E., & Lamb, M. J. (2005). *Evolution in four dimensions: Genetic, epigenetic, behavioral, and symbolic variation in the history of life*. Cambridge, MA: MIT Press.

Jung, C. G. (1954). *Answer to Job*. Cleveland, OH: World Publishing Company.

Kauffman, S. A. (1983). Developmental constraints: Internal factors in evolution. In B. C. Goodwin, N. Holder, & C. C. Wylie, (Eds.), *Development and evolution*. New York: Cambridge University Press.

Kauffman, S. A. (2008). *Investigations*. New York: Oxford University Press.

Kauffman, S. A., & Clayton, P. (2006). On emergence, agency, and organization. *Biology and Philosophy*, **21**, 501–521.

Lerner, R. L. (2002). *Concepts and theories of human development* (3rd ed.). Mahwah NJ: Erlbaum.

Levins, R. (1974). The qualitative analysis of partially specified systems. *Annals New York Academy of Sciences*, 123–138.

Levins, R., & Lewontin, R. (1985). *The dialectical biologist*. Cambridge, MA: Harvard University Press.

Lewontin, R. C. (1986). How important is genetics for an understanding of evolution? American Zoologist, **26**, 811–820.

Mayr, E. (1982). *The growth of biological thought: Diversity, evolution, and inheritance*. Cambridge, MA: Belknap Press.

McGowan, P. O., Sasaki, A. D'Alessio, A. C., Dymov, S. Labonté, B., Szyf, M., et al. (2009). Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nature Neuroscience*, **12**, 342–348.

Mead, G. H. (1932). *The philosophy of the present*. Chicago: University of Chicago Press.

Morgan, C. L. (1925). *Life, Mind, and Spirit*. London: Henry Holt.

Morowitz, H. (2002). *The emergence of everything: How the world became complex*. New York: Oxford University Press.

Müller, G. B., & Streicher, J. (1989). Ontogeny of the syndesmosis tibiofibularis and the evolution of the bird hindlimb: A caenogenetic feature triggers phenotypic novelty. *Anatomical Embryology*, **179**, 327–330.

Nakamichi, M., Kato, E. Kojima, Y., & Itoigawa, N. (1998). Carrying and washing of grass roots by free-ranging Japanese macaques at Katsuyama. *Folia Primatologia*, **69**, 35–40.

Needham, J. (1937). *Integrative levels: A reevaluation of the idea of progress*. Oxford: Clarendon Press.

Needham, J. (1945). A note on Dr. Novikoff's article. *Science*, **101**, 582.

Nijhout, H. F. (1990). Metaphors and the role of genes in development. *Bioessays*, **12**, 441–446.

Novikoff, A. B. (1945). The concept of integrative levels and biology. *Science*, **101**, 209–215.

Popper, K. (1974). Scientific reduction and the essential incompleteness of all science. In F. Ayala & T. Dobzhansky (Eds.), *Studies in the philosophy of biology: Reductionism and related problems*. Berkeley: University of California Press.

Raff, R. A. (1996). *The shape of life: Genes, development, and the evolution of animal form*. Chicago: University of Chicago Press.

Rose, S. P. R. (1981). From causations to translations: What biochemists can contribute to the study of behavior. In P. P. G. Bateson & P.H. Klopfer (eds.), *Perspectives in ethology: Advantages of diversity*. New York: Plenum Press.

Rosenblatt, J. S., & Mayer, A. D. (1995). An analysis of approach/withdrawal processes in the initiation of maternal behavior in the laboratory rat. In K. Hood, G. Greenberg, & E. Tobach (eds.), *Behavioral development: Concepts of approach-withdrawal and integrative levels. The T. C. Schneirla Conference Series. Vol.5*, New York: Garland Press.

Salthe, S. (1985). *Evolving hierarchical systems: Their structure and representation*. New York: Columbia University Press.

Sameroff, A. (1994). Developmental systems and family functioning. In R. D. Parke & S. G. Kellam (Eds.), *Exploring family relationships within other social contexts*. Hillsdale, NJ: Erlbaum.

Sapolsky, R.,A natural history of peace. *Foreign Affairs*. Retrieved March 24, 2010 from (2006). http://www.foreignaffairs.org/20060101faessay85110/robert.m.sapolsky.

Schneirla, T. C. (1949; 1972). A biological survey of integrative levels. In R. W. Sellars, V. J. McGill, & M. Farber (Eds.), *Philosophy for the future*. New York: Macmillian. Also in Aronson, L. R., Roach, E., Rosenblatt, J. S., & Lehrman, D. S. (Eds). (1972). *Selected writings of T. C. Schneirla*. San Francisco: W. H. Freeman and Company.

Schneirla, T. C. (1951; 1972). The levels concept in the study of social organization in animals. In Aronson, L. R., Roach, E., Rosenblatt, J. S., & Lehrman, D. S. (Eds). *Selected writings of T. C. Schneirla*. San Francisco: W. H. Freeman and Company.

Schneirla, T. C. (1965). Aspects of stimulation and organization in approach/withdrawal processes underlying vertebrate behavioral development. In D. S. Lehrman, R. Hinde, & E. Shaw (Eds.), *Advances in the study of behavior*, Vol. 1, New York: Academic Press.

Sennett, R. (2008). *The craftsman*. New Haven: Yale University Press.

Smuts, B. (2006). Emergence in social evolution: A great ape example. In P. Clayton & P. Davies (Eds.), *The re-emergence of emergence: The emergentist hypothesis from science to religion*. New York: Oxford University Press.

Sperry, R. W. (1969). Mental phenomena as causal determinants in brain function. In O. O. Globus, G. Maxwell, & I. Savodnik (Eds.), *Consciousness and the brain* (ch. 6, pp. 163–177). New York: Plenum Press. Reprinted (1976) in *Process Studies*, *5*, 247–256.

Sperry, R. W. (1980). Mind-brain interactionism: Mentalism, yes; Dualism, no. *Neuroscience*, **5**, 195–206.

Sporns, O., & Edelman, G. M. (1993). Solving Bernstein's problem: A proposal for the development of coordinated movement by selection. *Child Development*, **64**, 960–981.

Staddon, J. E. R. (1979a). Conservation and consequences – theories of behavior under constraint: An overview. *Journal of Experimental Psychology: General*, **108**, 1–3.

Staddon, J. E. R. (1979b). Operant behavior as adaptation to constraint. *Journal of Experimental Psychology: General*, **108**, 48–67.

Staddon, J. E. R. (1980). *Limits to action: The allocation of individual behavior*. New York: Academic Press.

Stearns, S. C. (1982). The role of development in the evolution of life histories. In J. T. Bonner (Eds.), *Evolution and development*. New York: Springer-Verlag.

Suzuki, Y., & Nijhout, H. F. (2008). Constraint and developmental dissociation of phenotypic integration in a genetically accommodated trait. *Evolution and Development*, **10**, 690–699.

Szyf, M., Weaver, I., & Meaney, M. (2007). Maternal care, the epigenome and phenotypic differences in behavior. *Reproductive Toxicology*, **24**, 9–19.

Thelen, E., & Smith, L. B. (1998). Dynamic systems theories. In R. M. Lerner (Ed.), *Theoretical models of human development*. Vol. 1 *in Handbook of Child Psychology* (5th ed.),W. Damon, Ed.) New York: Wiley.

Thompson, E. (2008). *Mind in life: Biology, phemomenology and the sciences of mind*. Cambridge, MA: Harvard University Press.

Tobach, E., & Schneirla, T. C. (1968). The biopsychology of social behavior in animals. In R. R. Cook (Eds.), *The biological basis of pediatric practice*. New York: McGraw-Hill.

Waddington, C. H. (1939). *An introduction to modern genetics*. London: Allen and Unwin.

Waddington, C. H. (1942). Canalization of development and the inheritance of acquired characters. Nature (London), **150**, 563.

Waddington, C. H. (1957). *The strategy of the genes*. London: Allen and Unwin.

Waddington, C. H. (1968). Toward a theoretical biology. *Nature*, **218**, 525–527.

West, M., & King, A. (1987). Settling nature and nurture into an ontogenetic niche. *Developmental Psychobiology*, **20**, 549–562.

Whitehead, A. N. (1978). *Process and reality*. New York: The Free Press.

Xie, H., Swift, D. J. Cairns, B. D., & Cairns, R. B. (2003). Aggressive behaviors in social interaction and developmental adaptation: A narrative analysis of interpersonal conflicts during early adolescence. *Social Development*, **11**, 205–224.

12

Nonhuman Primate Research Contributions to Understanding Genetic and Environmental Influences on Phenotypic Outcomes across Development

Allyson J. Bennett and Peter J. Pierre

Identification of genetic, experiential, and environmental risk factors that contribute to vulnerability for untoward health outcomes is a major target of behavioral and biomedical research aimed at understanding individual differences in lifespan health trajectories. The developmental systems theoretical perspective set forth by Gilbert Gottlieb (see Introduction within this volume) provides a fundamentally important and influential framework for this research. It has driven progress in the challenging work of understanding the pathways in which a constellation of interacting biological, experiential, and social factors influence individual differences in health. The theoretical perspective emphasizes the complexity of bidirectional and coactional processes across not only genes and environments, but also across multiple domains of function (behavioral, biological, neural) – all within a developmental context. This conceptualization offers invaluable perspective for productive consideration and integration of diverse research findings. In doing so, it provides firm footing for navigating the wide-ranging domains that play a role in Gottlieb's developmental systems theory. It provides traction, and also offers a kind of "bird's eye view" of the ground that will need to be traversed in order to move forward in the iterative process by which essential questions about lifespan developmental trajectories are addressed. When current knowledge is placed into the framework Gottlieb identified, the resulting outlook highlights insightful and hypothesis-driven paths for research undertaken

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from a systems perspective. At the same time – and perhaps as important to guiding the incremental progress that builds strong foundations for discovery – the coactional perspective also calls attention to soft spots in the platforms of basic data that must be addressed in order to bridge points in the framework.

This chapter reviews current nonhuman primate research aimed at uncovering routes of genetic and environmental interplay that contribute to individual differences in behavioral, biological, and neural phenotypes. One of important goals of this line of research – and, ultimately, a major key to achieving improvements in human health – is to uncover factors that contribute to individual differences in risk and resilience to a range of environmental and experiential threats to healthy development. Better mapping of divergent health pathways is requisite to progress in developing novel treatment, intervention, and prevention strategies aimed at changing risk pathways across the lifespan. Early life experiences are a particularly important target because they may be pivotal in shaping lifetime risk of disease and other adverse outcomes. Childhood impoverishment, stress, and adversity are part of a risk pathway for a broad range of deleterious health outcomes across the lifespan. Uncovering and describing how these early experiences generate increased risk and variation in health trajectories is important for a number of reasons. Among them is the potential to identify multiple windows for intervention and recovery. Equally crucial is increased understanding of the mechanisms by which early experiences alter risk of adverse health outcomes. In each of these goals, however, is another question, which is what genetic factors are also associated with individual differences in risk and resilience to these early events? And, specifically, how does genetic and environmental interplay shape the lifespan trajectory of health (Figure 12.1)?

Animal models are essential to this research. Relative to humans, the telescoped maturation of nonhuman animals facilitates more rapid assessment of long-term effects of early life events and longitudinal studies that can span multiple periods of development in a shorter timeframe. Among the important opportunities afforded by animal research is the possibility of controlled experimental approaches to identify the effect of environmental manipulations on a range of outcome measures. These strengths are essential to research aimed at understanding dynamic and complex interplay between genes, environments, and development of multiple interacting systems.

Nonhuman primate studies play an important role in many types of research and the review here is not meant to be exhaustive, rather it focuses on a specific and long-standing animal model used in the translational study of human psychopathology and, primarily, on a specific aspect of genetic variation. A series of studies in rhesus monkeys has now linked genetic variation to individual differences in a range of phenotypes measured across different periods in the lifespan. Exploration of the effects of specific genetic variation, or ''candidate'' genes, both alone, and in combination with environment (e.g., early infant environment, prenatal drug exposure) or subject characteristics (e.g., social dominance status), has grown

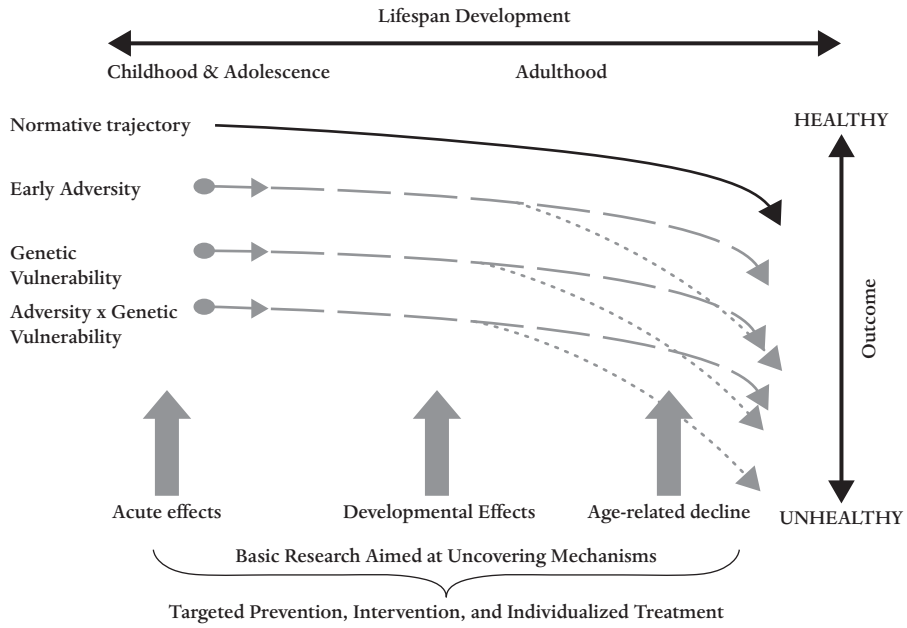

Figure 12.1. Simple model illustrating potential avenues for genetic and environmental factors to contribute to individual differences in trajectories of health across the lifespan.

steadily over the past decade. As well, the number of studies using other approaches (e.g., heritability studies, whole genome linkage) to studying genetic influences has increased. Both the phenotypes under study, as well as the development period in which measurements occur, vary across this emerging literature. In this chapter, the findings from these studies are reviewed from the perspective of identifying areas of convergence as well as gaps in knowledge and opportunities for hypothesis-driven research based on current data from work in both human and nonhuman primate populations.

The majority of review and discussion centers on a well-studied polymorphism in the promoter region of the serotonin transporter gene (serotonin transporter linked polymorphic region; 5HTTLPR) in order to foster understanding of the role nonhuman primate behavioral genetics studies play in basic and biomedical research. Review of the literature is organized in a manner meant to parallel aspects of the structure of Gottlieb's influential conceptualization and facilitate comparison across developmental periods and broad phenotypic domains. Examples from studies on the effects of early rearing environments and genetic variation in nonhuman primates, considered separately and in interaction, illustrate some of the current issues, challenges, and opportunities for integrative developmental research aimed at understanding individual differences.

New opportunities for nonhuman primate behavioral genetic research have increased rapidly over the past decade with major progress in developing tools and

knowledge for sophisticated analysis of the mechanisms of genetic effects. Combined with the rich phenotypic resources available from many years of careful behavioral analysis of captive nonhuman primates, this genetic information provides many avenues for fruitful study. Although the body of literature and emerging findings from nonhuman primate behavioral genetics studies continues to grow, significant gaps in knowledge remain as challenges to our understanding and opportunities for new research. A summary of these soft areas in the platforms that are needed as bridges for continued progress concludes the chapter. Through review of the current nonhuman primate literature and consideration of its potential future directions, the intent of this chapter is to illustrate how the developmental systems theoretical model has fostered experimental approaches to gaining a foot-hold in the complex set of interactions at its center.

Nonhuman Primates in Developmental Research

A range of animal models provide unique opportunities for research that contributes to understanding dynamic contributions of genetic and environmental variation to individual differences in development across the lifespan. For example, rodents are critical for more rapid assessment, high volume, and mechanistic studies. By contrast, nonhuman primate studies comprise a very small percentage of animal research overall, and a relatively small part of research on the consequences of early life experiences. Special ethical, pragmatic, and fiscal challenges to using nonhuman primates in research preclude some lines of investigation and enforce careful consideration of their selection for only those studies aimed at significant questions that could not be addressed in other animals. Nonetheless, nonhuman primate studies are an essential bridge between human studies and those of other animals.

Nonhuman primates are particularly valuable for research aimed at understanding complex neurobiological and behavioral pathways that play an integral role in shaping development. Similar to humans, nonhuman primates undergo relatively long maturational periods and experience congruent developmental changes in complex social behavior and cognitive abilities (Machado & Bachevalier, 2003). Experimental control and telescoped maturation, along with behavioral, neurobiological, genetic, and other similarities to humans, are the features that make nonhuman primates unique for studies that can address essential questions about how complex and interacting factors influence different aspects of development over extended periods (Nelson & Winslow, 2009). In turn, the results of these studies can guide more mechanistic and interventional studies that cannot be conducted in humans. Ultimately, this process of discovery offers an important route for work that can inform development of individualized prevention, intervention, and treatment strategies for significant public health issues (Capitanio & Emborg, 2008).

Many nonhuman primate genetic studies focus on phenotypic variation during the early life periods. Although approximate, the development of macaque monkeys scaled to humans is estimated at 1 year of monkey age to 3–4 years of human age (Sackett, Gunderson, & Baldwin, 1982). Thus, the first year of life for a macaque is roughly equivalent to the human early childhood maturational period. Later life periods, particularly those defined by rapid change either in growth (e.g., adolescence) or major decline (e.g., senescence), are far less well-described in the laboratory animal populations upon which much of this research is based. There is a wealth of knowledge about multiple measures of maturation in both humans and other animals that can guide comparisons between them and identification of equivalence in broad developmental categories (Bolter & Zihlman, 2007). Broad comparisons between humans, nonhuman primates, and rats are illustrated in Figure 12.2. It is important to remember however, that some of the key pieces of integration from which to align maturational stages between humans and other animals are missing (see Dahl, 2004; Spear, 2000a for discussion) and should be the object of continued investigation. For example, studies of physiological development in macaques have a long history and have provided important and detailed data on the time course of maturation from the pre-pubertal to adult period; however, discrete age points for the onset and end of adolescence in nonhuman primates (and other species) are the subject of some debate (Spear, 2000a). As well, description of the range of individual variation, species, and sex differences are relatively sparse. Figure 12.2

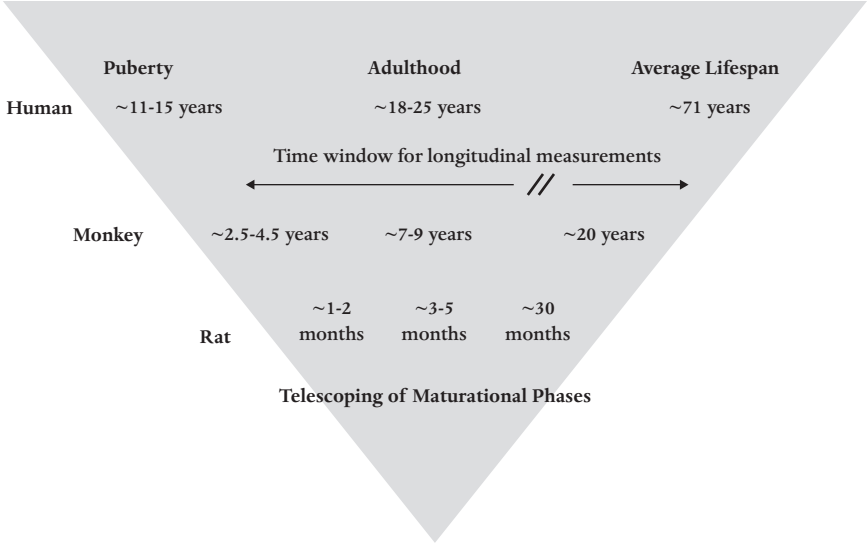

Figure 12.2. Comparative cross-species of development highlighting the relative time window for the broad developmental periods of puberty, adulthood, and the lifespan. The figure emphasizes that telescoping of maturational periods from human to monkey to rat affords different opportunities for measuring individual differences in developmental trajectories.

On average, puberty in rhesus macaques (Van Wagenen & Catchpole, 1954) occurs at roughly 2–3 years of age for females and 3–4 years of age for males (Bercovitch & Goy, 1990). Considered together with developmental data on the emergence of cognitive abilities (Sackett et al., 1982), these data have been used to suggest that monkeys mature at a rate three to four times that of humans so that 3 year old monkeys are roughly equivalent to 9–12 year old humans. The timing and factors influencing pubertal development have been well-studied in macaques (Resko, Goy, Robinson, & Norman, 1982; Wilson, 1989). Less is known about subsequent transitions in maturation from the post-pubertal to fully adult period. In many studies monkeys that are reproductively capable (3–7 years old) are classified as adult. By contrast, sexual maturity in humans is not generally considered the only marker for full adulthood. The average human lifespan is 71. For macaques it is approximately 20, with few surviving beyond 25 years of age and reported maximum lifespans of 30–35 (Tigges, Gordon, McClure, Hall, & Peters, 1988). Monkeys are considered aged at roughly 20–25 years old. In many cases, monkeys ranging in age from 5–20 years old are classified in studies as adult without further discrimination between maturational periods that in humans span late adolescence through the adult period in which the onset of age-related decline emerges.

The degree of importance attached to precision in measurement and categorization of maturational status varies widely. Chronological age, rather than maturational status, is often the only information available in research reports. How closely maturational stages must be mapped in order to guide productive comparison depends in part on the type of question asked. In some cases broad categorization (i.e., infant, juvenile, adolescent, adult, aged) is adequate – at least initially. Among the reasons for more careful consideration of development across the lifespan are several of special relevance here. First, one of the keys to understanding individual differences in lifespan health trajectories lies in identifying the normal range of change evidenced during maturational periods, both in terms of developmental change in specific systems as well as relationships between them. In the context of research aimed at determining how specific genes and environments together contribute to phenotypic variance, the developmental perspective is important for many reasons. Particularly relevant to the interpretation of results and to shaping ongoing comparative behavioral genetic research is the potential for differences in the presence, or direction, of genetic effects across developmental periods. Sensitive periods for environmental influences on behavior, physiology, neurobiology, and other types of function are well-documented. Similar sensitive periods for coactional genetic and environmental influences would be expected.

A strong foundation of knowledge about development, including accurate identification of maturational phases and individual differences, is requisite to research that can encompass questions about sensitive periods and fluctuations from the range of typical maturational change. Early life periods, from prenatal through early childhood in particular, have received much attention in terms of sensitivity to environmental influences. Other periods of rapid change are also

of interest, however, particularly from the perspective of public health questions. Adolescence is one of these. In humans, the period from puberty to adolescence and young adulthood is often marked by initiation of alcohol and drug use and other behaviors that pose risks to healthy development (Dahl, 2004; Giedd, 2008; Spear, 2000b; Volkow & Li, 2005; Witt, 1994; 2007). The rapid and cascading biological, behavioral, social, and cognitive changes that occur during the transition from puberty to adulthood underscore the range of systems that may be vulnerable to disruption during that developmental period. Taken together, these considerations emphasize the importance of basic descriptive work to better characterize lifespan development and provide tools for accurate identification of maturational stages. This type of basic information and the development of strong research tools have facilitated human research (e.g., Tanner Stages; Marshall & Tanner 1969, 1970). In a study that emphasizes the value of this approach, recent research in adolescent humans found that physiological maturation was a significant predictor of risk for problems with alcohol (Costello, Sung, Worthman, & Angold, 2007). Costello and her colleagues included measurement of multiple potential risk factors for initiation of alcohol use and for alcohol use disorders, including familial, peer, and individual characteristics. As well, each individual's physiological maturation was measured by Tanner Staging in order to examine the role that pubertal maturation plays in problems with alcohol use. The study's results demonstrated that individual differences in maturation were important to addiction risk, with early physiological maturation predicting alcohol use in both boys and girls, as well as alcohol use disorders in girls.

Increased attention to individual-based maturation is also essential to inform comparative research. While cross-sectional comparisons aimed at uncovering similarities and differences related to age can be straightforward within a single species, or even genus, comparisons across species or phyla is more difficult. In many ways, progress in comparative developmental research hinges upon the ability to specify study subjects' maturational status in a manner that provides a strong framework for aligning comparison groups across species. Identifying the appropriate "age translation" for comparison poses continued challenges (Sackett et al., 1982; Spear, 2000a for discussion). In part, this is because the translation depends heavily on the availability of normative data from a large enough sample to provide reasonable estimates of the age range for emergence of a developmental stage and the amount of inter-individual variability. Thus, at this time, there are soft aspects of the platform for drawing parallels between significant maturational stages needed for broad consideration of research findings from studies of humans and other animals.

The clear potential for gaining a richer understanding of how genes and environments exert influence over a broad range of individual differences and phenotypic changes across the lifespan underscores the importance of trying to place findings within a developmental context. The extent to which this is (or can be) achieved in behavioral genetic studies varies greatly. For the most part there are few empirical investigations in primates that can fully incorporate

developmental, environmental, and genetic factors to trace routes of interplay on a set of phenotypes related at a systems level. Of specific interest here is how nonhuman primate studies have incorporated a developmental perspective, what their findings show, and where gaps in knowledge and opportunities for integration are apparent. From a practical perspective, identification of age-related changes can form the initial basis for further work that employs a longitudinal approach to evaluate developmental change. Thus, while there are very few nonhuman primate studies that take a longitudinal approach to tracing the effects of interplay between specific aspects of genetic and environmental variation on one or more phenotypes, it is useful to compare the results of cross-sectional comparisons across age groups within the same species. Such comparisons can reveal not only genotype–phenotype association patterns that may be consistent across the lifespan, but may also shed light on the longevity of effects on early life events that serve to deflect an individual's developmental trajectory from the norm. Conversely, comparison of results from populations examined at different maturational periods may point to effects with limited duration, or those with potential for recovery. In sum, current findings from nonhuman primate studies are reviewed here with an eye towards illuminating not only what they tell us about genes, environments, and development, but also what avenues they suggest for continued efforts to integrate developmental perspectives based on longitudinal analysis.

Environmental Manipulations in Nonhuman Primate Behavioral Genetic Research

Among the important opportunities afforded by animal research is the possibility of controlled experimental approaches to identify the effect of environmental manipulations on a range of outcome measures. Of the wide range of environmental variables and interventions studied in humans and animals, there are three that have received the majority of attention in nonhuman primate studies aimed at disentangling genetic and environmental contributions to a range of phenotypic variation. Each is rooted in research programs that are explicitly driven by an attempt to model aspects of experience identified as risk factors in humans and is accompanied by a strong research platform demonstrating relevance of its key features to human environmental variables. One of these manipulations is pharmacological, two are manipulations of the social environment. The three manipulations occur at different maturational periods.

The largest number of studies in this area have examined the effect of early infant rearing environment and genetic variation on subsequent behavioral, neurochemical, neuroendocrine phenotypes (Barr, Newman, Becker, Champoux, et al., 2003, Barr, Newman, Becker, Parker, et al., 2003, Barr, Newman, Lindell, et al., 2004, Barr, Newman, Schwandt, et al., 2004, Barr, Newman, Shannon, et al., 2004;

Bennett et al., 2002; Champoux et al., 2002; Kinnally, Lyons, Abel, Mendoza, & Capitanio, 2008; for additional references and review, see below). Schneider and her colleagues have examined interplay between specific genetic variation and an early environmental manipulation with high clinical significance, prenatal alcohol exposure (Kraemer, Moore, Newman, Barr, & Schneider, 2008). Beyond the infant and prenatal period, two other social environments have been studied in interaction with candidate genes. One is an experimental intervention in which the investigators manipulate the stability of the animals' social group (Capitanio et al., 2008). The other approach examines the interactive effects of an animal's relative social rank (i.e., dominant vs subordinate), either by studying animals in existing stable groups (Jarrell et al., 2008), or by creating new groups in which animals assume new ranks (Wilson & Kinkead, 2008).

Among the primary objectives of these studies of relative social dominance is to capture the effects of a chronically stressful environment, that experienced by a subordinate animal. Chronic stress associated with subordinate status in monkeys has been widely studied and shown to result in a constellation of adverse health outcomes, although its effects vary with gender and in interaction with social group stability (for review, Kaplan, Chen, & Manuck, 2009). For the most part, studies of monkeys' social status focus on measurements taken in adulthood. Their aims are in the same vein, however, as those studies that experimentally manipulate the infant monkeys' social environment. In all cases, the overarching goal is to model the type of adversity and stress that are part of a risk pathway for a broad range of deleterious health outcomes across the lifespan in humans.

Each of these experimental models has been successful in research to identify both immediate and long-term effects of stress and impoverishment on outcome measures that range widely. Their results have also mapped well to findings from human studies of chronic stress or childhood adversity. Thus, these models provide much of the basis for the initial nonhuman primate studies that have begun to uncover patterns of interplay between specific genetic variation and environmental conditions in both infancy and adulthood. It is informative to consider in more detail the experimental model that has been used in many of these studies. What follows is a brief review to highlight aspects of the history, findings, and rationale for a long-standing nonhuman primate model of childhood adversity. Its purpose is not only to provide a backdrop for considering the significance of nonhuman primate studies in relationship to questions about human health, but also to highlight those areas and questions that remain opportunities as yet not fully realized for new research.

Nonhuman Primate Model for Early Childhood Adversity

Understanding how early adverse life experiences alter developmental trajectories is a central and long-standing target of research that spans many disciplines and

employs diverse research approaches and models. Among the early experiences intensively examined in humans and investigated in other animals is relative social and environmental impoverishment. Childhood stress and deprivation are potent risk factors contributing to a range of negative health outcomes. A growing number of reports document the significant deleterious effects of early adverse experiences on psychological and physical well-being in human children (Ames, Fraser, & Burnaby, 1997; Beckett et al., 2006; Flaherty et al., 2006; O'Connor, Rutter, Beckett, Keaveney, & Kreppner, 2000; Rutter, 1998). The consequences of early stress, trauma, and adversity are carried well beyond childhood, with complex effects evidenced into middle- and older age (Perry et al., 2005). For example, a strong link between adverse childhood experiences and the development of affective and substance abuse disorders, including alcoholism later in life, is evident in humans (Bremner, Southwick, Johnson, Yehuda, & Charney, 1993; De Bellis et al., 2002; Widom, Ireland, & Glynn, 1995). More broadly, childhood abuse and household dysfunction increase risk for many causes of death in adulthood ranging from psychopathology to smoking to obesity (Felitti et al., 1998; McEwen, 2003).

Epidemiological estimates of childhood abuse, neglect, trauma, and parental loss underscore the urgency and public health significance of identifying strategies of prevention, intervention, and treatment. The range of other types of childhood stress and adversity at levels that jeopardize development into healthy adulthood is broad and varies in severity. It includes not only disruption of attachment relationships through parental loss, childhood neglect, and abuse, but also other stressors with long-term adverse effects. These include both catastrophic punctate events – for example, naturally-occurring disasters or violent crime – as well as chronic stressors, such as homelessness, poverty, familial conflict, or disruption of child–caregiver attachment relationships. Together these considerations underscore the compelling need for and significance of research aimed at understanding the consequences and developing treatment or intervention strategies for those affected by a spectrum of early adverse experiences.

Animal studies are necessary for progress in this effort. Animal studies provide opportunities for controlled research that can disentangle the effects of specific aspects of adverse environments. For example, animal studies allow for separation of the effects of impoverished diet from social impoverishment. They also provide the experimental means for determining whether the effects of an intervention or treatment varies as a function of the developmental window in which it occurs. As well, animal studies provide a critical avenue for evaluating groups that differ in early experience with subsequent environments held relatively constant to obviate the confound of early stress followed by different forms of impoverishment in adolescence and adulthood. Increasing awareness and accumulating data on the profound negative consequences of low socioeconomic status on a full range of health outcomes (for example, Melchior, Moffitt, Milne, Poulton, & Caspi, 2007) and, particularly, on trajectories of aging (Karlamangla, Singer, McEwen, Rowe, &

Seeman, 2002; Seeman et al., 2004) highlight not only the importance of isolating different components of the environment, but also the rich opportunities for translational research centered on environmental contributions to health.

Concern for the deleterious outcomes affecting children reared in impoverished social environments (Johnson, 2000) has driven decades of animal research aimed at addressing the role that early adverse experiences play in healthy development. Experimental manipulations that disrupt or alter early social relationships are primary to several lines of research in both rodents (Denenberg, Brumaghim, Haltmeyer, & Zarrow, 1967; Hofer, 1970; Johnson, 2000; Liu et al., 1997) and nonhuman primates (Denenberg et al., 1967; Harlow & Harlow, 1965a,b; Harlow & Zimmermann, 1959; Hofer, 1970; Johnson, 2000; Kaufman & Rosenblum, 1969; Liu et al., 1997; Rosenblum & Kaufman, 1968; Rosenblum & Paully, 1984; Suomi, DeLizio, & Harlow, 1976). Congruence between the findings from research using different animals is an important aspect of this work because it provides a strong basis for parallel studies designed to use the animal model most appropriate to address specific questions at different levels of analysis.

Five decades of research has clearly shown that various forms of social impoverishment in early life have long-lasting consequences in nonhuman primates (Harlow & Harlow, 1965a,b; Harlow & Zimmermann, 1959; Seay & Harlow, 1965). Initial observations of profound alterations in development following the early manipulation of mother–infant relationships and other social experiences, provided the impetus for research that has documented effects of early adverse experiences on a wide range of behavioral, socio-emotional, physiological, and neural processes in monkeys (for review see Lyons, Kim, Schatzberg, & Levine, 1998; Kaufman & Rosenblum, 1969; Kraemer, 1992; Machado & Bachevalier, 2003; Sackett, 1965; Sackett, 1984; Sanchez, Ladd, & Plotsky, 2001; Suomi, 1997). One of the primary experimental models in this research uses the manipulation of removing the infant from its mother at birth and subsequently rearing it through infancy in a nursery (nursery-rearing; NR).

Complete discussion of the experimental evolution and variations of early social environmental manipulations used in nonhuman primate research is beyond the scope of this chapter (for comprehensive review, see Sackett, Ruppenthal, & Elias, 2006), however, the variation is noteworthy. Sources of heterogeneity in nursery-rearing as an experimental manipulation include differences in the relative sensory impoverishment of the nursery experience, the duration of exposure, and the presence, number, and duration of time spent with social partners. These variations bear careful consideration both in terms of conceptual implications, as well as their meaning for interpretation of results and comparison of findings across studies.

Similarly, it is important to bear in mind that other experimental manipulations of the mother–infant relationship may not be closely similar in either the aspects of infant experience that are manipulated, or in deviations between the experimental

group and those reared in an undisrupted mother-infant dyad. For example, the model discussed at length here, separation of the infant from its mother at birth and subsequent nursery-rearing through infancy, differs from other experimental manipulations of the mother–infant relationship in ways that may be fundamentally important. Animals reared for experimental purposes in a nursery have no early experience with either their mothers, nor with other conspecific adults. By contrast, monkeys subjected to repeated, but temporary, separations of the mother-infant dyad in a manner parallel to a manipulation widely-used in rodent studies (maternal separation; see chapter X, this volume) are reared primarily by their mothers and, typically, also with social groups of conspecifics. The age at which animals are separated from their mothers is also crucial (Sabatini et al., 2007).

The extent to which these different experimental manipulations produce similar and divergent effects remains a productive area for continued exploration and poses questions that are important from the perspective of drawing parallels to aspects of human experiences. It is also critical to guiding rigorous, hypothesis-driven research that can continue to advance our understanding of the consequences of early adversity within an animal model that can, in turn, serve as a platform for essential work to develop prevention, intervention, or treatment strategies for humans.

Relative to their mother-reared counterparts, nursery-reared monkeys show alterations in an array of physiological and behavioral processes. Some of the findings from these studies are strikingly robust and well-replicated. Overwhelming social and affective deficits first observed under the most stringent deprivation conditions in monkeys (Harlow & Harlow, 1965a; Harlow, Plubell, & Baysinger, 1973; Harlow & Suomi, 1974; Harlow & Zimmermann, 1959; Seay & Harlow, 1965), for example, have been followed by consistent observations of social and affective impairment (albeit less severe) in nursery-reared monkeys given some form of social contact and full sensory stimulation (Capitanio, 1984; Capitanio, 1985; Clarke & Snipes, 1998; Suomi, 1997).

Beyond deficits in core features of social and affective function, nursery-reared monkeys also differ from their mother-reared counterparts in aspects of learning, cognition, neurobiology, and neurochemistry. In many ways, the consequences of early infant environmental and social impoverishment in monkeys are reminiscent of problems experienced by humans with stressful early experiences. For example, among the consequences of early childhood adversity in humans are impairments in learning and cognition (Nelson, 2000). Animal research has also clearly shown that early environmental impoverishment results in impairments in various aspects of learning, cognition, and memory (Renner & Rosenzweig, 1987). In rhesus monkeys there is clear convergent evidence for deficits in aspects of learning and cognition following nursery-rearing (Beauchamp & Gluck, 1988; Beauchamp, Gluck, Fouty & Lewis, 1991; Gluck & Sackett, 1976; Gluck, Harlow & Schiltz, 1973; Griffin & Harlow, 1966; Harlow, Schiltz, & Harlow, 1969; Kraemer & Bachevalier, 1998; Sanchez, Hearn, Do, Rilling, & Herndon, 1998).

Comparisons of nursery- and mother-reared monkeys have also provided unique insights into the neurobiology of early stress, especially as revealed by indirect measures of the neurochemical and neuroendocrine systems (Clarke, 1993; Clarke, Kraemer, & Kupfer, 1998; Clarke & Schneider, 1993; Clarke et al., 1996; Clarke, Ebert, Schmidt, McKinney, & Kraemer, 1999; Higley, Suomi, & Linnoila, 1996a; see also below). Fewer studies have directly addressed neuroanatomical differences between mother- and nursery-reared animals. Those studies that have evaluated neuroanatomical endpoints have consistently (with the exception of Ginsberg, Hof, McKinney, & Morrison, 1993) demonstrated effects of early differential rearing on various measures of brain morphology and composition, including differences in the caudate-putamen (Ichise et al., 2006; Martin, Spicer, Lewis, Gluck, & Cork, 1991), hippocampus (Siegel et al., 1993) and corpus callosum (Sanchez et al., 1998). The pattern of early rearing group differences in monkeys parallel findings from neuroimaging studies of human populations with histories of early stress, maltreatment, and trauma (Cohen et al., 2006; De Bellis et al., 1999; De Bellis & Kuchibhatla, 2006; Kitayama et al., 2007; Seckfort et al., 2008; Teicher et al., 2003). Together, these findings point to a strong potential for research that taps the unique strengths of nonhuman primate models, including neural and cognitive complexity closely similar to humans, to illuminate the mechanisms for deleterious consequences of early adversity. A much more robust literature describing rodent models (Anand & Scalzo, 2000; Caldji et al., 1998; Liu et al., 1997; Miura, Qiao, & Ohta, 2002; Plotsky & Meaney, 1993) and human populations (see above), emphasizes by contrast, however, that many questions remain to be addressed both in order to understand the neurobiology of early stress (Gunnar & Quevedo, 2007) and to provide adequate foundation for research that fully incorporates multiple levels of analysis in order to address their interplay.

Childhood trauma also consistently emerges as a profound risk factor for developing affective disorders, substance-abuse disorders, and alcoholism (Bremner et al., 1993; Widom et al., 1995; for review, De Bellis, 2002). There are few studies that have disentangled the contribution of early adversity to neurobiological and behavioral changes that might underlie increased risk of loss of control over alcohol and drug consumption. Two key observations suggest that nursery-reared monkeys provide an important model in which the effects of early adversity on risk for alcoholism may be assessed within a hypothesis-driven framework that can encompass links between affective behavior, neural contributors, and individual differences. First, Higley and his colleagues (Fahlke et al., 2000; Higley, Suomi, & Linnoila, 1991; Higley et al., 1996a,b) have demonstrated that nursery-reared monkeys consume more alcohol than their mother-reared counterparts. Second, increased alcohol consumption in monkeys is associated with both increased cortisol and decreased turnover of brain serotonin as indicated by decreased CSF 5HIAA (Fahlke et al., 2000). In turn, some evidence suggests that CSF 5HIAA concentrations in nursery-reared monkeys are typically decreased relative to their

mother-reared counterparts (Higley et al., 1992; although see Bennett et al., 2002; Kraemer, Ebert, Schmidt, & McKinney, 1989). These findings are discussed in greater detail in the next section in conjunction with recent research that links individual differences in most of these measurements not only to the early infant environment, but also to specific genetic variation.

In summary, previous research in rhesus monkeys has provided ample demonstration that maternal separation followed by nursery-rearing produces an array of behavioral, physiological, and neurobiological deficits in the early life maturational periods. These data demonstrate that the experimental model is appropriate and promising for successful work aimed at understanding complex interplay between genes, environments, neural, biological, and behavioral systems across the entire lifespan. A wealth of behavioral and physiological data that have been accumulated over five decades supports the relevance and significance of this animal model to understanding the outcome of childhood adversity in humans. There are also unmistakable gaps in knowledge. The characterization of the neural consequences of nursery-rearing for instance, requires further effort in order to assume a role in nonhuman primate behavioral genetics studies that reflects its importance in the developmental pathway.

Nonhuman Primate Research on Specific Genetic Variation

One of the important goals of this line of research – and, ultimately, a major key to achieving improvements in human health – is to uncover factors that contribute to individual differences in risk and resilience to adverse early experiences. Genetic variation is one source of individual differences. While early manipulation of social experiences in monkeys has detrimental consequences for many different aspects of biobehavioral development, individual differences emerge even within the very controlled early environment (Suomi, 1987). Observation of sex (Sackett, 1972) and species differences (Sackett, Ruppenthal, Fahrenbruch, Holm, & Greenough, 1981) point to some sources for this variation. Although individual variation has long suggested the likelihood of genetic factors that could convey vulnerability or resilience to the environmental manipulation, identifying specific genes or routes of genetic influence is difficult in nonhuman primates. Some of the ideal experimental approaches to address this kind of question are not as well-suited for studies employing laboratory primates as for rodents. For example, selective breeding, genetic manipulation, and cross-fostering are all less feasible in nonhuman primates than rodents for a host of reasons including pragmatic, ethical, and fiscal considerations. As a result, relatively fewer studies have previously been able to directly address the question of how genetic factors influence and interact with responses to the early rearing environment in nonhuman primates. This is true not only for studies of nursery-reared monkeys, but also for a range of other

manipulations that are used in nonhuman primate research and that reveal individual differences in vulnerability and resilience to environmental factors. Variation in response to social stress, drug or toxicant exposure, or diet all serve as examples.

Advances in molecular biology and reproductive technology have opened new avenues for moving into areas that were previously less accessible. Mapping specific genes and identifying their variation across nonhuman primates allows for examination of the relationship between that genetic variation and specific phenotypes. Evaluating the presence of association between specific genotypes and phenotypes is a common approach in human studies. Research that has the broad goal of identifying pathways by which genes and environments interact to influence phenotypic development at neural, biological, and behavioral levels has increased tremendously in the past decade. Assessment of association studies in human populations are the topic of other chapters. The number of nonhuman primate studies is relatively small. What they offer, however, is unique and has played a significant role in shaping perspectives across many domains of study.

Association Studies in Nonhuman Primates

In the following sections studies in nonhuman primates, primarily rhesus macaques (*Macaca mulatta*), are used to illustrate avenues of this research. One general strategy for molecular behavioral genetic research is a hypothesis-driven, systems-based approach in which genes of interest are identified as targets based on convergence of evidence from the results of previous studies of the phenotype under study. In brief, genotypes and phenotypes are selected for initial association analysis with the goal of providing the appropriate data for subsequent prediction, hypothesis-generation, and then experimental intervention studies that can test those hypotheses. Selecting genotypes and phenotypes for study can be accomplished in many ways. One is to narrow the range by choosing a biological phenotype that serves as a common pathway linked to variation in a range of behavioral or other phenotypic variation (also sometimes referred to as an endophenotype, see Gottesman & Hanson, 2005). Aspects of neurochemistry, for example, can serve in this role.

Studies aimed at uncovering routes of interplay between genetic, environmental, and biological variation in rhesus monkeys have an important foundation in previous research that can guide selection of specific phenotypes from the broad array of processes that are altered following differential early rearing experiences (Bennett, 2008). For example, as discussed above, differences both in serotonin turnover and in behavioral deficits linked to serotonergic function (Higley and Bennett, 1999; Higley and Linnoila, 1997; Higley, Linnoila, & Suomi, 1994) are evident between nursery- and mother-reared monkeys. Serotonin (5-hydroxy-

tryptamine; 5-HT) is widely involved in behavioral and physiological functions including: motor activity, food intake, sleep, reproductive activity, temperature regulation, cognition, and emotional states. In monkeys, greater impulsivity and higher alcohol consumption appear linked to serotonergic function. Heritability studies have demonstrated a genetic contribution to serotonin function in primates (Clarke et al., 1995; Higley et al., 1993; Rogers et al., 2004).

Polymorphisms within serotonin system genes are obvious targets for association with serotonergic phenotypes. Those polymorphisms whose allelic variants are demonstrated to yield functional differences in their transcriptional efficiency, as well as previous evidence of association with serotonin-related phenotypes in humans, are particularly strong candidates for analysis. The serotonin-transporter (5-HTT) gene linked polymorphic region (5-HTTLPR), a polymorphism first identified in humans (Heils et al., 1996), is one such candidate. The serotonin transporter is one of many integral components that affect regulation of neuronal uptake of 5-HT and serotonergic neurotransmission. Thus, genetic variation that affects function of the serotonin transporter may play a role in creating individual variation in serotonergic turnover. Allelic variation in the 5-HTTLPR that results in variation of serotonin transporter expression (Heils et al., 1996) is associated with individual differences in a wide-range of phenotypes in humans. Reported genotype–phenotype associations include personality traits such as aggression and anxiety (Lesch et al., 1996; although also inconsistent see Lesch et al., 2002 for review), risk of affective disorders (Caspi et al., 2003; also see Kendler & Prescott, 2006; Serretti, Calati, Mandelli, & De Ronchi, 2006, for review), altered CNS 5HT function (Greenberg et al., 1999; Manuck, Flory, Ferrell, & Muldoon, 2004; also see Naylor et al., 1998), and differential response to pharmacological agents that target the serotonin transporter (for review see Hahn & Blakely, 2007; Lesch & Gutknecht, 2005; Murphy et al., 2008; Veenstra-VanderWeele, Anderson, & Cook, 2000).

As with other research using candidate gene and association study approaches, the literature on the 5-HTTLPR is marked by inconsistent findings. Inconsistency in the findings is not unexpected and may result from focusing on genetic influences in absence of consideration of interactive influences with environmental factors that may play an important role. Other potential sources of inconsistency aside, in the case of genetic studies mixed findings may well indicate the presence of underlying variance associated with environmental modulation, gene-gene interactions, or epistatic effects on the phenotype of interest (for review, Taylor & Kim-Cohen, 2007; Uher & McGuffin, 2008). Animal studies provide an important research avenue in this regard because they afford a high level of experimental and environmental control. Thus, when description of the 5-HTTLPR was extended from humans (Lesch et al., 1996) to nonhuman primates (Lesch et al., 1997), it provided new opportunities for comparative study. The locus for this polymorphism is different for great apes and humans as compared to Old World monkeys (rhesus macaques and baboons); however, the number of alleles and the difference in *in vitro*

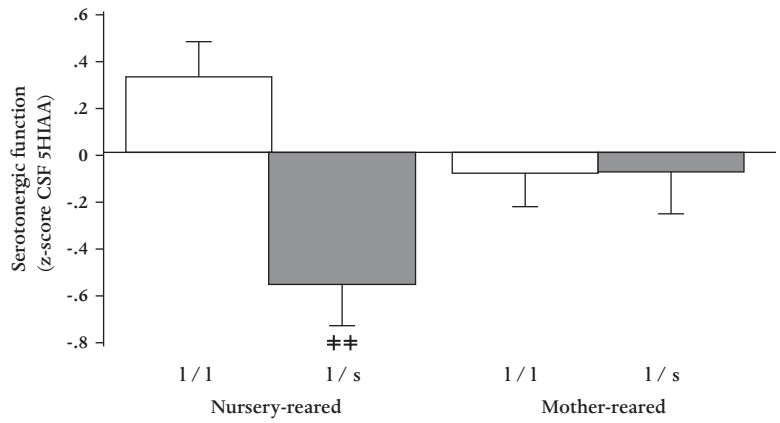

Figure 12.3. Interactive effect of rh5HTTLPR allelic variation and early rearing experience on central nervous system serotonin function in rhesus monkeys. *Source*: Adapted from Bennett et al. 2002.

transcriptional activity of those alleles is similar between human and rhesus monkey (Bennett et al., 2002, see below). The 44 base pair insertion/deletion polymorphisms are, most commonly, biallelic and designated as long (l) and short (s). In humans, the short allele of the 5-HTTLPR is associated with a lower expression of 5-HTT sites and reduced efficiency of 5-HT reuptake (Heils et al., 1996; Little et al., 1998).

Our initial report (Bennett et al., 2002; see Figure 12.3) demonstrated an interactive effect between specific genetic variation and an environmental variable on a biological phenotype in nonhuman primates. The results of the study identified an early experience-dependent association between the rh5-HTTLPR and a measure of serotonergic function in rhesus monkeys, cerebrospinal fluid concentrations of the serotonin metabolite 5-hydroxyindoleacetic acid (CSF 5-HIAA). The short allele resulted in reduced transcriptional activity *in vitro* and an early environment-dependent decrease in cerebrospinal fluid concentrations of CSF 5-HIAA, such that nursery-reared monkeys carrying a copy of the short, low-activity 5-HTTLPR allele had lower CSF 5-HIAA than their homozygote long allele counterparts. While nursery-reared monkeys differed as a function of their genotype, their mother-reared counterparts did not.

Review of 5HTTLPR Findings in Macaques

A series of studies in rhesus monkeys have now linked the 5HTTLPR polymorphism to variation in a range of phenotypes and have included detection of genotype–phenotype associations with, and without, environment-dependent components. Both the phenotypes under study, as well as the development period

in which measurements occur, vary across this emerging literature. In the next section, the findings from these studies are reviewed from the perspective of identifying areas of convergence as well as gaps in knowledge and opportunities for hypothesis-driven research based on current data from work in both human and nonhuman primate populations. The majority of the review and discussion center on the 5HTTLPR polymorphism in order to foster understanding of the role nonhuman primate behavioral genetics studies might play in basic and biomedical research. There are also a limited number of nonhuman primate studies of other candidate genes and these are summarized briefly.

The 5HTTLPR is arguably the only polymorphism that has received sufficient study in nonhuman primates to support initial evaluation of the pattern of findings across phenotypes, age groups, and study populations. It is also one of the few for which *in vitro* assays have demonstrated functional differences between alleles (Bennett et al., 2002; see also MAOALPR below). The *in vitro* results, showing that the two alleles differ in transcriptional activity, are important as supportive evidence of a route by which allelic variation can be linked to *in vivo* functional differences in serotonergic function. Knowledge of allele frequencies is also important to guiding selection of polymorphisms and to evaluating the feasibility of subsequent association studies. Simply stated, it is difficult to evaluate genotype–phenotype association if the allele frequency is such that there is little variation in genotypes across a population. Although rare alleles are informative for some types of genetic study, other types are precluded. One of the stumbling blocks to this type of nonhuman primate research is the absence of known frequencies for allelic variation in genes that are likely to be related to behavioral phenotypes for which nonhuman primates can readily be characterized.

Allele Frequencies and Comparative Considerations

In the case of the 5HTTLPR, nearly a decade of study has produced a fairly robust description of allele frequencies across different populations of rhesus monkeys. Allele frequencies drawn from published reports are summarized in Table 12.1. The table is provided for illustration, along with a cautionary note. In many cases multiple published reports are based on the same set of animals, which poses some difficulty to reliably discerning the number of non-overlapping genotypes. What the data show is that although there is variation across populations (or colonies) of animals, the frequency of the short allele is roughly 25% and the long allele 75% in rhesus monkeys. To our knowledge, there are no studies from wild rhesus monkey populations that would truly address whether these allele frequencies have been influenced by founder effects, colony management, or other aspects of breeding in captivity.

Although rhesus macaques are most commonly studied in the laboratory, they are only one of 20 extant macaque species that differ in a range of geographic,

Table 12.1. rh5HTTLPR Allele frequencies for six macaque species

Species (population/colony)	*N*	*Long allele (%)*	*Short allele (%)*
M. mulatta (NIHAC)[1]	**134**	**83.30**	**16.40**
Bennett et al., 2002			
M. mulatta (NIHAC)[1]*	**193**	**85.00**	**15.00**
Barr et al., 2004			
M. mulatta (CNPRC)[2]	**49**	**83.70**	**16.30**
Kinnally et al., 2008			
M. mulatta (CNPRC)[2]*	**52**	**81.70**	**18.30**
Capitanio et al., 2008			
M. mulatta (HPL, WNPRX)*	**173**	**76.44**	**23.56**
Rogers et al., 2008			
M. mulatta (YNPRC)*	**179**	**72.91**	**27.09**
Hoffman et al., 2007 female only			
M. mulatta (CPRC)*	**532**	**71.00**	**29.00**
Trevilov et al., 2000			
M. mulatta (ONPRC 2004)*	**128**	**61.00**	**39.00**
Bethea et al., 2004			
M. mulatta (GPRC)*	**154**	**66.00**	**34.00**
Lesch et al., 1997			
Non-overlapping total and means*	*1411.00*	*73.44*	*26.56*
M. fascicularis (CIBA Strasbourg and AAP Sanctuary)	**35**	**100.00**	**0.00**
Wendland et al., 2005			
M. nemestrina (various, see below)[3]	**12**	**100.00**	**0.00**
Wendland et al., 2005			
M. sylvasus (various, see below)[4]	**87**	**100.00**	**0.00**
Wendland et al., 2005		(XL)	
M. thibetana (CP Strasbourg)	**3**	**0.00**	**100.00**
Wendland et al., 2005			(XS)
M. tonkeanna (CP Strasbourg)	**28**	**100.00**	**0.00**
Wendland et al., 2005			

[1]Subjects in these two studies overlap.
[2]Subjects in these two studies overlap.
[3]Subjects were from AAP Sanctuary, Almere, Netherlands and CP Strasbourg.
[4]Subjects were from AAP Sanctuary in Almere, Netherlands; Naturzoo Rheine, Germany, Affenberg Salem, Germany, and Wildpark Daun, Germany
*Asterisk denotes the study used to calcualate the total number and overall average allele frequency.

morphometric, behavioral, and other variables. As such, comparison of the macaques provides opportunities for insight into the factors that contribute to the origin and maintenance of allelic variation in specific genes. Thus, identifying whether these species are polymorphic at the 5HTTLPR locus and, if so, their allele

frequencies could inform research aimed at understanding the role of specific serotonin genetic variation. Attempts to identify other macaque species with allelic variation in the 5HTTLPR similar to that shown in rhesus has, for the most part, failed. As shown in Table 12.1, other macaque species are monomorphic, with either only long alleles (*M. fascicularis*, *M. nemestrina*, *M. tonkeanna*; Wendland, Lesch, Newman, Timme, Gachot-Neveu, Thierry & Suomi, 2006), extra long alleles (*M. sylvanus*, Wendland et al., 2006), or extra short alleles (*M. thibetana*; Wendland et al., 2006). In ongoing research, however, we have demonstrated that bonnet macaques (*Macaca radiata*) show length variation in the gene-linked promoter region of the serotonin transporter (Bennett et al, 2004). Our preliminary data now show that the allele frequency for the bonnet macaques may be very similar to most previous reports from rhesus macaques (72% long; 28% short).

Continued efforts to identify interspecific differences in these genes are important to uncovering clues about the evolution of genetic variation linked to biobehavioral adaptation and individual differences among and between primates. Identification of a second nonhuman primate species that is polymorphic in the 5HTTLPR is important because the 5HTTLPR is one of the few – if not only – identified candidate loci with *in vivo*, *in vitro* analogy to the human specific genetic variation. Taken together with the rapidly growing literature demonstrating the 5HTTLPR's relevance to disorders of human health for which many laboratory nonhuman primates serve as models in biomedical research, as well as the clear demonstration of this candidate gene's environmental sensitivity, this finding underscores unique and valuable – if yet unrealized – opportunities for comparative studies.

Organization of Review of 5HTTLPR Macaque Studies

Organized in a manner meant to parallel aspects of the structure of Gottlieb's influential conceptualization and facilitate comparison across developmental periods and broad phenotypic domains, the primary findings from rhesus macaque 5HTTLPR association studies are summarized in Table 12.2. Placing the major findings within a matrix that encompasses not only a wide range of phenotypic domains, but also the entire lifespan, immediately draws attention to gaps in knowledge (shaded blocks) that remain for future study. Also highlighted, however, is emerging evidence of convergent findings across domains, populations, and life periods. It is important to consider the results of association studies within this kind of context, rather than as a series of disparate findings. Considered alone, the results of association studies can appear to have relatively little value. This is particularly true in areas and studies that give the appearance of a tendency to explore association of a specific polymorphism with a broad range of available phenotypes, including those with only tenuous links to a hypothesis based in available evidence.

Table 12.2. Summary of rh5HTTLPR allele associations with varying phenotypes and different maturational periods. (Findings shown as l/s or s/s phenotype compared to l/l, except where noted)

	Age/Maturational period			
Phenotype	*Birth - 1 year (infancy)*	*1-3 years (juvenile)*	*3-7 years (adolescence)*	*8 years and older (adult)*
Behavior	↑ reactivity basal, challenge (G□[1], G■[2], GxIE▲[3,4], GxPE◀[5]) ♀ ♂ No difference in behavioral inhibition (G ◀[5,6]) ♀ ♂	?	?	↑ aggression (G■[7], GxSG◇[8]) ♀ ♂ ↑ abusive mothering (G ■[2]) ♀
Neuroendocrine	↑ LHPA reactivity to challenge (G■[2], GxIE▲[9], GxPE◀[5]) ♀ ♂	No difference am cortisol (MR■[10]) ♀	?	↓ response glucocorticoid - feedback (G■[11]) ♀
Neurochemical	↓ 5HT (GxIE ▲[12]) ♀ ♂ No difference 5HT (G ◇[13] □[1]) ♀ ♂	↑ brain reactivity - challenge (G◀[14]) ♀ ♂	?	↓ 5HT activity (G■[11]) ♀
Alcohol-related	na	?	↑ sensitivity to alcohol (G▲[15]) ♀ ♂ ↑ alcohol consumption (GxIE▲[16]) ♀	?
Metabolic	?	↓ GH, leptin (GxSD■[10]) ♀	?	↓ body weight, leptin, fasting insulin (G■[11], GxSD■[7]) ♀

(*Continued*)

Table 12.2 (*Continued*)

	Age/Maturational period			
Phenotype	*Birth - 1 year (infancy)*	*1-3 years (juvenile)*	*3-7 years (adolescence)*	*8 years and older (adult)*
Immune	?	?	?	↓ T-cell numbers (GxSD■[17]) ♀ ↑ platelet counts (GxSD■[17]) ♀
Reproductive	na	↑ age first ovulation (GxSD■[10]) ♀	↓ age emigration s/s males (G●[18]) ♂	↓ ovulatory cycles (G■[11]) ♀ ↑ offspring in middle vs early/late life (l/s vs s/s & l/l) (G●[19]) ♂

Key:
G = main effect genotype
GxIE = infant environment-dependent genetic effect
GxPE = prenatal environment-dependent genetic effect
GxSD = gene x social dominance status effect
GxSG = gene x stable vs unstable social group effect
5HT = Serotonin (5-hydroxytryptamine)
LHPA = Limbic-hypothalamic-pituitary-adrenal axis

Monkey Population:
▲ Laboratory of Comparative Ethology, National Institute on Child Health and Human Development
● Caribbean Primate Research Center
◀ Harlow Primate Center and Wisconsin National Primate Research Center
♢ California National Primate Research Center
■ Yerkes National Primate Research Center
□ Oregon National Primate Research Center

[1]Bethea et al. (2004); [2]McCormack et al. (2009); [3]Champoux et al. (2002); [4]Spinelli et al. (2008); [5]Kraemer et al. (2008); [6]Rogers et al. (2008); [7]Jarrell et al. (2008); [8]Capitano et al., (2008); [9]Barr et al. (2004a); [10]Wilson & Kinkead (2008); [11]Hoffman et al. (2007); [12]Bennett et al. (2002); [13]Kinnally et al. (2008); [14]Kalin et al. (2008); [15]Barr et al. (2003a); [16]Barr et al. (2004b); [17]Paiardini et al. (2009); [18]Trefilov et al. (2000; [19]Krawczak et al. (2005).

Considered within a broader conceptual context and taken together, the findings of association studies can produce meaningful insights into sources and routes of individual phenotypic variation. They also illuminate potential avenues for new research. Thus, as with most research, the value of association studies lies not only in the discovery of new information, but more importantly in the extent to which this information can address and generate meaningful and testable hypotheses that stimulate progress. By emphasizing coactional processes between factors that shape development, one of the fundamental contributions that Gottlieb and others have made to behavioral genetic research is to ensure that the framework from which the research is viewed explicitly includes the range of factors and the assumption of dynamic processes.

As it has developed over the past decade, the current body of findings from nonhuman primate studies of specific genetic variation shows significant progress. Together the results of these studies, along with their parallels from studies of humans and other animals, point to several conclusions as well as many considerations for further thought and research. Significant differences associated with the 5HTTLPR polymorphism are evidenced not only across maturational periods, but also across phenotypic domains. In Table 12.2, directional findings are represented with up arrows to indicate greater values for the short allele group compared to the long allele group, and conversely, lower values in the short allele groups shown as down arrows. The lower frequency of the short allele in rhesus monkeys (see Table 12.1 and discussion above) and the small number of individuals homozygous for the short allele – particularly in studies with a small sample – has often been addressed by the exclusion of those with the s/s genotype, or by combining the l/s and s/s genotypes. Thus, most findings are based on comparison of monkeys with one or two copies of the short allele (l/s or s/s genotype) versus those with two copies of the long allele (l/l genotype).

Associations between the 5HTTLPR polymorphism and a range of phenotypes are evident both from consideration of main effects of genotype (denoted G in Table 12.2) as well as via interactive effects of genotype with an environmental variable or subject characteristic. The nonhuman primate studies can be divided into those where prenatal and infant environment is experimentally manipulated (e.g., nursery-rearing; IE; prenatal alcohol exposure, PE in Table 12.2) and those without experimental manipulation early in life. Interaction between specific genetic variation and experimental manipulation of the adult social environment has also been examined (stability of the social group; SG in Table 12.2), as has the role of a subject characteristic, relative social dominance rank (SD in Table 12.2). Examination of interplay between each of these environmental or experiential variables and the 5HTTLPR has successfully identified instances in which the presence or direction of genetic influences is dependent upon the environment.

In Table 12.2 the results are organized to facilitate evaluation of points of convergence and divergence across studies, developmental periods, and phenotypic domains. Placing the findings in this type of organizational framework

necessarily emphasizes some facets of the research while neglecting others. Bereft of some detail, it is an approach that facilitates summary, but compromises full consideration of other issues. Important caveats to bear in mind include variance in the number of subjects used in different studies and the use of the same population for multiple studies with multiple types of measurements. The number of subjects per genotype group varies widely across studies. Some studies have samples as large as 580 monkeys, with no less than 50 within a single genotype group. More typical, however, are studies with a much smaller number of subjects. When divided into comparison groups by genotype, experimental manipulation (e.g., rearing environment, prenatal treatment, social dominance status), and gender, these smaller samples can result in very few animals per group. At the lower limits, for example, are studies with 3–4 animals per comparison group (Barr, Newman, Lindell, et al., 2004; Capitanio et al., 2008; Jarrell et al., 2008; Wilson & Kinkead, 2008). Although nonhuman primate studies offer a major advantage of experimental and environmental control at levels not possible in human studies and, as a result that may be reasonably conducted with far fewer subjects, it is still important to realize that some of the studies reviewed here are based on comparison of very small numbers of animals.

From the developmental perspective there are also caveats that emerge from the literature and from the methods used to conduct these studies. For example, study results are organized and reported within the context of major phenotypic domains placed into broad maturational categories. With few exceptions (e.g., Wilson & Kinkead, 2008), however, the animals in these studies are classified only by chronological age. As discussed above, using chronological age as a surrogate for assessment of individual's maturational status is imperfect and its use in initial studies should eventually be complemented by subsequent research that incorporates individual-based assessment with objective measurements. In some cases the age groups used likely span quite different developmental stages (e.g., 7–32 months of age, Rogers, Shelton, Shelledy, Garcia, & Kalin, 2008). Given sex differences in maturation, this issue may be most relevant during the pubertal and adolescent period where males and females are tested at the same chronological age (e.g., Barr et al., 2003; Kalin et al., 2008).

New data from longitudinal studies point to interactive effects of genotype and monkeys' social status on pubertal maturation in females (Wilson & Kinkead, 2008) and genotype effects on important aspects of reproductive life history in males (Trefilov, Berard, Krawczak, & Schmidtke, 2000). These data underscore the importance of explicitly including accurate measurement of maturation and suggest that its inclusion may not only have explanatory value, but may also generate new insights. As presented here, the division of results into basic age/maturational categories serves to illustrate the major patterns of findings with respect to different periods of the lifespan. What is especially promising is that there is evidence for differences between genotype groups in each of the major life periods. Together these results should encourage continued research aimed at

evaluating age-related differences, and particularly the use of longitudinal research approaches that can address the influence of a specific genetic variation and its interplay with environmental factors on lifespan trajectories.

The population of monkeys evaluated in each study is indicated in Table 12.2 by symbols following each finding. Subject population is noted for two reasons. First, as in human studies and other research areas, where findings are replicated across populations they may provide especially strong supportive evidence. In the case of nonhuman primates, populations studied at different primate facilities are unlikely to consist of overlapping individuals. Conversely, a cautionary note may be warranted where a set of findings have arisen from studies of the same population. Studies based on animals within the same facility are likely, but do not always, to have genetically-related or overlapping subject pools. Some studies directly address relatedness among their subjects through pedigree-based analytic techniques meant to discern the role of specific genetic effects from other shared genetic contributions. Other studies use animals that are not closely related, while still others simply attend less to the issue.

The second reason that the study population merits consideration is that the type of environmental manipulation, or animal model, under study is often specific to only one or two research groups and one or two populations of animals. For instance, the great majority of studies of nursery-reared monkeys are based on animals reared in the laboratories of Stephen Suomi and J. Dee Higley and their colleagues (LCE, NICHD), while the majority of studies incorporating analysis of social dominance status are based on animals at the Yerkes National Primate Research Center (YNPRC). These issues warrant attention in terms of their implications for interpreting the findings and comparing them across the literature as a whole. At the same time, it is unrealistic to expect that the same kind of large-scale studies and cross-population replications that are possible with rodents or humans will be accomplished in nonhuman primate given the ethical, pragmatic, and fiscal constraints inherent in nonhuman primate research. Thus, the issues might be considered instead to guide the process of identifying the most critical questions and thoughtful design of studies that can span models and populations to address hypotheses originating from common questions.

Major Findings across 5HTTLPR Macaque Studies

Where different models and populations have been used to address common questions, a general pattern has emerged both from studies in which genotype–phenotype relationships are examined without consideration of variation in early experience, as well as from those in which the effect of early experiential manipulations are considered. The strongest finding to emerge from these studies is evidence of increased behavioral and neuroendocrine reactivity during the infant

and early juvenile period (Bethea et al., 2004; Champoux et al., 2002; Kraemer et al., 2008; McCormack, Newman, Higley, Maestripieri, & Sanchez, 2009; Spinelli et al., 2007). Neuroendocrine function is one of the few measures for which there are reports across multiple life periods. In this case, there is evidence of increased reactivity of the limbic-hypothalamic-pituitary adrenal-axis (LHPA) in response to challenge in infancy (above), no difference in morning cortisol during the juvenile to pubertal period (females only; Wilson & Kinkead, 2008), and evidence of dysregulation in adulthood (females only; Hoffman, Kaplan, Kinkead, Berga, & Wilson, 2007).

Differences in behavioral inhibition expressed in the context of either a novel environment or ecologically-relevant threat did not appear as a function of genotype (Rogers et al., 2008). Differences in patterns of brain reactivity when exposed to the same challenges, however, revealed significant effects of genotype, with higher reactivity in animals with the short allele (Kalin et al., 2008). Together these studies support a relationship between increased stress-reactivity in monkeys with the short allele, particularly in combination with nursery-rearing, with genotype differences apparent in some – but not all – measures. The findings are consistent with a growing number of studies in humans that demonstrate a link between the serotonin transporter polymorphism, affective behavior, and underlying differences in amygdala reactivity (Hariri et al., 2005).

A cursory view of the remaining findings represented in Table 12.2 quickly reveals that the literature is not yet developed sufficiently to support strong evaluation or confident conclusions. For the most part, findings are isolated, with only one study representing assessment of a particular phenotypic domain. There are, however, links between some of these domains that are supported by other lines of evidence and bodies of literature. These links form the basis for hypothesizing that the phenotypes selected for study were linked to serotonergic genetic variation (see above). For example one of the important constellations of behaviors forming a risk pathway in human psychopathology includes aggression, impulsivity, and excessive alcohol consumption – all of which are linked to serotonergic function (Higley & Bennett, 1999 for review). Our initial study (Bennett et al., 2002) identified early-environment dependent association between the 5HTTLPR and a measure of serotonergic function, but included animals across age groups. Two other studies have evaluated aspects of serotonin neurotransmission. One failed to detect genotype effects on serotonin transporter expression measured peripherally (Kinnally et al., 2008). The other found decreased serotonin activity, as measured by prolactin response to citalopram, in adult females with a short allele (Hoffman et al., 2007).

Although none of the studies described here has evaluated impulsivity, aspects of aggression are often linked to impulsivity (although see Winstanley, Dalley, Theobald, & Robbins, 2004 for a review and discussion of the components of impulsivity and relationships between them). In adulthood, there is some evidence for increased aggression in animals with the short allele, both as a main effect

(Jarrell et al., 2008) and in a complex interactive pattern with the stability of the animals' current social group (Capitanio et al., 2008). At the behavioral extreme, there is an association between the short allele and abusive patterns of maternal behavior in adult females (McCormack et al., 2009).

Impulsivity, as a trait (or temperament) characteristic, is hypothesized to create risk for alcoholism. As well, low serotonin functioning is hypothesized as a risk factor of excessive drinking (Higley & Linnoila, 1997; Lesch & Gutknecht 2005). Nonhuman primates provide an important part of the translational model for alcohol research (Grant & Bennett, 2003). Monkeys can not only exhibit the entrenched, abusive patterns of alcohol drinking that characterize human alcoholism, but they also show variation in drinking patterns (Grant et al., 2008). Furthermore, the first risk factors identified for excessive alcohol drinking in monkeys – male gender (Vivian et al., 2001) and early stress (Higley, Hasert, Suomi, & Linnoila, 1991) – are important risk factors in humans. Thus, nonhuman primates provide unique opportunities for controlled experimental approaches to identify aspects of the course and contour of interplay between developmental, genetic, and environmental risk factors for excessive drinking (for review and discussion see Barr, Schwandt, Newman, & Higley, 2004; Grant & Bennett, 2003; Macri, Spinelli, Adriani, Dee Higley, & Laviola, 2007; Witt 1994, 2007).

Two alcohol-related phenotypes have been examined for association with the 5HTTLPR and both showed significant relationships. Both studies were with the population of animals at the LCE, NICHD. Monkeys with the short allele were assigned higher intoxication ratings by experienced human observers (Barr, Newman, Becker, Champoux, et al., 2003), providing evidence of increased sensitivity to alcohol during the adolescent period. As well, nursery-reared female monkeys with the short allele showed greater alcohol preference (Barr, Newman, Lindell, et al., 2004). Together, these results are consistent with the idea that the low-activity, short allele of the 5HTTLPR is associated with alterations in a constellation of phenotypes relevant to risk for excessive alcohol consumption. Whether the genotype is associated with excessive drinking in nonhuman primates, or with alcohol-related phenotypes expressed in adulthood has not yet been addressed. Furthermore, as with all of the studies discussed here, however, strong conclusions await replication in additional populations.

A second set of phenotypes investigated for association with the 5HTTLPR includes measurements of metabolic health and immune function. These studies were all conducted at YNPRC with female monkeys. The pattern of results emerging from this series of recent studies provides intriguing evidence of multiple negative health outcomes in animals with the short allele, including decreased body weight, leptin, fasting insulin, and immune measures. These associations occurred as main effects of genotype and as an interactive effect with subordinate social dominance status (Jarrell et al, 2008; Paiardini et al., 2009; Wilson & Kinkead, 2008). Although caution is warranted by a relatively small number of subjects from only one colony, these results are of great interest because they incorporate rigorous

physiological measurements of health and provide a converging line of evidence for increased sensitivity to environmental, or experiential, influences in carriers of the short allele. With one exception (Wilson & Kinkead, 2008), these results are from adult animals. It remains for future study to determine whether differences in metabolic and other physiological aspects of health are influenced by genotype or environment in a similar way during earlier developmental periods.

The final phenotypic domain shown in Table 12.2 includes measures of both reproductive function and reproductive strategy. First, in pubertal females, Wilson and Kinkead (2008) report a later age at first ovulation and longer pubertal tempo in subordinate females with the short allele. In adult females, Hoffman et al. (2007) report fewer ovulatory cycles in females with the short allele. In males, there is strong evidence from large longitudinal studies that demonstrate an association between 5HTTLPR genotypes and differences in male monkeys' reproductive strategy and life history (Krawczak et al., 2005; Trefilov, Berard, Krawczak, & Schmidke, 2000). Both of these studies were conducted with animals at the Caribbean Primate Research Center (Cayo Santiago). Adolescent males with two copies of the short allele emigrated from their natal troop at younger ages than their heterozygous or homozygous long counterparts (Trefilov et al., 2000). When reproductive output of this population was examined there was no difference as a function of genotype; however, an elegant analysis of the timing of reproduction revealed that heterozygous animals produced more offspring during the intermediate age (10–13 years) than during younger or older periods. The authors interpret the finding in terms of differences in age of dispersal from the natal group and provide intriguing discussion about the implications for genotype-linked differences in dispersal and reproductive strategies.

In summary, the findings reviewed here provide evidence of an emerging pattern of association between specific genetic variation and aspects of behavior, neural function, metabolic and immune function, and reproduction. The strongest findings are in convergent (but not entirely consistent) evidence of greater behavioral and LHPA reactivity in animals with the short allele, particularly when coupled with environmental adversity. There is also evidence for decreased serotonergic function that has found parallel in human studies. For example, Manuck and his colleagues (Manuck et al., 2004) found that the 5HTTLPR genotype interacts with socioeconomic status (SES) to influence a measure of serotonergic function, prolactin-response to fenfluramine challenge. Individuals with low SES and the low-activity short allele had significantly lower serotonergic function than either their long allele, low SES counterparts. Individuals with high SES were relatively undifferentiated by genotype. Not only is there evidence that the short allele can confer vulnerability to serotonergic alterations in the presence of relative impoverishment, but a separate study has demonstrated that the short allele is associated with increased risk of depression following stressful life events (Caspi et al., 2003). Increased aggression, heightened sensitivity to alcohol, and greater preference for alcohol also showed association with the 5HTTLPR in

nonhuman primates. A combination of the 5HTTLPR short allele and low social status was found to confer increased risk of negative effects in measures of metabolic and immune function. Finally, measures of reproductive maturation and timing showed variation with 5HTTLPR genotype.

Other Genes

In parallel to the rh5HTTLPR findings, both human and nonhuman primate studies of specific variation in other genes have also produced evidence of both genotype–phenotype association, as well as interactive effects of genes and early environment. Individual differences in response to novelty, social and affective cognition, and personality characteristics are closely linked to variation in dopaminergic and serotonergic neurotransmission, as evidenced by both clinical and experimental studies. Evidence of heritability of these traits is also strong. Furthermore, Rogers and his colleagues (2004) have demonstrated that overlapping sets of genes influence monoamine neurotransmission in Old World monkeys. Together, these lines of evidence have formed the basis for targeted exploration of polymorphisms within not only serotonin genes, but also monoamine oxidase A (MAOA) and dopamine genes in monkeys.

MAO is an enzyme that catalyzes the oxidative deamination of biogenic amines and is central to catecholamine and indolamine neurotransmitter function. Its common role in function of these different neurotransmitters places it within the risk pathway for apparently disparate neuropsychiatric disorders and underscores its importance to a host of neurobehavioral, physiological, and developmental processes. A polymorphism in the regulatory region of the MAOA gene (Sabol, Hu, & Hamer, 1998), with functional allelic differences in *in vitro* transcriptional efficiency as shown by luciferase assay (Deckert et al., 1997), was shown by Caspi and his colleague to interact with childhood experience of maltreatment to predict variation in risk for antisocial behavior (Caspi et al., 2002). In rhesus monkeys, the MAOA polymorphism is associated with early rearing environment-dependent differences in aggressive behavior (Newman et al., 2005). This result was replicated and extended in a large study ($n = 473$) of the interactive effects of the MAOA genotype on multiple aspects of socioaffective behavior expressed under challenge (Karere et al., 2009). In an elegant demonstration of the interplay between specific genetic variation and ''broader contextual features'' of the social environment, Karere et al. showed that both genotype and the size of the social group in which mother-reared monkeys were raised contributed to aggression and anxiety. This study is the first to detect significant interactive effects between genotype and variation in the size (and thus, social complexity) of the social group in which mother-infant dyads live. It is an important finding as a demonstration of a broad environmental influence interacting with specific genetic factors to modify

individual differences in behavior and also one that bears consideration for its potential implications for other nonhuman primate research.

Variation in dopaminergic neurotransmission is linked to variation in the normal range of human personality characteristics associated with response to novelty, sensation-seeking, and risk-taking, and is also a key contributor to an array of neuropsychiatric disorders, including schizophrenia, substance-abuse, and attention deficit/hyperactivity disorder. Hence, studies aimed at identifying specific genotypes associated with both personality differences, as well as risk for these disorders, have focused on genes that play a role in dopaminergic function. A functional polymorphism in one of these genes, the dopamine receptor 4 (Van Tol et al., 1992) has been associated with individual differences in personality traits linked to novelty-seeking, attentional processes, and ADHD (for review Bobb, Castellanos, Addington, & Rapoport, 2006). In contrast to a relatively large human literature, little is known about whether association between the DRD4 exon 3 VNTR polymorphism and measures of temperament are also present in nonhuman primates. A recent report in a large sample of vervets (*Cercopithecus aethiops;* $n = 452$), an Old World monkey, found an association between the DRD4 exon 3 VNTR polymorphism and response to novelty (Bailey et al., 2007).

There are several other reports of association between variation in other genes and a range of phenotypes. These include reports on genetic association with neuroendocrine function and its role in temperament and alcohol consumption (e.g., cortiotropin-releasing hormone haplotype, Barr, Dvoskin, et al., 2008), alcohol response (mu opioid receptor gene, Barr et al., 2007), infant attachment behavior (mu opioid receptor gene, Barr, Schwandt, et al., 2008), and susceptibility to the side effects of neuroleptic drugs (dopamine 3 receptor, Werge, Elbaek, Andersen, Lundbaek, & Rasmussen, 2003). Taken together, these findings provide strong evidence that evaluation of specific genetic variation is a useful research avenue for uncovering sources of individual variation in a broad range of behavioral and biological phenotypes.

Summary and Conclusions

Nonhuman primate studies have provided new insights into the interplay between genes, environments, and biological and behavioral phenotypes. Together they suggest many paths for subsequent study aimed at uncovering the mechanisms by which monkeys' early environment and specific genes interact to produce differential associations on behavior and physiology, as well as their underlying neural substrates. For example, evidence that the interaction between genotype and the rearing environment is manifest within the first months of life after exposure to the environmental manipulation (Champoux et al., 2002; Karere et al., 2009; Kraemer et al., 2008), may be among the most significant clues these results hold for future

mechanistic studies. The emergence of this effect so early in life helps to narrow, and thus facilitate, definition of the set of specific factors by which the environment induces genotype-dependent effects on neurobehavioral development. Even within the relatively controlled experimental environments manipulated in nonhuman primate and other animal studies, there is a broad range of specific factors and interacting influences that may contribute to overall environment effects. For example, diet, temperature, type and amount of contact and experience with conspecifics, all vary between mother- and nursery-reared animals. Nonetheless, the set of factors that differ between the environments of these groups in infancy is relatively small in comparison to older animals. In older animals, disentangling contributions of specific environmental influences becomes even more complex as variation in lifetime experiences increases and includes both exposure to a greater range of environmental variation, as well as the cumulative effects of interplay between biological and behavioral systems, genes, and both early and later environmental influences.

Convergence between the findings of human and nonhuman primate is encouraging and also raises many questions and challenges for further progress in moving from observation of significant interplay between genes and environments to hypothesis-driven experimental research that can further illuminate these complex relationships. The human literature surrounding the effects of not only the 5HTTLPR polymorphism, but also polymorphisms in genes related to other aspects of neurochemical function (e.g., DRD4, MAOA) has grown rapidly. It now includes many demonstrations of interplay between allelic variation and a range of phenotypes. These findings have stimulated discussion and re-examination of the nature of many of the aspects of the environment that are under study (Shanahan & Hofer, 2005). In the case of the 5HTTLPR, for example, careful consideration has occurred in part as a means to identify testable hypotheses that could account for a pattern of results that point to broad susceptibility, or environmental sensitivity, associated with the short allele (for review and discussion, Kendler & Prescott, 2006; Reiss & Leve, 2007).

The results of nonhuman primate studies have also begun to provoke closer attention and re-examination of specific aspects of the experimental models used to manipulate the environment (Bennett, 2008; Karere et al., 2009), as well as to parallels between them and the human conditions they are meant to model. Congruence between human and nonhuman primate studies suggest the need for further consideration of the specific features of the experimental manipulations shown to interact with genetic variation. One primary question is whether aspects of the experimental manipulation may be directly similar to the human environmental conditions. This may not be the case. The parallel may not be in a specific aspect of the environment. It may be that the experimental manipulation induces biological effects that result in movement towards a pathway in common to the human environment which is paralleled. For example, in the case of nursery-reared animals, a number of different specific environmental factors could be responsible

for alterations in aspects of neurochemistry and other associated physiological and behavioral processes. The environmental factors contributing to shifts in the biobehavioral system may differ substantially between this animal model of early adversity and the conditions experienced by humans. Convergence in the biological and behavioral consequences of the early experiences, however, suggests common pathways of effect for a broad set of environmental influences. These are questions that must be addressed in order to translate research findings into hypotheses accessible for experimental study.

The value of animal research lies precisely in this niche. Part of its strength lies in the opportunities that it offers for experimental studies that can address the mechanisms by which genetic variables and specific environmental variables play together to influence the expression of these genes and individual differences in phenotypes. The challenge is not only to describe early environmental conditions with common features or effects that might produce similar results in human and nonhuman primates, but also to specify those aspects of the environment in a manner that permits further experimental study. Although possibilities have been suggested (Barr, Schwandt, et al., 2004; Suomi, 2006), very little work in the area has begun in which hypotheses generated by the findings are explicitly tested with experimental studies that can be conducted with nonhuman primates and other animals. Thus, what remains for future work is to take advantage of the experimental opportunities offered by these animal models in an effort to begin to uncover the mechanism by which early experience can modify the expression of genetic influences on biology and behavior.

The early controlled environment of nursery-reared monkeys offers an avenue for tracking the effect of specific genetic variation on resilience or vulnerability to the deleterious effects of this early social impoverishment. Equally important, however, the controlled environment grants opportunities for tracing the pathways of interplay between genetic influences and those related to parental care. In monkeys, as in other animals, the parental-offspring relationship is a major influence on development. Variation in parental care can be explicitly studied and deliberately influenced by experimental manipulations (Lyons et al., 1998; Rosenblum and Paully, 1984). As such, it provides an important avenue for research. At the same time, studies aimed at understanding genotype–phenotype relationships in development also benefit from removing this source of variation by rearing offspring in standard conditions. This is particularly true given that the genetic influences under study for their effects on infant behavior may also be those that contribute to differences in parental care. One of the areas that remains relatively unexplored in nonhuman primate research is the role of genetic variation in maternal care. McCormack and her colleagues (2009) have demonstrated an association between the short 5HTTLPR allele and infant abuse in rhesus monkeys. This finding, along with a rich history of nonhuman primate studies of parental care, suggest a productive potential avenue for research aimed at understanding the interactive effects of offspring and maternal genotypic and phenotypic variation.

Animal models provide unmatched opportunities for controlled studies that can accelerate knowledge about the impact of both specific and broad genetic and environmental factors on the many integrated systems that contribute to healthy development across the lifespan. However, while animal models are ideally suited to address questions undertaken from the developmental systems theoretical framework, there are critical gaps in foundational knowledge that inhibit the iterative process. Review of the current literature from both human and nonhuman primate developmental behavioral genetics research points to four areas in particular that pose important challenges for future work. In each of these areas, there is an essential need for basic descriptive work. This foundational work is critical to ensure that a solid platform is in place to support hypothesis-driven research to address questions that require bridges between the different domains of function that play a role in development (e.g., behavioral, biological, neural, genetic). First, as outlined above, further refinement of the experimental models, particularly continued progress in discriminating specific components of the environment that alter phenotypic development, is needed.

Second, longitudinal research that addresses the entirety of the lifespan health trajectory in nonhuman primates is relatively sparse. In particular, there is a dearth of information about how very early experiences alter development and health across the middle- and later-life periods. Thus, despite its promise as an animal model that could be used to examine the life-long consequences of early adversity, only a handful of studies have systematically compared nursery- and mother-reared monkeys later in life. Studies that have examined older animals (14–24 years; approximate scale 41–68 human years) have found evidence of long-lasting effects of early rearing experience, including earlier mortality and alterations in immune function (Lewis, Gluck, Petitto, Hensley, & Ozer, 2000) as well as deficits in measures of learning (Beauchamp & Gluck, 1988; Beauchamp et al., 1991). There are no longitudinal studies that have made use of noninvasive technologies to examine the effects of early rearing group differences in brain morphology and tissue composition either early in life, or as the animals age. Absence of a comprehensive evaluation of the longevity of early rearing effects in nonhuman primates represents a serious gap in our understanding. Addressing this gap is an important goal because it will greatly expand possibilities for evaluating developmental trajectories across the entire lifespan, rather than a truncated period that fails to encompass aging. Following animals through the later life periods affords opportunities for addressing how individual differences in the slope of age-related decline are influenced by events that occurred early in life and their interplay with genetic factors. As well, lifespan longitudinal studies provide opportunities to understand how combinations of genes and different types of early and later experience affect outcomes.

Third, identifying both the range of individual variation and points of correspondence and divergence across species in normal maturational processes beyond the early life periods is an important target for nonhuman primate research. It is

part of a workable platform from which to make full use of comparative studies to discover common pathways by which genes, environments, neural, behavioral, and other systems and influences interact across developmental periods to influence individual's lifespan health trajectory. One of the major challenges to incorporating a developmental perspective into any study is that its potential for success is proportional to the strength of the underlying platform of basic knowledge about development. Weaknesses and gaps in foundational information rapidly emerge as obstacles to hypothesis formation, experimental design, and interpretation of results. For studies whose target is description of similarities and differences across either closely-related or distantly-related species, part of the requisite foundation is a reliable grasp of how the important features of maturation compare. Where animals are employed as models – or surrogates – for humans, capturing maturationally-equivalent time points is likely to be essential to the interpretation, translation, and progress of research. Similarly, identifying the normal range, extent of individual variation, and hallmark features of maturational periods is important to determining whether the effects of an event, treatment, or intervention vary according to the maturational period in which it is delivered. Where outcome measures or phenotypes are expected to vary across development, weaknesses in understanding their pattern of change, as well as similarity and differences across species, will produce corresponding soft areas in the research platform. While it is clear that identifying deviations from healthy development across the lifespan requires basic knowledge about normative health trajectories, it is equally apparent that for some areas of study much of this basic data is either sparse or missing entirely.

Finally, knowledge about nonhuman primate brain development across the lifespan, including not only individual differences, but also its relationships to other aspects of development including behavioral and biological systems, is currently uneven and – in many ways, insufficient from a developmental perspective. This is an area where rapid progress is possible given that advances in noninvasive methods largely eliminate what previously stood as obstacles to longitudinal study. A rapidly growing literature on human longitudinal studies using neuroimaging has demonstrated robust developmental changes in brain from childhood through young adulthood (Almli, Rivkin, & McKinstry, 2007; Courchesne et al., 2000; Lenroot & Giedd, 2006; Paus et al., 2001; Shaw et al., 2008). To our knowledge, there is only one longitudinal study of nonhuman primate brain morphology (Malkova, Heuer, & Saunders, 2006). In that study, Malkova and colleagues showed significant changes across the infant to adolescent period in rhesus macques. Cross-sectional studies using neuroimaging to evaluate age-related change in aspects of brain morphology in macaques have also demonstrated parallels to humans (Pierre, Hopkins, Taglialatela, Lees, & Bennett, 2008). Together these findings all point to strong potential for rapid progress of research that can provide a sound foundation of basic descriptive information about brain development in nonhuman primates. In turn, the importance of this foundation

can be realized by the opportunities that it will provide to integrate neurodevelopment more strongly into animal models exploring the interplay between genes, environments, and other aspects of the developmental systems theoretical framework.

In summary, the emerging body of literature provides promising evidence that continued nonhuman primate research guided by Gottlieb's developmental systems theoretical perspective will yield important insights into how individual differences in lifespan health trajectories emerge. Attention to what is required to achieve this is essential and must include careful consideration of the developmental context, as it is essential to integration of current findings and navigation of new avenues of research. At the same time, rapid advances in knowledge and technology, as well as exciting convergence in discoveries in human and animal studies, underscore remarkable new opportunities to address and trace the routes of individual differences across lifespan trajectories.

References

Almli, C. R., Rivkin, M. J., & McKinstry, R. C. (2007). The NIH MRI study of normal brain development (Objective-2): Newborns, infants, toddlers, and preschoolers. *Neuroimage*, **35**, 308–325.

Ames, E. W., Fraser, S., & Burnaby, B. C. (1997). *The development of Romanian orphanage children adopted to Canada*. British Columbia: Simon Fraser University.

Anand, K. J., & Scalzo, F. M. (2000). Can adverse neonatal experiences alter brain development and subsequent behavior? *Biology of the Neonate*, **77**, 69–82.

Bailey, J. N., Breidenthal, S. E., Jorgensen, M. J., McCracken, J. T., & Fairbanks, L. A. (2007). The association of DRD4 and novelty seeking is found in a nonhuman primate model. *Psychiatry and Genetics*, **17**, 23–27.

Barr, C. S., Dvoskin, R. L., Yuan, Q., Lipsky, R. H., Gupte, M., Hu, X., et al. (2008). CRH haplotype as a factor influencing cerebrospinal fluid levels of corticotropin-releasing hormone, hypothalamic-pituitary-adrenal axis activity, temperament, and alcohol consumption in rhesus macaques. *Archives of General Psychiatry*, **65**, 934–944.

Barr, C. S., Newman, T. K., Becker, M. L., Champoux, M., Lesch, K. P., Suomi, S. J., et al. (2003). Serotonin transporter gene variation is associated with alcohol sensitivity in rhesus macaques exposed to early-life stress. *Alcohol Clinical and Experimental Research*, **27**, 812–817.

Barr, C. S., Newman, T. K., Becker, M. L., Parker, C. C., Champoux, M., Lesch, K. P., et al. (2003). The utility of the non-human primate; model for studying gene by environment interactions in behavioral research. *Genes, Brain, and Behavior*, **2**, 336–340.

Barr, C. S., Newman, T. K., Lindell, S., Shannon, C., Champoux, M., Lesch, K. P., et al. (2004). Interaction between serotonin transporter gene variation and rearing condition in alcohol preference and consumption in female primates. *Archives of General Psychiatry*, **61**, 1146–1152.

Barr, C. S., Newman, T. K., Schwandt, M., Shannon, C., Dvoskin, R. L., Lindell, S. G., et al. (2004). Sexual dichotomy of an interaction between early adversity and the serotonin transporter gene promoter variant in rhesus macaques. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 12358–12363.

Barr, C. S., Newman, T. K., Shannon, C., Parker, C., Dvoskin, R. L., Becker, M. L., et al. (2004). Rearing condition and rh5-HTTLPR interact to influence limbic-hypothalamic-pituitary-adrenal axis response to stress in infant macaques. *Biological Psychiatry*, **55**, 733–738.

Barr, C. S., Schwandt, M., Lindell, S. G., Chen, S. A., Goldman, D., Suomi, S. J., et al. (2007). Association of a functional polymorphism in the mu-opioid receptor gene with alcohol response and consumption in male rhesus macaques. *Archives of General Psychiatry*, **64**, 369–376.

Barr, C. S., Schwandt, M. L., Lindell, S. G., Higley, J. D., Maestripieri, D., Goldman, D., et al. (2008). Variation at the mu-opioid receptor gene (OPRM1) influences attachment behavior in infant primates. *Proceedings of the National Academy of the Sciences USA*, **105**, 5277–5281.

Barr, C. S., Schwandt, M. L., Newman, T. K., & Higley, J. D. (2004). The use of adolescent nonhuman primates to model human alcohol intake: Neurobiological, genetic, and psychological variables. *Annals of the New York Academy of Sciences*, **1021**, 221–233.

Beauchamp, A. J., & Gluck, J. P. (1988). Associative processes in differentially reared monkeys (*Macaca mulatta*): Sensory preconditioning. *Developmental Psychobiology*, **21**, 355–364.

Beauchamp, A. J., Gluck, J. P., Fouty, H. E., & Lewis, M. H. (1991). Associative processes in differentially reared rhesus monkeys (*Macaca mulatta*): Blocking. *Developmental Psychobiology*, **24**, 175–189.

Beckett, C., Maughan, B., Rutter, M., Castle, J., Colvert, E., Groothues, C., et al. (2006). Do the effects of early severe deprivation on cognition persist into early adolescence? Findings from the English and Romanian adoptees study. *Child Development*, **77**, 696–711.

Bennett, A. J. (2008). Gene environment interplay: Nonhuman primate models in the study of resilience and vulnerability. *Developmental Psychobiology*, **50**, 48–59.

Bennett, A. J., Kaiss, K. M., Laudenslager, M. L., Kaplan, J. R., Robbins, K., Ma, R., et al. (2004). Comparison of allele frequencies in *Macaca fascicularis*, *M. mulatta*, *M. nemestrina*, and *M. radiata* using an enhanced method for detecting the short and long promoter alleles of the *Macaca*. *American Journal of Primatology*, **62**(Supplement 1), 100–101.

Bennett, A. J., Lesch, K. P., Heils, A., Long, J. C., Lorenz, J. G., Shoaf, S. E., et al. (2002). Early experience and serotonin transporter gene variation interact to influence primate CNS function. *Molecular Psychiatry*, **7**, 118–122.

Bercovitch, F. B., & Goy, R. W. (1990). The socioendocrinology of reproductive development and reproductive success in macaques. In T. E. Ziegler (Ed.), *Socioendocrinology of primate reproduction* (pp. 59–93). New York: Wiley-Liss.

Bethea, C. L., Streicher, J. M., Coleman, K., Pau, F. K., Moessner, R., & Cameron, J. L. (2004). Anxious behavior and fenfluramine-induced prolactin secretion in young

rhesus macaques with different alleles of the serotonin reuptake transporter polymorphism (5HTTLPR). *Behavior Genetics*, **34**, 295–307.

Bobb, A. J., Castellanos, F. X., Addington, A. M., & Rapoport, J. L. (2006). Molecular genetic studies of ADHD: 1991 to 2004. *American Journal of Medical Genetics (Neuropsychiatric Genetics)*, **141B**, 551–565.

Bolter, D., & Zihlman, A. (2007). Primate growth and development: A functional and evolutionary approach. In C. J. Campbell, A. Fuentes, K. C. MacKinnon, M. Panger, & S. K. Bearder (Eds.), *Primates in perspective* (pp. 408–422). New York: Oxford University Press.

Bremner, J. D., Southwick, S. M., Johnson, D. R., Yehuda, R., & Charney, D. S. (1993). Childhood physical abuse and combat-related posttraumatic stress disorder in Vietnam veterans. *American Journal of Psychiatry*, **150**, 235–239.

Caldji, C., Tannenbaum, B., Sharma, S., Francis, D., Plotsky, P. M., & Meaney, M. J. (1998). Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 5335–5340.

Capitanio, J. P. (1984). Early experience and social processes in rhesus macaques (*Macaca mulatta*): I. Dyadic social interaction. *Journal of Comparative Psychology*, **98**, 35–44.

Capitanio, J. P. (1985). Early experience and social processes in rhesus macaques (*Macaca mulatta*): II. Complex social interaction. *Journal of Comparative Psychology*, **99**, 133–144.

Capitanio, J. P., Abel, K., Mendoza, S. P., Blozis, S. A., McChesney, M. B., Cole, S. W., et al. (2008). Personality and serotonin transporter genotype interact with social context to affect immunity and viral set-point in simian immunodeficiency virus disease. *Brain, Behavior, and Immunity*, **22**, 676–689.

Capitanio, J. P., & Emborg, M. E. (2008). Contributions of non-human primates to neuroscience research. *Lancet*, **371**, 1126–1135.

Caspi, A., McClay, J., Moffitt, T. E., Mill, J., Martin, J., Craig, I. W., et al. (2002). Role of genotype in the cycle of violence in maltreated children. *Science*, **297**, 851–854.

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., et al. (2003). Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science*, **301**, 386–389.

Champoux, M., Bennett, A., Shannon, C., Higley, J. D., Lesch, K. P., & Suomi, S. J. (2002). Serotonin transporter gene polymorphism, differential early rearing, and behavior in rhesus monkey neonates. *Molecular Psychiatry*, **7**, 1058–1063.

Clarke, A. S. (1993). Social rearing effects on HPA axis activity over early development and in response to stress in rhesus monkeys. *Developmental Psychobiology*, **26**, 433–446.

Clarke, A. S., Ebert, M. H., Schmidt, D. E., McKinney, W. T., & Kraemer, G. W. (1999). Biogenic amine activity in response to fluoxetine and desipramine in differentially reared rhesus monkeys. *Biological Psychiatry*, **46**, 221–228.

Clarke, A. S., Hedeker, D. R., Ebert, M. H., Schmidt, D. E., McKinney, W. T., & Kraemer, G. W. (1996). Rearing experience and biogenic amine activity in infant rhesus monkeys. *Biological Psychiatry*, **40**, 338–352.

Clarke, A. S., Kammerer, C. M., George, K. P., Kupfer, D. J., McKinney, W. T., Spence, M. A. et al. (1995). Evidence for heritability of biogenic amine levels in the cerebrospinal fluid of rhesus monkeys. *Biological Psychiatry*, **38**, 572–577.

Clarke, A. S., Kraemer, G. W., & Kupfer, D. J. (1998). Effects of rearing condition on HPA axis response to fluoxetine and desipramine treatment over repeated social separations in young rhesus monkeys. *Psychiatry Research*, **79**, 91–104.

Clarke, A. S., & Schneider, M. L. (1993). Prenatal stress has long-term effects on behavioral responses to stress in juvenile rhesus monkeys. *Developmental Psychobiology*, **26**, 293–304.

Clarke, A. S., & Snipes, M. (1998). Early behavioral development and temperamental traits in mother- vs peer-reared rhesus monkeys. *Primates*, **39**, 433–448.

Cohen, R. A., Grieve, S., Hoth, K. F., Paul, R. H., Sweet, L., Tate, D., et al. (2006). Early life stress and morphometry of the adult anterior cingulate cortex and caudate nuclei. *Biological Psychiatry*, **59**, 975–982.

Costello, E. J., Sung, M., Worthman, C., & Angold, A. (2007). Pubertal maturation and the development of alcohol use and abuse. *Drug and Alcohol Dependence*, **88**(Suppl 1), S50–S59.

Courchesne, E., Chisum, H. J., Townsend, J., Cowles, A., Covington, J., Egaas, B., et al. (2000). Normal brain development and aging: Quantitative analysis at in vivo MR imaging in healthy volunteers. *Radiology*, **216**, 672–682.

Dahl, R. E. (2004). Adolescent brain development: A period of vulnerabilities and opportunities. Keynote address. *Annals of the New York Academy of Sciences*, **1021**, 1–22.

De Bellis, M. D. (2002). Developmental traumatology: A contributory mechanism for alcohol and substance use disorders. *Psychoneuroendocrinology*, **27**, 155–170.

De Bellis, M. D., Keshavan, M. S., Clark, D. B., Casey, B. J., Giedd, J. N., Boring, A. M., et al. (1999). Developmental traumatology Part II: Brain development. *Biological Psychiatry*, **45**, 1271–1284.

De Bellis, M. D., Keshavan, M. S., Frustaci, K., Shifflett, H., Iyengar, S., Beers, S. R., et al. (2002). Superior temporal gyrus volumes in maltreated children and adolescents with PTSD. *Biological Psychiatry*, **51**, 544–552.

De Bellis, M. D., & Kuchibhatla, M. (2006). Cerebellar volumes in pediatric maltreatment-related posttraumatic stress disorder. *Biological Psychiatry*, **60**, 697–703.

Deckert, J., Catalano, M., Heils, A., Di, B. D., Friess, F., Politi, E., et al. (1997). Functional promoter polymorphism of the human serotonin transporter: Lack of association with panic disorder. *Psychiatry and Genetics*, **7**, 45–47.

Denenberg, V. H., Brumaghim, J. T., Haltmeyer, G. C., & Zarrow, M. X. (1967). Increased adrenocortical activity in the neonatal rat following handling. *Endocrinology*, **81**, 1047–1052.

Fahlke, C., Lorenz, J. G., Long, J., Champoux, M., Suomi, S. J., & Higley (2000). Rearing experiences and stress-induced plasma cortisol as early risk factors for excessive alcohol consumption in nonhuman primates. *Alcohol Clinical and Experimental Research*, **24**, 644–650.

Felitti, V. J., Anda, R. F., Nordenberg, D., Williamson, D. F., Spitz, A. M., Edwards, V., et al. (1998). Relationship of childhood abuse and household dysfunction to many of the

leading causes of death in adults. The Adverse Childhood Experiences (ACE) Study. American Journal of Preventive Medicine, **14**, 245–258.

Flaherty, E. G., Thompson, R., Litrownik, A. J., Theodore, A., English, D. J., Black, M. M., et al. (2006). Effect of early childhood adversity on child health. *Archives of Pediatric Adolescent Medicine*, **160**, 1232–1238.

Giedd, J. N. (2008). The teen brain: Insights from neuroimaging. *Journal of Adolescent Health*, **42**, 335–343.

Ginsberg, S. D., Hof, P. R., McKinney, W. T. & Morrison, J. H. (1993). The noradrenergic innervation density of the monkey paraventricular nucleus is not altered by early social deprivation. *Neuroscience Letters*, **158**, 130–134.

Gluck, J., Harlow, H. F., & Schiltz, K. A. (1973). Differential effect of early enrichment and deprivation on learning in the rhesus monkey (*Macaca mulatta*). *Journal of Comparative and Physiological Psychology*, **84**, 598–604.

Gluck, J., & Sackett, G. (1976). Extinction deficits in socially isolated rhesus monkeys (*Macaca mulatta*). *Developmental Psychology*, **12**, 173–174.

Gottesman, I. I., & Hanson, D. R., (2005). Human development: Biological and genetic processes. *Annual Review of Psychology*, **56**, 263–286.

Grant, K. A., & Bennett, A. J. (2003). Advances in nonhuman primate alcohol abuse and alcoholism research. *Pharmacology and Therapeutics*, **100**, 235–255.

Grant, K. A., Leng, X., Green, H. L., Szeliga, K. T., Rogers, L. S., & Gonzales, S. W., (2008). Drinking typography established by scheduled induction predicts chronic heavy drinking in a monkey model of ethanol self-administration. *Alcohol Clinical and Experimental Research*, **32**, 1824–1838.

Greenberg, B. D., Tolliver, T. J., Huang, S. J., Li, Q., Bengel, D., & Murphy, D. L. (1999). Genetic variation in the serotonin transporter promoter region affects serotonin uptake in human blood platelets. *American Journal of Medical Genetics*, **88**, 83–87.

Griffin, G. A., & Harlow, H. F. (1966). Effects of three months of total social deprivation on social adjustment and learning in the rhesus monkey. *Child Development*, **37**, 533–547.

Gunnar, M., & Quevedo, K. (2007). The neurobiology of stress and development. *Annual Review of Psychology*, **58**, 145–173.

Hahn, M. K., & Blakely, R. D. (2007). The functional impact of SLC6 transporter genetic variation. *Annual Review of Pharmacology and Toxicology*, **47**, 401–441.

Hariri, A. R., Drabant, E. M., Munoz, K. E., Kolachana, B. S., Mattay, V. S., Egan, M. F., et al. (2005). A susceptibility gene for affective disorders and the response of the human amygdala. *Archives of General Psychiatry*, **62**, 146–152.

Harlow, H. F., & Harlow, M. K. (1965a). Effects of various mother-infant relationships on rhesus monkey behaviors. In B. M. Foss (Ed.), *Determinants of infant behaviour* (pp. 15–36). London: Methuen.

Harlow, H. F., & Harlow, M. K. (1965b). The affectional systems. In A. M. Schrier (Ed.), *Behavior of Nonhuman Primates*, Volume 2, (pp. 287–334). New York: Academic Press.

Harlow, H. F., Plubell, P. E., & Baysinger, C. M. (1973). Induction of psychological death in rhesus monkeys. *Journal of Autism and Childhood Schizophrenia*, **3**, 299–307.

Harlow, H. F., Schiltz, K. A., & Harlow, M. K. (1969). *Effects of social isolation on the learning performance of rhesus monkeys* (Vol. 1,) New York: Karger.

Harlow, H. F., & Suomi, S. J. (1974). Induced depression in monkeys. *Behavioral Biology*, **12**, 273–296.

Harlow, H. F., & Zimmermann, R. R. (1959). Affectional responses in the infant monkey. *Science*, **130**, 421–432.

Heils, A., Teufel, A., Petri, S., Stober, G., Riederer, P., Bengel, D., et al. (1996). Allelic variation of human serotonin transporter gene expression. *Journal of Neurochemistry*, **66**, 2621–2624.

Higley, J. D., & Bennett, A. J. (1999). Central nervous system serotonin and personality as variables contributing to excessive alcohol consumption in non-human primates. *Alcohol and Alcoholism*, **34**, 402–18.

Higley, J. D., Hasert, M. F., Suomi, S. J., & Linnoila, M. (1991). Nonhuman primate model of alcohol abuse: Effects of early experience, personality, and stress on alcohol consumption. *Proceedings of the National Academy of Sciences of the United States of America*, **88**, 7261–7265.

Higley, J. D., & Linnoila, M. (1997). A nonhuman primate model of excessive alcohol intake. Personality and neurobiological parallels of type I- and type II-like alcoholism. *Recent Developments in Alcoholism*, **13**, 191–219.

Higley, J. D., Linnoila, M., & Suomi, S. J. (1994). Ethological contributions. In M. Hersen, R. T. Ammerman, & L. A. Sisson (Eds.) *Handbook of aggressive and destructive behavior in psychiatric patients* (pp. 17–32) New York: Plenum Press.

Higley, J. D., Mehlman, P., Taub, D., Higley, S. B., Suomi, S. J., Vickers, J. H., et al. (1992). Cerebrospinal fluid monoamine and adrenal correlates of aggression in free-ranging rhesus monkeys. *Archives of General Psychiatry*, **49**, 436–441.

Higley, J. D., Suomi, S. J., & Linnoila, M. (1991). CSF monoamine metabolite concentrations vary according to age, rearing, and sex, and are influenced by the stressor of social separation in rhesus monkeys. *Psychopharmacology*, **103**, 551–556.

Higley, J. D., Suomi, S. J., & Linnoila, M. (1996a). A nonhuman primate model of type II alcoholism? Part 2. Diminished social competence and excessive aggression correlates with low cerebrospinal fluid 5-hydroxyindoleacetic acid concentrations. *Alcoholism, Clinical and Experimental Research*, **20**, 643–650.

Higley, J. D., Suomi, S. J., & Linnoila, M. (1996b). A nonhuman primate model of type II excessive alcohol consumption? Part 1. Low cerebrospinal fluid 5-hydroxyindoleacetic acid concentrations and diminished social competence correlate with excessive alcohol consumption. *Alcohol Clinical and Experimental Research*, **20**, 629–642.

Higley, J. D., Thompson, W. W., Champoux, M., Goldman, D., Hasert, M. F., Kraemer, G. W., et al. (1993). Paternal and maternal genetic and environmental contributions to cerebrospinal fluid monoamine metabolites in rhesus monkeys (*Macaca mulatta*). *Archives of General Psychiatry*, **50**, 615–623.

Hofer, M. A. (1970). Physiological responses of infant rats to separation from their mothers. *Science*, **168**, 871–873.

Hoffman, J. B., Kaplan, J. R., Kinkead, B., Berga, S. L., & Wilson, M. E. (2007). Metabolic and reproductive consequences of the serotonin transporter promoter polymorphism (5-HTTLPR) in adult female rhesus monkeys (*Macaca mulatta*). *Endocrine Journal*, **31**, 202–211.

Ichise, M. Vines, D. C., Gura, T., Anderson, G. M., Suomi, S. J., Higley, J. D., et al. (2006). Effects of early life stress on [11C]DASB positron emission tomography imaging of serotonin transporters in adolescent peer- and mother-reared rhesus monkeys. *Journal of Neuroscience*, **26**, 4638–4643.

Jarrell, H., Hoffman, J. B., Kaplan, J. R., Berga, S., Kinkead, B., & Wilson, M. E. (2008). Polymorphisms in the serotonin reuptake transporter gene modify the consequences of social status on metabolic health in female rhesus monkeys. *Physiology and Behavior*, **93**, 807–819.

Johnson, D. E. (2000). Medical and developmental sequelae of early childhood institutionalization in eastern European adoptees. In C. A. Nelson (Ed.), *The effects of early adversity on neurobehavioral development* (pp. 113–162). Mahwah: Erlbaum.

Kalin, N. H., Shelton, S. E., Fox, A. S., Rogers, J., Oakes, T. R., & Davidson, R. J. (2008). The serotonin transporter genotype is associated with intermediate brain phenotypes that depend on the context of eliciting stressor. *Molecular Psychiatry*, **13**, 1021–1027.

Kaplan, J. R., Chen, H., & Manuck, S. B. (2009). The relationship between social status and atherosclerosis in male and female monkeys as revealed by meta-analysis. *American Joural of Primatology*, **71**, 1–10.

Karere, G. M., Kinnally, E. L., Sanchez, J. N., Famula, T. R., Lyons, L. A., & Capitanio, J. P. (2009). What is an ''adverse'' environment? Interactions of rearing experiences and MAOA genotype in rhesus monkeys. *Biological Psychiatry*, **65**, 770–777.

Karlamangla, A. S., Singer, B. H., McEwen, B. S., Rowe, J. W., & Seeman, T. E. (2002). Allostatic load as a predictor of functional decline MacArthur studies of successful aging. *Journal of Clinical Epidemiology*, **55**, 696–710.

Kaufman, I. C., & Rosenblum, L. A. (1969). Effects of separation from mother on the emotional behavior of infant monkeys. *Annals of New York Academy of Sciences*, **159**, 681–695.

Kendler, K. S., & Prescott, C. A. (2006). *Genes, environment, and psychopathology: Understanding the causes of psychiatric and substance use disorders*. New York: The Guilford Press.

Kinnally, E. L., Lyons, L. A., Abel, K., Mendoza, S., & Capitanio, J. P. (2008). Effects of early experience and genotype on serotonin transporter regulation in infant rhesus macaques. *Genes, Brain, and Behavior*, **7**, 481–486.

Kitayama, N., Brummer, M., Hertz, L., Quinn, S., Kim, Y., & Bremner, J. D. (2007). Morphologic alterations in the corpus callosum in abuse-related posttraumatic stress disorder: A preliminary study. *Journal of Nervous and Mental Disease*, **195**, 1027–1029.

Kraemer, G. W. (1992). A psychobiological theory of attachment. *Behavioral and Brain Sciences*, **15**, 493–541.

Kraemer, G. W., & Bachevalier, J. (1998). Cognitive changes associated with persisting behavioral effects of early psychosocial stress in rhesus monkeys: A view from psychobiology. In B. P. Dohrenwend (Ed.), *Adversity, stress, and psychopathology* (pp. 438–462) New York: Oxford University Press.

Kraemer, G. W., Ebert, M. H., Schmidt, D. E., & McKinney, W. T. (1989). A longitudinal study of the effects of different rearing environments on cerebrospinal fluid norepinephrine and biogenic amine metabolites in rhesus monkeys. *Neuropsychopharmacology*, **2**, 175–189.

Kraemer, G. W., Moore, C. F., Newman, T. K., Barr, C. S., & Schneider, M. L. (2008). Moderate level fetal alcohol exposure and serotonin transporter gene promoter polymorphism affect neonatal temperament and limbic-hypothalamic-pituitary-adrenal axis regulation in monkeys. *Biological Psychiatry*, **63**, 317–324.

Krawczak, M., Trefilov, A., Berard, J., Bercovitch, F., Kessler, M., Sauermann, U., et al. (2005). Male reproductive timing in rhesus macaques is influenced by the 5HTTLPR promoter polymorphism of the serotonin transporter gene. *Biology of Reproduction*, **72**, 1109–1113.

Lenroot, R. K., & Giedd, J. N. (2006). Brain development in children and adolescents: Insights from anatomical magnetic resonance imaging. *Neuroscience and Biobehavioral Reviews*, **30**, 718–729.

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S. et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*, **274**, 1527–1531.

Lesch, K. P., Greenberg, B. D., Higley, J. D., Bennett, A., & Murphy, D. L. (2002). Serotonin transporter, personality, and behavior: Toward a dissection of gene–gene and gene–environment interaction. In J. Benjamin, R. Ebstein, & R. H. Belmaker (Eds.), *Molecular genetics and the human personality* (pp. 109–135) Washington, DC: American Psychiatric Publishing.

Lesch, K. P., & Gutknecht, L. (2005). Pharmacogenetics of the serotonin transporter. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, **29**, 1062–1073.

Lesch, K. P., Meyer, J., Glatz, K., Flugge, G., Hinney, A., Hebebrand, J. et al. (1997). The 5-HT transporter gene-linked polymorphic region (5-HTTLPR) in evolutionary perspective: Alternative biallelic variation in rhesus monkeys. *Journal of Neural Transmission*, **104**, 1259–1266.

Lewis, M. H., Gluck, J. P., Petitto, J. M., Hensley, L. L., & Ozer, H. (2000). Early social deprivation in nonhuman primates: Long-term effects on survival and cell-mediated immunity. *Biological Psychiatry*, **47**, 119–126.

Little, K. Y., McLaughlin, D. P., Zhang, L., Livermore, C. S., Dalack, G. W., McFinton, P. R. et al. (1998). Cocaine, ethanol, and genotype effects on human midbrain serotonin transporter binding sites and mRNA levels. *American Journal of Psychiatry*, **155**, 207–213.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A. et al. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science*, **277**, 1659–1662.

Lyons, D. M., Kim, S., Schatzberg, A. F., & Levine, S. (1998). Postnatal foraging demands alter adrenocortical activity and psychosocial development. *Developmental Psychobiology*, **32**, 285–291.

Machado, C. J., & Bachevalier, J. (2003). Non-human primate models of childhood psychopathology: The promise and the limitations. *Journal of Child Psychology and Psychiatry*, **44**, 64–87.

Macri, S., Spinelli, S., Adriani, W., Dee Higley, J., & Laviola, G. (2007). Early adversity and alcohol availability persistently modify serotonin and hypothalamic-pituitary-adrenal-axis metabolism and related behavior: What experimental research on rodents and primates can tell us. *Neuroscience & Biobehavioral Reviews*, **31**, 172–180.

Malkova, L., Heuer, E., & Saunders, R. C. (2006). Longitudinal magnetic resonance imaging study of rhesus monkey brain development. *European Journal of Neuroscience*, **24**, 3204–3212.

Manuck, S. B., Flory, J. D., Ferrell, R. E., & Muldoon, M. F. (2004). Socio-economic status covaries with central nervous system serotonergic responsivity as a function of allelic variation in the serotonin transporter gene-linked polymorphic region. *Psychoneuroendocrinology*, **29**, 651–668.

Marshall, W. A., & Tanner, J. M. (1969). Variations in pattern of pubertal changes in girls. *Archives of Disease in Childhood*, **44**, 291–303.

Marshall, W. A., & Tanner, J. M. (1970). Variations in the pattern of pubertal changes in boys. *Archives of Disease in Childhood*, **45**, 13–23.

Martin, L. J., Spicer, D. M., Lewis, M. H., Gluck, J. P., & Cork, L. C. (1991). Social deprivation of infant rhesus monkeys alters the chemoarchitecture of the brain: I. Subcortical regions. *Journal of Neuroscience*, **11**, 3344–3358.

McCormack, K., Newman, T. K., Higley, J. D., Maestripieri, D., & Sanchez, M. M. (2009). Serotonin transporter gene variation, infant abuse, and responsiveness to stress in rhesus macaque mothers and infants. *Hormones and Behavior*, **55**, 538–547.

McEwen, B. S. (2003). Early life influences on life-long patterns of behavior and health. *Mental Retardation and Developmental Disabilities Research Reviews*, **9**, 149–154.

Melchior, M., Moffitt, T. E., Milne, B. J., Poulton, R., & Caspi, A. (2007). Why do children from socioeconomically disadvantaged families suffer from poor health when they reach adulthood? A life-course study. *American Journal of Epidemiology*, **166**, 966–974.

Miura, H., Qiao, H., & Ohta, T., (2002). Attenuating effects of the isolated rearing condition on increased brain serotonin and dopamine turnover elicited by novelty stress. *Brain Research*, **926**, 10–17.

Murphy, D. L., Fox, M. A., Timpano, K. R., Moya, P. R., Ren-Patterson, R., Andrews, A. M. et al. (2008). How the serotonin story is being rewritten by new gene-based discoveries principally related to SLC6A4, the serotonin transporter gene, which functions to influence all cellular serotonin systems. *Neuropharmacology*, **55**, 932–960.

Naylor, L., Dean, B., Pereira, A., Mackinnon, A., Kouzmenko, A., & Copolov, D. (1998). No association between the serotonin transporter-linked promoter region polymorphism and either schizophrenia or density of the serotonin transporter in human hippocampus. *Molecular Medicine*, **4**, 671–674.

Nelson, C. A. (2000). *The effects of early adversity on neurobehavioral development: The Minnesota Symposia on Child Psychology* (Vol. 31,) Mahwah, NJ: Erlbaum.

Nelson, E. E., & Winslow, J. T. (2009). Non-human primates: Model animals for developmental psychopathology. *Neuropsychopharmacology*, **34**, 90–105.

Newman, T. K., Syagailo, Y. V., Barr, C. S., Wendland, J. R., Champoux, M., Graessle, M. et al. (2005). Monoamine oxidase A gene promoter variation and rearing experience influences aggressive behavior in rhesus monkeys. *Biological Psychiatry*, **57**, 167–172.

O'Connor, T. G., Rutter, M., Beckett, C., Keaveney, L., & Kreppner, J. M. (2000). The effects of global severe privation on cognitive competence: Extension and longitudinal follow-up. *Child Development*, **71**, 376–390.

Paiardini, M., Hoffman, J., Cervasi, B., Ortiz, A. M., Stroud, F., Silvestri, G. et al. (2009). T-cell phenotypic and functional changes associated with social subordination and gene polymorphisms in the serotonin reuptake transporter in female rhesus monkeys. *Brain, Behavior, and Immunity*, **23**, 286–293.

Paus, T. Collins, D. L., Evans, A. C., Leonard, G., Pike, B., & Zijdenbos, A. (2001). Maturation of white matter in the human brain: A review of magnetic resonance studies. *Brain Research Bulletin*, **54**, 255–266.

Perry, J. C., Sigal, J. J., Boucher, S., Pare, N., Ouimet, M. C., Normand, J. et al. (2005). Personal strengths and traumatic experiences among institutionalized children given up at birth (Les Enfants de Duplessis – Duplessis' children) II: Adaptation in late adulthood. *Journal of Nervous and Mental Disease*, **193**, 783–789.

Pierre, P. J., Hopkins, W. D., Taglialatela, J. P., Lees, C. J., & Bennett, A. J. (2008). Age-related neuroanatomical differences from the juvenile period to adulthood in mother-reared macaques (*Macaca radiata*). *Brain Research*, **1226**, 56–60.

Plotsky, P. M., & Meaney, M. J. (1993). Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. *Brain Research Molecular Brain Research*, **18**, 195–200.

Reiss, D., & Leve, L. D. (2007). Genetic expression outside the skin: Clues to mechanisms of genotype x environment interaction. *Development and Psychopathology*, **19**, 1005–1027.

Renner, M. J., & Rosenzweig, M. R. (1987). *Enriched and impoverised environments: Effects on brain and behavior*. New York: Springer-Verlag.

Resko, J. A., Goy, R. W., Robinson, J. A., & Norman, R. L. (1982). The pubescent rhesus monkey: Some characteristics of the menstrual cycle. *Biology of Reproduction*, **27**, 354–361.

Rogers, J., Martin, L. J., Comuzzie, A. G., Mann, J. J., Manuck, S. B., Leland, M. et al. (2004). Genetics of monoamine metabolites in baboons: Overlapping sets of genes influence levels of 5-hydroxyindolacetic acid, 3-hydroxy-4-methoxyphenylglycol, and homovanillic acid. *Biological Psychiatry*, **55**, 739–744.

Rogers, J., Shelton, S. E., Shelledy, W., Garcia, R., & Kalin, N. H. (2008). Genetic influences on behavioral inhibition and anxiety in juvenile rhesus macaques. *Genes, Brain, and Behavior*, **7**, 463–469.

Rosenblum, L. A., & Kaufman, I. C. (1968). Variations in infant development and response to maternal loss in monkeys. *American Journal Orthopsychiatry*, **38**, 418–426.

Rosenblum, L. A., & Paully, G. S. (1984). The effects of varying environmental demands on maternal and infant behavior. *Child Development*, **55**, 305–314.

Rutter, M. (1998). Developmental catch-up, and deficit, following adoption after severe global early privation. The English and Romanian adoptees (ERA) study team. *Journal of Child Psychology and Psychiatry*, **39**, 465–476.

Sabatini, M. J., Ebert, P., Lewis, D. A., Levitt, P., Cameron, J. L., & Mirnics, K. (2007). Amygdala gene expression correlates of social behavior in monkeys experiencing maternal separation. *Journal of Neuroscience*, **27**, 3295–3304.

Sabol, S. Z., Hu, S., & Hamer, D. (1998). A functional polymorphism in the monoamine oxidase A gene promoter. *Human Genetics*, **103**, 273–279.

Sackett, G. P. (1965). Effects of rearing conditions upon the behavior of rhesus monkeys (*Macaca mulatta*). *Child Development*, **36**, 855–868.

Sackett, G. P. (1972). Exploratory behavior of rhesus monkeys as a function of rearing experiences and sex. *Developmental Psychology*, **6**, 260–270.

Sackett, G. P. (1984). A nonhuman primate model of risk for deviant development. *American Journal of Mental Deficiency*, **88**, 469–476.

Sackett, G. P., Gunderson, V., & Baldwin, D. (1982). Studying the ontogeny of primate behavior. In J. L. Fobes & J. E. King (Eds.), *Primate behavior* (pp. 135–169) New York: Academic Press.

Sackett, G. P., Ruppenthal, G.C., & Elias, K. (Eds.). (2006). *Nursery rearing of nonhuman primates in the 21st century*. New York: Springer.

Sackett, G. P., Ruppenthal, G. C., Fahrenbruch, C. E., Holm, R. A., & Greenough, W. T. (1981). Social isolation rearing effects in monkeys vary with genotype. *Developmental Psychology*, **17**, 313–318.

Sanchez, M. M., Hearn, E. F., Do, D. Rilling, J. K., & Herndon, J. G. (1998). Differential rearing affects corpus callosum size and cognitive function of rhesus monkeys. *Brain Research*, **812**, 38–49.

Sanchez, M. M., Ladd, C. O., & Plotsky, P. M. (2001). Early adverse experience as a developmental risk factor for later psychopathology: Evidence from rodent and primate models. *Development and Psychopathology*, **13**, 419–449.

Seay, B., & Harlow, H. F. (1965). Maternal separation in the rhesus monkey. *Journal of Nervous and Mental Disease*, **140**, 434–441.

Seckfort, D. L., Paul, R., Grieve, S. M., Vandenberg, B., Bryant, R. A., Williams, L. M. et al. (2008). Early life stress on brain structure and function across the lifespan: A preliminary study. *Brain Imaging and Behavior*, **2**, 49–58.

Seeman, T. E., Crimmins, E., Huang, M. H., Singer, B., Bucur, A., Gruenewald, T. et al. (2004). Cumulative biological risk and socio-economic differences in mortality: MacArthur studies of successful aging. *Socal Science and Medicine*, **58**, 1985–1997.

Serretti, A., Calati, R., Mandelli, L., De and Ronchi, D. (2006). Serotonin transporter gene variants and behavior: A comprehensive review. *Current Drug Targets*, **7**, 1659–1669.

Shanahan, M. J., & Hofer, S. M. (2005). Social context in gene–environment interactions: retrospect and prospect. *Journals of Gerontology*, **60B**(Spec No 1), 65–76.

Shaw, P., Kabani, N. J., Lerch, J. P., Eckstrand, K., Lenroot, R., Gogtay, N., et al. (2008). Neurodevelopmental trajectories of the human cerebral cortex. *Journal of Neuroscience*, **28**, 3586–3594.

Siegel, S. J., Ginsberg, S. D.,Hof, P. R., Foote, S. L., Young, W. G., Kraemer, G. W., et al. (1993). Effects of social deprivation in prepubescent rhesus monkeys: Immunohistochemical analysis of the neurofilament protein triplet in the hippocampal formation. *Brain Research*, **619**, 299–305.

Spear, L. P. (2000a). The adolescent brain and age-related behavioral manifestations. *Neuroscience and Biobehavioral Reviews*, **24**, 417–463.

Spear, L. P. (2000b). Modeling adolescent development and alcohol use in animals. *Alcohol Research and Health*, **24**, 115–123.

Spinelli, S., Schwandt, M. L., Lindell, S. G., Newman, T. K., Heilig, M., Suomi, S. J., et al. (2007). Association between the recombinant human serotonin transporter linked promoter region polymorphism and behavior in rhesus macaques during a separation paradigm. *Developmental Psychopathology*, **19**, 977–987.

Suomi, S. J. (1987). Genetic and maternal contributions to individual differences in rhesus monkey biobehavioral development. In N. A. Krasnegor, E. M. Blass, M. A. Hofer, & W. Smotherman,(Eds.), *Perinatal development: A psychobiological perspective* (pp. 397–419) Orlando: Academic Press.

Suomi, S. J. (1997). Early determinants of behaviour: evidence from primate studies. *British Medical Bulletin*, **53**, 170–184.

Suomi, S. J. (2006). Risk, resilience, and gene x environment interactions in rhesus monkeys. *Annals of the New York Academy of Sciences*, **1094**, 52–62.

Suomi, S. J., DeLizio, R., & Harlow, H. F. (1976). Social rehabilitation of separation-induced depressive disorders in monkeys. *American Journal of Psychiatry*, **133**, 1279–1285.

Taylor, A., & Kim-Cohen, J. (2007). Meta-analysis of gene–environment interactions in developmental psychopathology. *Development and Psychopathology*, **19**, 1029–1037.

Teicher, M. H. Andersen, S. L. Polcari, A. Anderson, C. M. Navalta, C. P., & Kim, D. M. (2003). The neurobiological consequences of early stress and childhood maltreatment. *Neuroscience and Behavioral Reviews*, **27**, 33–44.

Tigges, J., Gordon, T. P., McClure, H. M., Hall, E. C., & Peters, A. (1988). Survival rate and life span of rhesus monkeys at the Yerkes Regional Primate Research Center. *American Journal of Primatology*, **15**, 263–273.

Trefilov, A., Berard, J., Krawczak, M., & Schmidtke, J. (2000). Natal dispersal in rhesus macaques is related to serotonin transporter gene promoter variation. *Behavior Genetics*, **30**, 295–301.

Uher, R., & McGuffin, P. (2008). The moderation by the serotonin transporter gene of environmental adversity in the aetiology of mental illness: Review and methodological analysis. *Molecular Psychiatry*, **13**, 131–146.

Van Tol, H. H., Wu, C. M., Guan, H. C., Ohara, K., Bunzow, J. R., Civelli, O. et al. (1992). Multiple dopamine D4 receptor variants in the human population. *Nature*, **358**, 149–152.

Van Wagenen, G., & Catchpole, H. R. (1954). Physical growth of the rhesus monkey (*Macaca mulatta*). *American Journal of Physical Anthropology*, **14**, 245–273.

Veenstra-VanderWeele, J., Anderson, G. M., & Cook E. H., Jr. (2000). Pharmacogenetics and the serotonin system: Initial studies and future directions. *European Journal of Pharmacology*, **410**, 165–181.

Vivian, J. A., Green, H. L., Young, J. E., Majerksy, L. S. , Thomas, B. W., Shively, C. A. et al. (2001). Induction and maintenance of ethanol self-administration in cynomolgus monkeys (Macaca fascicularis): long-term characterization of sex and individual differences. *Alcohol Clinical and Experimental Research*, **25**, 1087–1097.

Volkow, N. D., & Li, T. K. (2005). Drugs and alcohol: Treating and preventing abuse, addiction and their medical consequences. *Pharmacology and Therapeutics*, **108**, 3–17.

Wendland, J. R., Lesch, K. P., Newman, T. K., Timme, A. ,Gachot-Neveu, H., Thierry, B., et al. (2006). Differential functional variability of serotonin transporter and monoamine oxidase a genes in macaque species displaying contrasting levels of aggression-related behavior. *Behavior Genetics*, **36**, 163–172.

Werge, T., Elbaek, Z., Andersen, M. B., Lundbaek, J. A., & Rasmussen, H. B. (2003). *Cebus apella*, a nonhuman primate highly susceptible to neuroleptic side effects, carries the GLY9 dopamine receptor D3 associated with tardive dyskinesia in humans. *The Pharmacogenomics Journal*, **3**, 97–100.

Widom, C. S., Ireland, T., & Glynn, P. J. (1995). Alcohol abuse in abused and neglected children followed-up: Are they at increased risk? *Journal of Studies on Alcohol*, **56**, 207–217.

Wilson, M. E. (1989). Relationship between growth and puberty in the rhesus monkey. In H. A. Delemarre van de Wall, T. M. Plant, G. P. van Rees, & J. Shoemaker (Eds.), *Control of the onset of puberty III* (pp. 137–149) Amsterdam: Elsevier Science.

Wilson, M. E., & Kinkead, B. (2008). Gene–environment interactions, not neonatal growth hormone deficiency, time puberty in female rhesus monkeys. *Biology of Reproduction*, **78**, 736–743.

Winstanley, C. A., Dalley, J. W., Theobald, D. E., & Robbins, T. W. (2004). Fractionating impulsivity: Contrasting effects of central 5-HT depletion on different measures of impulsive behavior. *Neuropsychopharmacology*, **29**, 1331–1343.

Witt, E. D. (1994). Mechanisms of alcohol abuse and alcoholism in adolescents: A case for developing animal models. *Behavioral and Neural Biology*, **62**, 168–177.

Witt, E. D. (2007). Puberty, hormones, and sex differences in alcohol abuse and dependence. *Neurotoxicology and Teratology*, **29**, 81–95.

13

Interactive Contributions of Genes and Early Experience to Behavioral Development

Sensitive periods and lateralized brain and behavior

Lesley J. Rogers

Introduction

Over recent years studies examining the interaction between genetic and epigenetic factors during the development of behavior have been, to a large extent, pushed aside by the focus on molecular genetics and the accompanying rise of first socio-biology and then evolutionary psychology (Bateson, 2005; Kaplan & Rogers, 2003). Despite this, some research on behavioral development has continued and now it is increasingly apparent, to neuroscientists in particular, that understanding behavior and the influence of experience is essential to expanding knowledge of brain function and development. Gottlieb's concept of "probabilistic epigenesis" is relevant to this understanding (Gottlieb, 2000, 2007; Gottlieb & Lickliter, 2007). It refers to the multiple and reciprocal influences between levels (genetic, neural, behavioral, social, etc.) on an organism's development and hence their contribution to the expression of its phenotype. In other words, his concept replaces the central dogma of molecular biology (e.g., Crick, 1970, and discussed by Lewontin, 1991), which sees causation from genes through proteins to structure and behavior as a unidirectional pathway, by a concept of bidirectionality both within and between levels. Gottlieb's conceptualization of the processes involved in development and expression of phenotype involves continual dynamic interactions between genes and environment

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(Gottlieb, 1998, 2000, 2002). Other authors too have drawn attention to the complex interactions between genetic and environmental influences and stressed the importance of moving away from reductionist thinking, meaning that the causation of complex behavior should not be reduced to unitary genetic explanations (e.g., Oyama, 1985; Rogers, 1999a; Rose, 1997). Despite their popularity, unitary genetic explanations of complex behavior are fallacious not only because they ignore the complex, multiple interactions between genetic expression and experience but also because they are a static view of the organism (Rose, 1997). This applies to the behavior of all species, from lower organisms to humans.

Some of the most revealing evidence for such interactions, to be discussed later, has come from studying the development of precocial species. These are species in which the offspring are at a relatively advanced stage of development at birth or hatching and must pass through a series of rather rapid phases of learning to acquire behavior necessary for survival. Such species include ducks, geese, chickens, quails and, in the mammalian class, guinea pigs, goats, sheep, and most ungulates. Avian embryos and newly hatched precocial birds are excellent model systems for empirical studies of the interaction of genes, hormones, and experience, as well recognized by Gottlieb and his colleagues.

Studies of development in precocial animals have been crucial in illustrating that experience/learning in early life can have over-riding effects on behavior in later life and, speaking generally, the findings apply to all vertebrate species, including humans (Bateson & Martin, 1999). Certain types of learning occur more readily and have longer-lasting effects at a particular stage of maturation than they do at any other stage. These stages are known as sensitive periods. There are many different kinds of sensitive periods, of different durations, when different developmental or learning effects take place on, and one sensitive period may not be independent of the others. As Bateson and Martin (1999) have stated, each sensitive period represents a dynamic interplay between the individual organism's internal organization and the external conditions.

An earlier notion of critical periods as fixed in time, or stage, of development and entirely or largely under genetic control has now been replaced by the concept of sensitive periods that can vary as a result of prior experience and experience during the sensitive period itself (summarized by Michel & Tyler, 2005). Recognition of this malleability does not, however, mean that sensitive periods do not exist even though the longer term effects of experience during the sensitive period can, in some cases, be over-ridden by subsequent experience. I hope the discussion to follow will clarify this point and show the importance of specific experiences during different stages of development.

Sensitive Periods of Development in Precocial Birds

Precocial birds are excellent for studying experience-dependent development because, from the early stages of embryonic development to at least the second

week of post-hatching life, they pass through a series of precisely timed, brief and discrete phases of neural and behavioral development (Rogers, 1995; Vallortigara, Andrew, Sertori, & Regolin, 1997). Of particular interest here are the periods during which experience has specific, marked, and long-lasting influences. As Bateson (1979) pointed out, these sensitive periods are stages of development when the organism is open to specific experiences, as if poised for change, and they remain open until that experience has occurred. Should the animal be denied the experience the sensitive period may remain open for a while longer but then will close without the crucial developmental step being taken and with long-lasting consequences.

Three distinct sensitive periods when experience has profound and long-lasting influences on brain structure and behavior have been well described in the developing chick (*Gallus gallus*). The first occurs during the final stages of incubation, 2 to 3 days before hatching, when exposure of the embryo to light for a brief period triggers the development of asymmetry of the visual pathways and facilitates the development of lateralized visual behavior after hatching. The second is that of critical early learning, mainly imprinting, which takes place around days 2 to 3 post-hatching. The third, as more recently discovered (see later), occurs on days 10 and 11 post-hatching and concerns processing of spatial information.

These three periods have several features in common. They are all of short duration, have long-lasting effects on behavior, involve modifications of neural connections and involve separate roles of the left and right brain hemispheres. In fact, lateralization of processing may be a common thread linking the three stages, as I will elaborate later. Each period will be introduced separately first.

Pre-Hatching Sensitivity to Light Exposure

During the final days of incubation, when the embryo has grown so large that it fills almost all of the internal volume of the egg, the embryo's head is turned to the side so that its body occludes its left eye (Rogers, 1990). The right eye is positioned next to the membranes of the air sac and can be stimulated by light that enters the egg through the shell and membranes (Rogers, 1999b).

This turning of the embryo would, it seems, be a flow on from positioning of the embryo at a much earlier stage of development. In fact, the embryo already expresses a side bias to turn to its left side on day 2 of incubation and it continues to lie with its left side on the yolk sac throughout the period of development before the yolk sac is reduced in size and repositioning takes place, culminating in the head-turned position described above. The early rotation of the embryo, as well as the side on which the heart and other organs develop, involves the unilateral expression of genes, such as *Shh*, *lefty* and *nodal* (e.g. Levin, Johnson, Sten, Kuehn, & Tabin, 1995; Meyers & Martin, 1999; see also Deng & Rogers, 2002a). It is unknown whether these genes are also involved in the late-stage leftward turning of the embryo's head or whether early stimulation has a role in this lateralized

development. Regardless of this, these early expressed genes should not be seen as having any over-riding influence on the lateralized behavior that is expressed after hatching because experience interacts with any influence these genes may have. I stress this point since expression of genes associated with cortical asymmetry in the human fetus has often been interpreted as evidence of genetic determinism of lateralization (e.g., Sun et al., 2005), despite the fact that controlled studies of animal models show that this is an incorrect extrapolation of the findings.

The head-turned position of the chick embryo is adopted just at the time when the visual connections to regions of the pallium (forebrain) become functional (summarized in Rogers, 1995). The development of these visual connections is not solely determined by the genetic program. In fact, it is radically influenced by visual experience at that time. Light reaching the right eye stimulates the development of neural projections to the pallium that receive input from that eye. As Figure 13.1 illustrates, the right eye sends its first relay projections to the opposite side of the midbrain, the left optic tectum and the left lateral geniculate nucleus (LGN), and

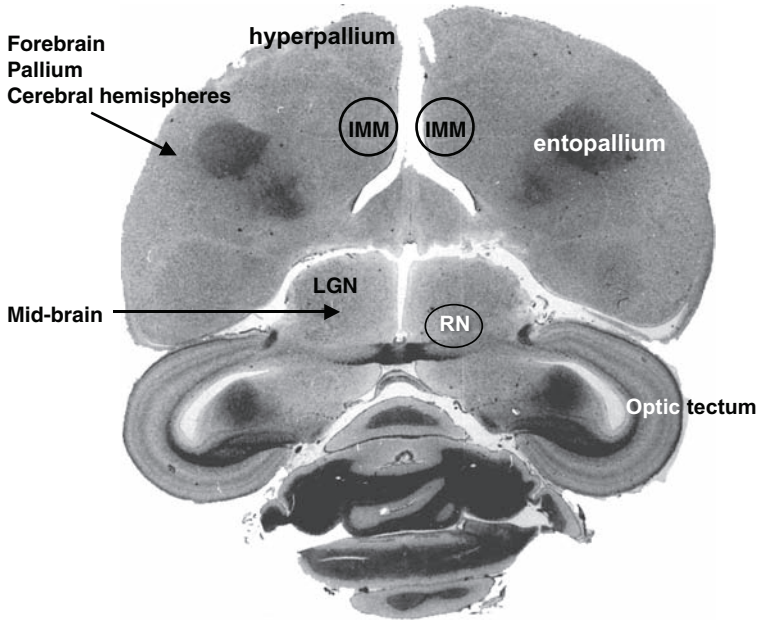

Figure 13.1. A horizontal section of the two-day-old chick brain showing the regions mentioned in the text. On the left side are labeled the regions of the visual pathway from the right eye to the lateral geniculate nucleus (LGN) to the hyperpallium in the forebrain. Connections from LGN on the left to the hyperpallial regions in both hemispheres are more numerous than their counterparts from the right LGN. On the right side, the regions of the other visual pathway are marked: this pathway goes from the eye to the optic tectum to the rotundal nucleus (RN) and then to the entopallium. The regions of the brain where imprinting memory is stored are marked as IMM, which stands for intermediate medial mesopallium.

from here second-order neurones project to the pallium, from the optic tectum to the entopallium and from the LGN to the hyperpallium. In the chick embryo it is the projections from LGN to the hyperpallial regions in both hemispheres that respond to the light stimulation. Compared to their equivalents receiving input from the left eye, and on the opposite side of the brain, the projections receiving inputs from the right eye are more numerous when examined on day 2 post-hatching (Rogers & Deng, 1999), provided that the embryo has been exposed to light for at least 2 hours at sometime during the last 2 to 3 days before hatching (Rogers, 1990). If the embryo fails to receive exposure to light during this sensitive period, no asymmetry develops in these visual projections (Rogers & Deng, 1999; Rogers & Sink, 1988).

The asymmetry of the visual pathways that develops following exposure to light persists throughout the first two weeks of the chick's life after hatching (Rogers & Sink, 1988) and appears to be instrumental in certain aspects of visual processing that are carried out differentially by the left and right hemispheres (Rogers, 1990). These lateralities are discussed further below. The effect of lateralized exposure to light is definitely confined to a sensitive period just before hatching because light exposure prior to day 17 or 18 has no effect on lateralization (Zappia & Rogers, 1983). The important point to mention here is that hatched chicks express different behavioral phenotypes depending on whether or not they have been exposed to light during the sensitive period prior to hatching. For example, light-exposed, lateralized chicks are able to perform two tasks, searching for food grains on the pebble floor and being vigilant for a model predator moving overhead, simultaneously, whereas chicks hatched from eggs incubated in the dark during the sensitive period have great difficulty in doing so (Rogers, Zucca, & Vallortigara, 2004). The lateralized chicks seem to be able to use one hemisphere (the left) to process the information needed to find the food grains and the other hemisphere (the right) to watch out for the predator. Chicks incubated in the dark perform poorly on both tasks, apparently due to confusion because, if they are tested on either of the tasks separately, they perform each task as well as the light-exposed chicks. Confusion is also indicated by the fact that the performance of the dark-incubated chicks on the dual task becomes increasingly impaired the longer they are asked to perform the two tasks simultaneously.

In fact, dark-incubated chicks, whether they are alone or in a group, are more distracted by the overhead predator than are light-exposed chicks (Dharmaretnam & Rogers, 2005; Wichman, Freire, & Rogers, 2009). Once they catch sight of the predator, dark-incubated chicks make more distress (fear) calls and observe it for longer than do light-exposed chicks (Dharmaretnam & Rogers, 2005). In fact, the effect of light exposure during the sensitive period on day 19 of incubation in reducing fear responses had been noted much earlier by Adam and Dimond (1971), although these researchers did not relate it to the development of lateralization, which had not then been discovered in chicks. They found that exposure of chick embryos on day 19 of incubation to light led to shorter approach times to an attractive visual stimulus, compared to chicks that had been exposed to light on either day 17 or 15 of incubation,

and less intense distress calling. Hence, the chicks exposed to light on day 19 of incubation express reduced fear compared to those not receiving light at this particular stage of development. All of these results are consistent with each other.

It seems as though the light-exposed chicks are able to suppress fear of novel objects, which may be predators, and can shift attention back to feeding, whereas dark-incubated chicks are not able to switch attention away from the predator once they have caught sight of it (Wichman, Freire, et al., 2009). In the natural habitat either one of these behavioral types may have an advantage depending on the level of predation, type of predators and the need to perform foraging and predator detection simultaneously.

A recent study by Chiandetti and Vallortigara (2009) has shown that dark-incubated chicks differ from light-exposed chicks in yet another way. Although dark-incubated chicks use landmarks as spatial cues as well as do light-exposed chicks, they show impaired use of cues that indicate left-right differences. For example, if the location of a food bowl in the corner of a cage is indicated by a blue wall on the chick's right side and a white wall on its left side, light-exposed chicks trained on this task will choose only this corner and not a corner with blue on the left and white on the right. Dark-incubated chicks do not distinguish between these two types of corner.

Light-exposed and dark-incubated chicks also attend differently to features of food objects and their location. Chiandetti, Regolin, Rogers, & Vallortigara (2005) trained chicks to peck at food grains inside small cones and to avoid empty cones. The loaded and empty cones were marked on their outer surface with different patterns, a grid or stripes. The food-loaded, striped cones were located on one side of a testing arena and the empty, grid-patterned cones on the opposite side. Then the chicks were tested with the now empty grid-patterned and striped cones located in reversed positions. The chicks could, therefore, choose to peck at cones using either object-specific cues (pattern) or position-specific cues (location). Monocular tests revealed that the chicks hatched from eggs incubated in the dark chose to approach and peck at the cones with the pattern that had been rewarded previously; that is, they attended to object-specific cues and ignored location. The light-exposed chicks made no clear choice, indicating that they attended to both object-specific and position-specific cues. Added to this, the light-exposed chicks tested monocularly could use the visual information processed in both hemispheres, whereas dark-incubated chicks used only the hemisphere receiving direct input from the non-occluded eye. This suggests that the light exposure before hatching might improve interhemispheric communication.

In these examples, development of the embryo and the behavior that the hatched chick expresses are due to an interaction between genetic determinants of the embryo's orientation and experience of non-patterned light stimulation. In the natural condition, such stimulation may be patterned in time, as the hen comes and goes from the nest, but not patterned in terms of features because light passing through the egg's shell and membranes lacks this property. Another series of experiments by Lickliter (1990) has shown that, in bobwhite quails (*Colinus*

virginianus), premature exposure of the late-stage embryo to patterned visual input, during the last 36 hours of incubation, affects behavior after hatching. By removing the shell and membranes from the air sac end of the egg just prior to the last days before hatching, the embryo was exposed prematurely to patterned light (visual stimuli) that was presented in pulses. This experience altered the chicks' responses to auditory and visual stimuli after hatching: newly hatched chicks that have not received this abnormal exposure to patterned visual input before hatching approach a sound source playing adult hen vocalizations without any requirement to see the visual stimulus of a hen, whereas chicks that have received the premature experience do not approach the sound source without being able to see a model of the hen at the same time. The relative balance of initial preferences is changed by the visual experience before hatching and this would alter the important early learning experience of imprinting on auditory and visual stimuli.

Hence, visual stimulation before hatching, which is lateralized as a consequence of the embryo's position, affects behavior and, in particular, learning after hatching. It even affects patterns of sleep: chicks exposed to light before hatching mainly open their right eye during monocular sleep, whereas this is reversed in chicks hatched from eggs incubated in darkness (Mascetti & Vallortigara, 2001). Such influences in early life are likely to have long-lasting effects on brain lateralization and behavior since any further development of brain and behavior may build on the initial asymmetries.

However, although there is some evidence that adult chickens show lateralized behavior (lateralized agonistic behavior; Rogers, 1991; MacKenzie, Andrew, & Jones, 1998), there has been no empirical investigation of the effects of light exposure before hatching on lateralization in adults. It is possible that the effects of light exposure before hatching are all transient, as in the case of the superior ability of chicks using the right eye to peck at grain and avoid pecking at small pebbles (Rogers, 1997a; Mench & Andrew, 1986) – this asymmetry is no longer present when the chicks are three weeks old (Rogers, 1991) – but light exposure before hatching is known to trigger the development of a left-eye / right hemisphere bias for agonistic responses (Rogers, 1982; Vallortigara, Cozzutti, Tommasi, & Rogers, 2001; Zappia & Rogers, 1983) and it seems that this effect persists into adulthood. Moreover, there are known asymmetries in the behavior of adult pigeons that depend on light exposure of the embryo, as discussed below.

Sensitive Period for Imprinting

The sensitive period during which a newly hatched, precocial bird forms an attachment to a conspicuous stimulus is referred to as the period of filial imprinting (reviewed by Bolhuis, 1991, and Bateson, 2000). It is a phase during which the young bird learns the features of its social environment. It forms an attachment to its mother and siblings and will follow them when they move. A later stage of

imprinting involves the establishment of a sexual preference under the guidance of the previously acquired filial imprinting (Bischof, 2003) but it will not be discussed here since it falls outside the first two weeks of post-hatching life.

As Gottlieb and Klopfer (1962) showed in the duck, filial imprinting on an auditory stimulus precedes that for a visual stimulus and may commence before hatching, although imprinting after hatching is facilitated by presenting combined auditory (maternal calls) and visual stimuli (Gottlieb, 1971). Imprinting on a visual stimulus usually begins about 12 hours after hatching (Ramsay & Hess, 1954) and extends into day 2 or 3. During at least the first day after hatching chicks raised by the hen spend most of their time sleeping and under the cover of the hen's body but, by day 2, they venture out often enough to view her head and imprint on her features (Bateson, 1987; Workman & Andrew, 1989; see summary in Rogers, 1995), especially those of her head and neck, which the chicks find particularly attractive (Horn, 1985).

Once an imprinting memory has formed, which requires about one hour of exposure to a conspicuous stimulus (summarized by Rogers, 1995), the sensitive period closes (Bateson, 1979, 1987). In other words, the ending of the sensitive period is experience-dependent and not merely due to the passing of a pre-programmed physiological state, as originally suggested by Lorenz (1935) and adhered to by Hess (1973).

If no conspicuous stimulus is available (e.g., if the chicks are reared in darkness), the sensitive period remains open for longer, thus allowing for a potential late appearance of the appropriate stimulus, as first shown by Sluckin (1962, 1966). Such an extension of the imprinting period is limited, however, since chicks kept in the dark for the first days after hatching will imprint up to about day 4 or 5 of life provided that they are then taken into the light and exposed to an imprinting stimulus but they will not imprint if held in the dark for longer (Parsons, 1994), unless they are treated with pharmacological agents that modulate the number or sensitivity of glutamate receptors in the brain (McCabe, Davey, & Horn, 1992; Parsons & Rogers, 1997, 2000). For example, chicks that have been treated with ketamine or MK-801, both drugs which act on glutamate receptors, within about 10 hours after hatching will imprint on day 8, whereas controls show no ability to imprint at this age (Parsons & Rogers, 2000).

Extension of the imprinting period by preventing exposure to conspicuous stimuli can also be achieved by fitting the young birds with translucent goggles. Using this technique, Moltz and Stettner (1961) were able to extend the imprinting period of ducklings to 48 hours after hatching, compared to the ending of the imprinting period at 24 hours in ducklings with no obstruction of their vision.

As Lickliter and Gottlieb (1986) showed so convincingly, ducklings imprint not only on the parent bird but also on their siblings, and the imprinting memory encodes not just visual cues but also auditory, olfactory, and tactile cues (Dyer, Lickliter, & Gottlieb, 1989; Lickliter, Dyer, & McBride, 1993). The young bird even remembers individual siblings, as shown by the fact that chicks raised with a cage mate will choose to approach that chick in preference to an unfamiliar chick

(Vallortigara & Andrew, 1991, 1994a). Furthermore, the memory of individuals is more specific if the chick has had more experience with different chicks of the same age (normally siblings), as shown by the fact that choice of a familiar over an unfamiliar chick is stronger if the test chick has had experience with a number of other chicks in the first 10 or so hours after hatching than it is if it has had experience with only one other chick (Deng & Rogers, 2002b). Possibly the experience of a greater number of chicks enhances attention to details and so improves the chick's ability to discriminate between a familiar and an unfamiliar chick.

Social experience with siblings in early life is clearly important to precocial species in a number of ways. As another example, Gottlieb (1993) showed that ducklings reared with siblings expressed higher levels of arousal than those reared in isolation and would imprint on a chicken's call, whereas those reared in isolation would not do so. He saw this as increased malleability as a consequence of social experience. It certainly demonstrates that prior experience can affect imprinting.

Imprinting memory is encoded in different locations in each hemisphere. Initially, up to some 3 hours after imprinting, it is encoded in the intermediate, medial mesopallial (IMM) regions of both hemispheres, as shown by the effects of placing a lesion in either one of these regions and by structural changes of the synapses in these regions (increased synaptic apposition length) that correlate with imprinting, as well as by localized enhancement of mRNA and protein synthesis in these regions (Horn & McCabe, 1984; summarized by Horn, 1985). Lateralization then emerges since the IMM in the right hemisphere is not involved in storage of the long-term memory of the imprinting stimulus, whereas the memory is still stored the IMM of the left hemisphere (Cipolla-Neto, Horn, & McCabe, 1982; Figure 13.1).

Recall of imprinting memory is also lateralized, as shown by Johnston and Rogers (1998). In chicks that had had the normal exposure of the right eye to light during the final stages of incubation, recall of imprinting memory two days after hatching was possible provided the left hemisphere was used but the right was not required: glutamate treatment of the left hemisphere prevented the memory recall but the same treatment of the right hemisphere had no effect. The reverse was found for chicks that had had the right eye occluded before hatching and the left eye, abnormally, exposed to light. This shows that exposure to light before hatching affects the lateralized recall of imprinting memory. In other words, the lateralization that develops as a result of light exposure of the embryo, affects the differential roles of the hemispheres in processing of imprinting memory. This could be a simple priming effect of light, since it has been known for many years that chicks imprint more readily if they have been exposed to light for a brief period (about 20 min.) after hatching and before the imprinting experience (Bateson & Seaburne-May, 1973).

Regardless of the mechanism involved, it is clear that the unihemispheric stimulation by light that results from the positioning of the late-stage embryo in the egg affects the processes involved in imprinting. The two sensitive periods are

linked. In fact, light stimulation of the embryo changes the lateralized levels of glutamate receptor binding (Johnston, Bourne, Stewart, Rogers, & Rose, 1997) and the levels of these receptors in IMM are important for imprinting memory formation (Johnston, Rogers, & Dodd, 1995; Johnston, Rogers, & Johnston, 1993; Parsons & Rogers, 2000).

Sensitive Period for Learning About Disappearance of Attachment Figures and Spatial Relationships

One of the crucial experiences of early life in most species is the temporary disappearance of an attachment figure. Since young birds that have imprinted will follow the imprinting stimulus and work to be able to see it (Bateson, 1987; Horn, 1985), we can conclude that absence of the imprinting stimulus is stressful. This is confirmed by the fact that chicks make distress (peep) calls in such circumstances and not when contact with the imprinting stimulus is re-established (Boakes & Panter, 1985), when instead they make pleasure (twitter) calls. At some stage of early life young chicks, and other precocial birds, have to learn that the disappearance of the hen behind a barrier does not mean that she no longer exists. Hence, the chick must be able to form a concept of where an imprinting stimulus is even when it is out-of-sight. In fact, even 3-day-old chicks are able to remember where the imprinting object has disappeared, for up to a few minutes at least, as shown by moving the imprinting stimulus behind one of two screens, each with a different pattern, and preventing the chick from approaching or following it until a period of delay during which it needs to remember the screen behind which the stimulus disappeared (Vallortigara, Regolin, Rigoni, & Zanforlin, 1998). Once released into the arena, the chick approaches the screen behind which the imprinting object is concealed. Chicks can also recognize an imprinting object when it is partially occluded by a barrier (Regolin & Vallortigara, 1995; Vallortigara & Regolin, 2002), meaning that they can perform amodal completion (summarized in Rogers, 1997b).

During the first 10 days of post-hatching life, separation of chicks from the hen and siblings occurs very rarely but, as Workman and Andrew (1989) and Vallortigara et al. (1997) showed, around day 11 individual chicks begin to move independently of the clutch and go out of sight from time to time. This emerging behavior coincides with a sensitive period when such going out of sight improves spatial memory. Freire, Cheng, and Nicol (2004) showed this by giving chicks the opportunity to move behind one or two opaque barriers placed in their home cage (2 chicks per cage) from day 8 to day 12 of life. The chicks had been imprinted on a tennis ball suspended in the middle of the cage and the ball could not be seen when the chick moved behind a barrier. The amount of time that the chicks spent out of sight of the imprinting stimulus and of each other was highest on day 11, compared to days 10 and 12. When tested on day 13, those chicks that had spent

more time "out of sight" made fewer orientation errors in a detour test, in which they had to go around an obstacle in order to approach the imprinting stimulus.

Further investigation of the effects of going out of sight of the imprinting stimulus compared chicks given opaque screens in the cage from days 10 to 12 with chicks given transparent screens (Freire & Rogers, 2005). Subsequent testing on a spatial task, in which they were able to find the imprinting stimulus using either proximal or distal visual cues, showed that chicks that had received the out-of-sight experience (opaque barriers) were significantly more likely to use distal visual cues (extraneous to the testing apparatus) in order to orient than were the chicks with no out-of-sight experience (transparent barriers).

Possibly it is not the experience of being out of sight of the imprinting stimulus that is in itself the main aspect of the effect but, as Gottlieb and Lickliter noted in discussion of mother-infant separation in humans (Gottlieb & Lickliter, 2004), the searching for and restoration of contact with the imprinting stimulus. The searching, in particular, might enhance attention to distal, spatial cues. Furthermore, it seems that the chicks do not always seek to go out of sight of the imprinting stimulus since they move around transparent barriers more than they do around opaque barriers (Wichman, Rogers, & Freire, 2009). At this age, therefore, chicks seem to be motivated by two competing systems: one to stay close to the imprinting stimulus and the other to explore novel stimuli, including hidden areas of their environment. It might be their heightened interest in novel objects and other novel stimuli that takes them out-of-sight of their attachment/imprinting stimulus and subsequently leads to active searching to find it, using spatial cues to do so.

Attention to novel stimuli and also to distal visual cues is a specialization of the right hemisphere (expressed when chicks are tested monocularly using the left eye), whereas the left hemisphere attends to proximal, landmark cues, as shown by Regolin, Garzotti, Rugani, Pagni, and Vallortigara (2005) and Tommasi and Vallortigara (2004). The out-of-sight experience enhances the specialization of the right hemisphere (Freire & Rogers, 2005) and this is important because the right hemisphere is in control of the chick's behavior on days 10 and 11 (Rogers, 1991, 1995; also discussed further below). Here it is important to point out that effective out-of-sight experience is limited to a sensitive period: as little as one day's experience of the opaque barriers on day 11 is sufficient to enhance the chick's attention to distal spatial cues, whereas the same experience on day 8 is ineffective (Freire & Rogers, 2007).

Hence, during the sensitive period of development that occurs around day 11 post-hatching, chicks move out-of-sight of the hen and each other and this experience opens their attention to distal spatial cues. We know that the effect of this experience on attention to distal cues lasts up to day 15 post-hatching but chicks older than this have not yet been tested. Furthermore, this sensitive period on day 11 for experience that affects attention to spatial cues has much in common with the imprinting period: it is of short duration, its effects are likely to be crucial to survival in the natural environment and it, apparently, has a long lasting effect on

subsequent behavior. In my opinion, it warrants the focus that has been given previously to the imprinting period to discover the nature of the effective perceptual experience; for example, whether it can be postponed by the absence of distal, spatial cues and so on.

We have yet to discover its neural correlates, although there is evidence that the right hippocampus of the chick has longer dendrites and more synapses per neurone than the left hippocampus (Freire & Cheng, 2004) and this is suggestive given the known role of the right hippocampus in spatial behavior of the chick (Tommasi, Gagliardo, Andrew, & Vallortigara, 2003). The findings in chicks are likely to have relevance to other species since, for example, this sort of egocentric ability to orientate is associated with the hippocampus in rats (Colombo & Broadbent, 2000) and other species.

Hemispheric Specializations

Before discussing the sensitive periods of development further and attempting to explain how they may be linked by a common feature of hemispheric lateralization, it is important to summarize the known functions that are lateralized in the chick brain. To do so I will assume that the "normal" condition is that of chicks hatched from eggs that have been exposed to light. Since the hen leaves the nest from time to time to feed and since as little as 2 hours of light exposure is optimum, this assumption seems to be reasonable (discussed further below).

Differential functions of the left and right hemispheres of the chick were first discovered by injecting cycloheximide into the left or right hemisphere on day 2 post-hatching, a treatment which impairs subsequent function of the injected hemisphere and apparently forces the chick to use the other, untreated hemisphere. Chicks treated in the left hemisphere and tested later on a range of tasks performed differently than those treated in the right hemisphere (Rogers & Anson, 1979). Later it became apparent that the hemispheric specializations could be revealed simply by testing the birds with a patch over one or the other eye (Mench & Andrew, 1986; Rogers, 1997a). For example, a chick tested with a patch over its left eye is primarily using its left hemisphere because inputs from the right eye are processed primarily, although not exclusively, by the left hemisphere, and vice versa. The results obtained by these two methods are consistent. For example, chicks treated with cycloheximide in the left hemisphere are unable to categorize food grains as distinct from small pebbles resembling them in size and range of colours, whereas treatment of the right hemisphere does not impair this ability (Rogers, 1982; Rogers & Anson, 1979). This suggests that this is a function of the left hemisphere. Consistent with this, chicks tested monocularly using their right eye are able to categorize grain from pebbles (i.e., using their left hemisphere), whereas those using their left eye (and right hemisphere) are unable to do so (Rogers, 1997a).

Instead of directing pecks at targets that could be categorized as grain as opposed to pebbles, chicks using the right hemisphere attended to individual grains and pebbles and directed pecks to each target as a single entity. In other words, information processed by the left hemisphere is ordered into categories, whereas that processed by the right hemisphere considers the details of each of the stimuli.

Other evidence that the left hemisphere processes stimuli in terms of categories and the right hemisphere attends to details has come from testing the ability of young chicks to choose between a familiar cage-mate and an unfamiliar chick (Vallortigara & Andrew, 1994a). In the task, mentioned earlier, the unfamiliar chick and the cage-mate were placed in compartments at each end of a runway and separated from it by a transparent panel. The test chick, with a patch covering one or the other eye, was placed in the centre of the runway and its choice to approach the stranger versus cage-mate was measured. Chicks tested using the right eye (left hemisphere) chose to approach and stay beside their cage-mate. Chicks tested using their left eye (right hemisphere) displayed no preference for the cage-mate or stranger: they simply approached any chick without making a category decision on the basis of familiar versus unfamiliar.

It would be incorrect to conclude that the right hemisphere is completely unable to attend to categories. Indeed, complete inability to categorize information would be an ineffective strategy. Instead, the hemispheric difference is a matter of degree, one in which the left hemisphere organizes stimuli according to rather tightly specified categories and the right hemisphere uses broad categories that can be generalized readily. In the pebble-grain test, the right hemisphere clearly attends to small objects (grain and pebbles) as opposed to other stimuli, which, of course, indicates some degree of categorization of information. Also, in the test of stranger versus cage-mate, it attends to chicks in general, presumably as opposed to other birds or other stimuli.

The right hemisphere is also vigilant for novel stimuli, including those that might be predators, and is used preferentially when such stimuli are being examined. For example, chicks interrupt pecking at grains to attend to a model of a hawk moving overhead more readily if the hawk approaches on their left side than on their right (Rogers, 2000) and they use the left eye to view the predator even if they have to turn their head to do so after initially catching sight of it with the right eye (Dharmaretnam & Rogers, 2005). Moreover, on hearing their species-typical alarm call signaling the presence of an aerial predator, chickens look up using the left eye (and right hemisphere) (Evans, Evans, & Marler, 1993). Use of the right hemisphere also means that the chick is more readily distracted by auditory stimuli and less able to habituate to repeated presentations of the same sound: sound presentations, for example, repeatedly inhibit pecking and the chick freezes in alert attention (Rogers & Anson, 1979). These observations show that the right hemisphere shifts the chick's attention from performance of one task to attend to a distracting stimulus that could be a predator. It monitors for threat and is able to make marked shifts from one behavior to another.

Additional evidence shows that attention to novel stimuli is a fundamental aspect of the right hemisphere's specialization. Birds usually turn their head to view novel stimuli with the left eye but, even when they cannot turn the head (i.e., in an experimental situation), they still focus the image on the left retina. Recent evidence from detailed measurements of eye movements of the zebra finch shows that, when identical novel stimuli are presented simultaneously in the left and right monocular fields, the bird turns its eyes in a conjugated movement so that the stimulus in the left field is viewed in detail on the most cone-rich area of the retina, whereas the stimulus on the right side is moved to a part of the retina where it might be monitored but cannot be seen in any detail (Voss & Bischof, 2009).

The left hemisphere is the hemisphere that switches the chick's attention from one category to another related category (e.g., from pecking at grains of one type to grains of another type) while the chick continues the same, on-going behavior. Attention switching of this type, or its converse, which has been called "attentional persistence," has been tested by raising chicks to prefer red-dyed grains of chick mash and then testing them with a choice of red grains and undyed (yellow) grains of mash (Rogers, 1986). Untreated chicks and control chicks treated intracranially with physiological saline peck at both red and yellow grains in short runs: they readily switch attention between the two categories (Andrew & Rogers, 1972). Note that they do not peck at random (i.e., without attention to color cues). Forced use of the left hemisphere only, caused by treatment of the right hemisphere with cycloheximide, has no effect on attention switching between red and yellow grains but forced use of the right hemisphere, caused by cycloheximide treatment of the left hemisphere, prevents the attention switching (Rogers, 1986; Rogers & Anson, 1978). The chicks using their right hemisphere peck in very long runs at red food and, if they do on occasion shift to yellow food, they peck in a long run on that too.

To consider these differences between the hemispheres further, it seems that the left hemisphere attends to categories that have, usually, been established by learning, whereas the right hemisphere operates using responses that are less specific but are essential in emergency situations. It could be said that the right hemisphere responds to releasers, as Andrew, Dharmaretnam et al. (2009) have pointed out, and hence triggers response to both predators and conspecifics. The right hemisphere's preferential role in controlling attack and copulation responses, also shown in chicks (Rogers, 1982; Rogers, Zappia, & Bullock, 1985), is another example of this specialization for response to releasers and use in emergency situations.

As a general summary of the differences between the hemispheres MacNeilage et al. (2009) have concluded that the left hemisphere is specialised to carry out routine, well-established patterns of behaviour, which might be best described as quotidian, and the right hemisphere is specialized to respond to novel stimuli or unexpected stimuli and to take charge of emergency behaviour. The left hemisphere is not easily distracted from the task it is performing, whereas the right hemisphere is easily distracted.

This division of function between the hemispheres is consistent with that shown for a wide range of vertebrate species, including humans (Andrew and Rogers, 2002; MacNeilage, Rogers, & Vallortigara, 2009) and it applies to all modalities, although

vision and audition have been the most studied so far. The basic differences between the hemispheres seem to have evolved as early as the first vertebrates (Andrew, 2002) and to have been elaborated on throughout vertebrate evolution. To give another example to illustrate this point, left hemisphere specialization to process or produce species-typical, non-stressed vocalizations has been found in frogs (Bauer, 1993), songbirds (Nottebohm, 1977), mice (Ehret, 1987), dogs (Siniscalchi, Quaranta, & Rogers, 2008), primates (Hauser & Andersson, 1994; Hook-Costigan & Rogers, 1998) and, of course, humans. This left hemispheric specialization fits with its role in routine functions (for further discussion see MacNeilage et al., 2009).

The right hemisphere is specialized to process and express intense and negative emotions (Andrew & Rogers, 2002; Rogers, 2002). For example, it is used when vocalizations elicit fear reactions (Siniscalchi et al., 2008) and to express fear and threat (Hauser, 1993; Hook-Costigan & Rogers, 1998). It is also known that, in a number of vertebrate species, the right hemisphere is specialized to process global spatial cues, which include geometric and distal cues (reviewed by Vallortigara, Pagni, & Sovrano, 2004).

Sequential Changes in Hemispheric Dominance

Over the first 12 days of the chick's post-hatching life, one or the other hemisphere assumes prominence in the control of bird's behavior. The first evidence of this was obtained by injecting cycloheximide into the left or right hemisphere at different ages after hatching and then testing the chicks on day 14 (Rogers, 1991, 1995; Rogers & Ehrlich, 1983). The test used was categorization of grain as distinct from pebbles. As mentioned above, treatment of the left hemisphere on day 2 after hatching impairs performance of this task: the chicks are forced to use the right hemisphere and so do not categorize grain as differing from pebbles. Since cycloheximide treatment of the left but not the right hemisphere on day 2 disrupts the development of normal behavior, apparently some critical developmental process, involving protein synthesis or a change in amino acid pools (Hambley & Rogers, 1979), is proceeding in the left hemisphere at this age. The same result is found if the treatment is given on any day after day 2 up until day 5 or 6 (Rogers & Ehrlich, 1983; Figure 13.2).

Prior to this period of left hemisphere vulnerability (i.e. on day 1), cycloheximide has no effect on either hemisphere and the same is true on days 7 and 9. Day 8 is another day when the left hemisphere is susceptible and represents a very brief period of left hemisphere dominance. On days 10 and 11 the right hemisphere becomes vulnerable for the first time. Precisely timed phases of development are manifested in all of these results (Figure 13.2). Here I would like to note the three phases of unihemispheric vulnerability and point out that these are phases when one hemisphere or the other takes dominant control of the chick's behavior. It seems they are periods when specific learning occurs in one or the other hemisphere.

The first phase concerns the left hemisphere and it covers day 2 to day 5 or 6. Since chicks have been tested on day 5, when treatment of the left hemisphere only

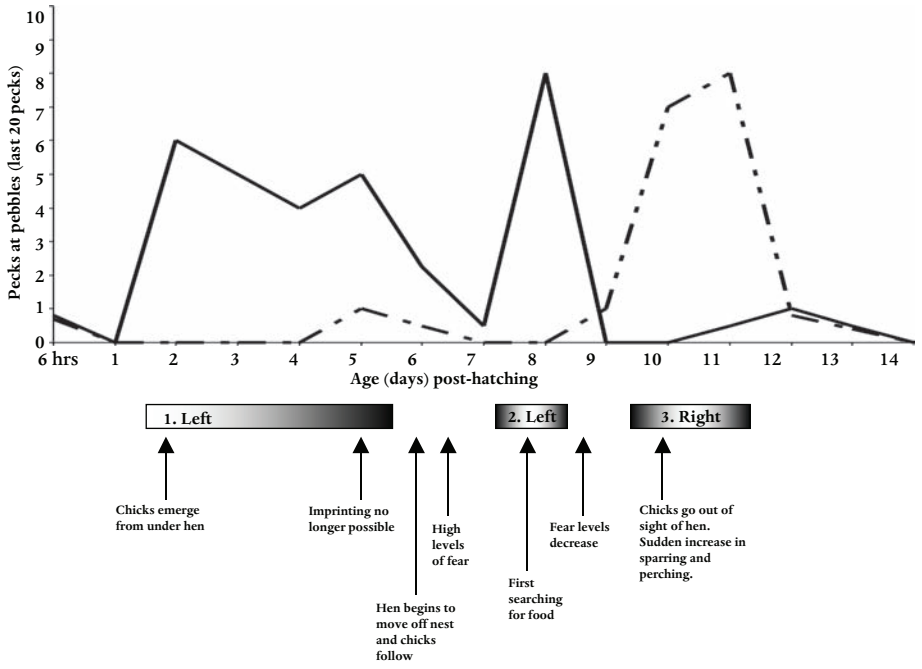

Figure 13.2. An illustration of the periods of hemispheric dominance and key steps in the development of the domestic chick in the first two weeks post-hatching. The effect of treating the left hemisphere (continuous line) or the right hemisphere (dashed line) with cycloheximide on performance tested on day 14 is plotted to show which hemisphere is susceptible at the different ages. The behavioral measure of performance is the number of pecks at pebbles (errors) in the last 20 pecks of a task in which the chick is allowed a total of 60 pecks to find grains and avoid pecking at pebbles stuck down to the floor. Higher scores show impairment of this ability caused by injecting one or the other hemisphere with cycloheximide (data from Rogers, 1991). The higher the score on the Y-axis the more impaired by the cycloheximide-treatment is the chick's performance. Three periods discussed in the text are marked in the boxes showing which hemisphere is dominant in each period. Developmental stages marked come from Workman and Andrew (1989).

is effective, but not on day 6, it is not possible to say precisely whether this sensitive period extends into day 5 or 6. Its onset on day 2 coincides with the age at which chicks begin to emerge from under the hen and display filial imprinting, following the hen when she moves away from the nest. It seems that the left hemisphere is in control of behavior during this period.

Up to day 5 or 6, chicks are learning what to eat under instruction by the hen, as seen by the fact that she performs titbitting, pecking at food grains with the beak closed (Workman & Andrew, 1989), and we know that this is under left hemisphere control (Rogers, 1986; 1997a).

Since the left hemisphere categorizes stimuli and it is also the hemisphere involved in decisions about approaching stimuli, such as attractive objects or the hen (Andrew & Rogers, 2002, and shown recently in Australian magpies by Koboroff, Kaplan, & Rogers, 2008), it is not surprising that it is the hemisphere in charge during the period when imprinting occurs.

The end of this phase of left-hemisphere dominance (day 5 or 6) also coincides, or almost does so (precise data are not available), with the closure of the period during which it is possible to imprint chicks that have been reared in darkness (see above). Starting on day 5 and particularly on day 6, the hen begins to show more locomotion than before (Workman & Andrew, 1989), possibly initiating changes in the chicks' behavior, since they now follow her to wherever she moves. Fear levels of unfamiliar stimuli increase at this age (summarized in Rogers, 1995).

A second and brief phase of susceptibility of the left hemisphere to cycloheximide occurs on day 8. This day is notable as the first day on which chicks begin to search actively for food independently of the hen (Workman & Andrew, 1989) and this means they make use of the left hemisphere's ability to categorize grain types. It is a day of clear right eye preference and of left hemispheric control of behavior (Andrew, 1988; Andrew & Dharmaretnam, 1991) and a day on which chicks look fixedly at each other using the right eye (Workman & Andrew, 1989). This one-day period ends sharply when on day 9 neither hemisphere is affected by cycloheximide treatment and chicks now show deceased levels of fear (Andrew & Brennan, 1983).

Days 10 and 11 make up the third phase of hemispheric dominance and at this time the right hemisphere (left eye) assumes control of behavior. On day 10 the chick begins to explore the environment more than before (Andrew, 1988). As Workman and Andrew (1989) found by observing hens raising chicks in the farm yard environment, the chicks start to run ahead of the hen on this day and often move out of her sight. This behavior remains high on day 11 too (Workman & Andrew, 1989). This period also coincides with, on day 11, a switch to left eye preference for viewing the hen (Andrew & Dharmaretnam, 1991) and for viewing humans, as well as increased sparring and frolicking (Workman & Andrew, 1989). As discussed earlier, during this phase of right hemisphere control the chick pays attention to global spatial cues and undergoes a special period of learning about spatial relationships using distal cues.

The discrete phases in development during which the behavior of chicks changes, sometimes from one day to the next, appear to depend on control by one hemisphere or another, with intervening times (e.g. on days 7 and 9; Rogers, 1991) when neither hemisphere takes dominant control and other behavior is expressed. Although, not fully investigated so far, there are sex differences in the time-course of these phases of development. The timings of the phases that I have discussed so far have been delineated using male chicks, as is the case for most of the research on chicks, but we do know that females follow a somewhat different pattern of unihemispheric susceptibility to cycloheximide treatment. As in males, cycloheximide treatment of the left hemisphere of the female on day 2 disrupts the

chick's ability to categorize grain from pebbles but this period extends only to day 4 (Rogers, 1991). The second period of susceptibility to cycloheximide that occurs in males on day 8 is absent in females and so is the right hemisphere susceptibility on days 10 and 11 (Rogers, 1991). Day 12 in the female is a unique stage when both hemispheres are mildly susceptible to treatment with cycloheximide. An absence of right hemispheric dominance on days 10 and 11 in females is also indicated by the fact that they spend less time out of sight of the hen than do males (Workman & Andrew, 1989) and by the fact that females using their left eye are less interested in changes in the spatial position of objects (Andrew & Brennan, 1984). Consistent lesser attention to distal spatial cues by females compared to males might well be explained by an absence of right hemisphere dominance during the critical stage of development on days 10 and 11.

Relevance to Other Species

The chick has been discussed as a model system for studying the interaction between genetic determinants and experience and, before leaving this topic, it is relevant to ask whether the model generalizes to other species. The widespread similarity across vertebrate species of the functions specialized to be carried out by the left and right hemispheres has been covered in detail previously (Rogers, 2002; Rogers & Andrew, 2002) and is not of primary interest *per se* here. Instead, the developmental stages and the influence of experience on the development of lateralization will be considered.

Light Exposure and the Development of Lateralization

Light exposure also influences the development of asymmetry of the visual projections of the pigeon, an altricial species: following exposure of the embryo to light, asymmetry develops in the cell sizes of particular neurones of the optic tectum (Güntürkün, 1993) and in the visual pathway from the optic tecta to rotundal nuclei (RN) (Güntürkün, 2002), in comparison to asymmetry in the pathway from the lateral geniculate nuclei to the hyperpallial regions of the forebrain in the chick (Figure 13.1). The light-dependent asymmetries that develop in the pigeon are manifested in behavioral asymmetries that can be revealed by testing the birds monocularly (Güntürkün, 2002), as is the case in the chick. These findings suggest that the pre-hatching exposure to light might influence the development of visual lateralization in the majority of avian species, apart from those that incubate their eggs in burrows, tree trunks, or mounds of earth.

The interesting question now is whether light stimulation plays a critical role in the development of lateralization in non-avian species. In fact, recent evidence shows that it does so in zebrafish (Andrew, Osorio, & Budaev, 2009). Zebrafish are

lateralized for functions very similar to those of the chick (Andrew, Dharmaretnam, et al., 2009) and light experience during embryonic development is essential for some of these asymmetries to develop. If the zebrafish have been exposed to light throughout embryonic development, the fry show a left eye preference for viewing conspecifics and stimuli that might offer them a refuge from predators, whereas this lateralization is not seen in fry that have developed in the dark (Andrew, Osorio, et al., 2009). In fact, light versus dark raised zebrafish fry express different behavioral phenotypes: fry that have been exposed to light during development exhibit elevated boldness compared to those that have developed in darkness (Budaev & Andrew, 2009), just as found in the chick (Adam & Dimond, 1971).

It is likely that the mechanisms by which light exposure affects lateralization in fish and birds differ. In birds the lateralized functions that develop as a result of light exposure of the embryo are controlled by visual regions of the forebrain and depend on asymmetry in the visual pathway. In fact, examination of my earlier results obtained for monocular testing of copulation responses of young chicks exposed to light before hatching has shown that 20 chicks out of 21 tested were asymmetrical on this score (copulation scores higher when the left eye was in use than when the right eye was in use: mean $\pm$ SEM, 14.57 ± 1.47 with left eye in use and 4.05 ± 1.40 with right eye in use). The remaining chick exhibited a high score for copulation when tested with either eye in use. This strength of asymmetry of behavior is consistent with the known rarity of chick embryos that orient in the reverse direction so that their left eye, rather than the usual right, is exposed to light (according to Kovach (1968), over 99% of chick embryos orient so that their right eye is exposed to light). In other words, the behavioral asymmetry appears to have a one-to-one relationship with orientation of the embryo and exposure to light. However, it must be said that the strength of the directional asymmetry in a group/population depends on the power of the behavioral task used to measure it, as shown by the fact that lateralization of attack responses in the chick are not as strongly directionally biased as those of copulation. The same 21 chicks tested for lateralization of copulation were also tested for lateralization of attack and in this case only 13 showed elevated attack scores, 8 chicks with higher attack scores when using the left eye than when using the right eye and 5 the other way around.

Further support for the direct role of the asymmetry in the visual pathway with behavioral lateralization in the chick has come by treating localized regions of the chick forebrain with glutamate and testing lateralized visual performance (Deng & Rogers, 1997). The left hyperpallium, the recipient region for the visual projections from the LGN, was found to be the site essential for categorizing grain from pebbles and for copulation and, somewhat less specifically, attack responses. Hence, the evidence shows clearly that light affects lateralization in birds via the visual projections to the forebrain.

Andrew, Dharmaretnam, et al. (2009) suggest that, in fish, the effect of light on the development of visual asymmetry may be located in the diencephalon, where it depends on the light-sensitive pineal gland and habenular nuclei. The lateral

habenular nucleus is larger on the left side of the zebrafish's brain and the medial habenular on the right side (Aizawa et al., 2005; Halpern, Guntürkun, Hopkins, & Rogers, 2005), and similar asymmetries are common in other lower vertebrates (Braitenberg & Kemali, 1970) and birds (Concha & Wilson, 2001). Since the eggs of zebrafish and many other piscine species are transparent, it is improbable that light exposure would influence the development of lateralization via unequal stimulation of the eyes, as it does in birds. However, since the habenular nuclei are asymmetrical in structure, light stimulation during embryonic development could enhance asymmetries already present (Andrew, Dharmaretnam et al., 2009).

It remains an open question whether light stimulation affects the development of any asymmetries in the mammalian brain but it is not beyond possibility since light can penetrate the body wall to some extent and we know that the human fetus, at least, is positioned asymmetrically *in utero* during the final stages of gestation. Influence of other forms of stimulation on the development of lateralization should also be considered. Much research on rats has shown the importance of "handling" (separation of the pups from their mother and each other for a brief period each day) in neonatal life for the development of certain lateralized patterns of behavior, such as lateralized control of activity (Denenberg, 1984, 2005), and this is associated with development of the corpus callosum (summarized by Cowell & Denenberg, 2002). Experiential effects on the development of lateralization in humans are possible. Indeed, Previc (1991) proposed that asymmetrical stimulation of the fetal vestibular system, resulting from the lateralized positioning of the fetus *in utero* just prior to birth and the forward walking of the mother, could possibly influence the development of cerebral lateralization but this idea has not been tested in humans or any other species. Along similar lines, Michel (1981) indicated that the position of the fetus in the uterus, head orientation after birth and hand preference are all associated (see also, Michel & Goodwin, 1979). In humans these measures are all related to hemispheric specialization but, in apes and other primates, hand preference is independent of hemispheric specialization, except for difficult tasks (Rogers, 2009). Of course, different types of stimulation may influence the development of lateralization in different species but this topic awaits much further research.

Imprinting

Imprinting also occurs in fish, as known in the spectacular case of olfactory/taste imprinting of salmon on their natal stream (Hasler & Schols, 1988; Nevitt & Dittman, 1999) and also shown recently in the zebrafish (Gerlach, Hodgins-Davis, Avolio, & Schunter, 2008). On day 6 post-fertilization zebrafish larvae learn to recognize their kin using olfactory/chemical cues. Exposure to kin on day 6 leads to olfactory imprinting, whereas exposure before or after this age has no effect. Given that it is well established that zebrafish have lateralized brains, at various levels of

organization (Barth et al., 2005; Halpern et al., 2005), it is possible that this imprinting process is lateralized, as in the chick, but this has not yet been investigated.

Visual and auditory imprinting in precocial birds has been mentioned above. Imprinting also occurs in other modalities. Olfactory imprinting is also known to occur in chicks (Burne & Rogers, 1995; Vallortigara & Andrew, 1994b) and it is also lateralized (Burne & Rogers, 2002; Vallortigara & Andrew, 1994b). However, that is all that is known. Olfactory imprinting occurs in many mammalian species, including rodents (Miller & Spear, 2008), rabbits (Patris, Ferrier, Schaal, & Coureaud, 2008; Serra & Nowak, 2008) and humans (e.g., Schaal, Hummel, & Soussignan, 2004) but much more research needs to be done.

The imprinting period during which the young animal acquires important information about its social environment, parent, and siblings, has been discussed with an emphasis on precocial avian species. However, altricial avian species undergo a similar phase of learning although it occurs over a longer period of time. As studied extensively using the zebra finch as a model, the acquisition phase of social/sexual imprinting occurs from about day 10 to 60 post-hatching, with a peak at day 20 (Bischof, Geissler, & Rollenhagen, 2002; summarized by Bischof, 2003). After this there is a second phase, from day 70 to 150, although this varies according to the social environment in which the birds are reared (Bischof, 2007), during which sexual preferences are consolidated. This involves several areas of the brain, including lateralized expression of the early gene, c-*Fos*, in the hippocampal area (Bischof, 2003). *Fos* levels are elevated in the left hippocampus and not the right. This particular finding is mentioned since it demonstrates again lateralization of an important learning process, although here it is not obviously related to the known function of the hippocampus in spatial learning, unless it can be considered to indicate that spatial features are encoded as part of the sexual imprinting memory.

In songbirds, which are altricial, these phases have a parallel in song learning (Bischof, 2003, 2007; Nottebohm, 2000). The first phase of song learning, during which the bird learns its song by hearing song produced by a conspecific tutor (e.g., Bertin, Hausberger, Henry, & Richard-Yris, 2007), can be considered to be equivalent to the phase of filial imprinting in precocial birds. The second phase of song learning occurs later and it involves the bird producing its own song modeled on the template formed in the first phase. Much has been published on this topic. Here I wish only to emphasize that each hemisphere has a different role in song perception and production. In many species the higher vocal centre in the left hemisphere has a predominant role not only in song production (e.g., Nottebohm, 1977) but also in processing visual input related to communication during singing (George, Hara, & Hessler, 2006). For perception of song, it is known that zebra finches discriminate whole songs one from another using the left hemisphere and between harmonic profiles using the right hemisphere (Cynx, Williams, & Nottebohm, 1992).

Parallels have been made between the sensitive periods for song learning in birds and for language learning in humans (e.g., Bischof, 2007). It is, of course, well

recognized that sensitive periods are as much a part of the development of humans as they are of any other species (see Bateson & Martin, 1999) but whether there are direct similarities may rely on further evidence of the corresponding cellular and subcellular events, as well as more detailed study of the experiential processes involved and the subsequent expression of behavior.

It is beyond the scope of this chapter to discuss sensitive periods in humans further and it is well recognized that it is difficult to obtain information about the cellular and subcellular correlates of the sensitive period in humans. Moreover, the various sensitive periods in humans have not been as well delineated as they have in animals, perhaps because they are more malleable depending on experience, although this is not yet clear. We do know, however, that social deprivation of human infants leads to long-lasting changes in the neuropeptides associated with social behavior and in the functioning of the pituitary-adrenal system (Wismer Fries, Shirtcliff, & Pollak, 2008), as it does in rats, and that it has lasting, if not permanent, affects on social behavior. There is also other evidence of sensitive periods in humans, especially for development of perceptual skills (see examples in Maurer, 2005).

Disappearance of Attachment Figures

Most likely, in a wide range of social species, the temporary disappearance of an attachment figure triggers similar emotional and learning processes as it does in the chick. Certainly, prolonged separation of young animals from their mothers can have marked adverse consequences on subsequent attachment formation and other behavior, as recognized for human infants particularly by Freud (1974) and Bowlby (1973, 1980). To merely touch on the large literature on separation of mother and infant in humans, it is worth mentioning, in the light of the studies on chicks, that once the mother-infant bond has been established the infant must experience separation and re-instatement, for example, each time the mother leaves the room. Before the infant can crawl or walk this separation experience would be passive and, when the infant is mobile, it could be active. The passive stage should involve the ability for amodal completion, known to occur in infants as young as 2 months old (Vallortigara & Regolin, 2002), as it does in 3-day-old chicks. The active stage would have more in common with the 10-11 day-old chick and, based on the experiments with chicks and barriers (discussed above), one could predict that experience at this age might involve the infant's right hemisphere and shift attention to global spatial cues. In my opinion, this would be worth testing in humans and I say this while being fully aware of the pitfalls in moving from a precocial to an altricial species and in extrapolating to humans from a species removed so far in evolutionarily terms. In fact, any differences between the species could be as informative as any similarities (see Gottlieb & Lickliter, 2004, for further discussion of this point).

Changes in Hemispheric Dominance During Development

Evidence from research on animals indicates that lateralization of brain and behavior changes across early life and probably across the life span, although the notion of fixed lateralization is commonly assumed, especially for humans (for further discussion see Cowell & Denenberg, 2002). Some evidence of age-dependent shifts in hemispheric dominance is available for humans. For example, Thatcher, Walker, and Giudice (1987) found asynchronous changes in the electroencephalogram activity of the two hemispheres in developing children aged between 3 and 10 years. Other researchers have suggested that maturation of each hemisphere may differ in timing and, coupled with the effects of experience, that this may have a role in the development of both individual and sex differences in behavior. (Galaburda, Corsiglia, Rogers, & Sherman, 1987; see also Rogers, 1999a). Measurement of hemispheric activity of 3-month old infants using fMRI has shown that the left hemisphere is activated more than the right in response to presentation of many sounds, not just speech sounds, which may suggest left hemisphere dominance in neonatal life (i.e. left hemisphere first, as in the chick) but responses in other modalities need to be tested (Dehaene-Lambertz, Hertz-Pannier, & Dubois, 2006). Nevertheless, such a left hemisphere bias may well relate to early stages of language learning, as Dehaene-Lambertz et al. (2006) say, with widespread repercussions for later developments not only in language but also in other functions.

Do These Epigenetic Influences Occur During Development in the Natural Environment?

As Gottlieb (2002) pointed out, natural selection favors animals that have followed particular developmental trajectories that are not restricted to genetic causes but include "normally occurring embryonic sensory stimulation." Determining stimulation that occurs during "normal" development in the animal's natural habitat is, therefore, important. Although the effects of stimulation can be investigated with precision in laboratory studies by manipulating the developing animal's sensory experience and/or the timing at which it occurs, it is equally important to study the naturally occurring developmental processes, as Miller (1981) pointed out, since they may vary from the situation in the laboratory.

Turning now to the influence of light stimulation of the chick embryo on the development of certain asymmetries in visual behavior, we may ask whether such stimulation is a normally occurring event. I argue that it is likely to be so under normal non-stressful conditions of breeding in jungle fowl in their natural habitat since the hen leaves the nest at some periods of the day and because as little as two hours of light stimulation is maximally effective in establishing the lateralization

(Rogers, 1990). On the other hand, there may well be conditions of breeding in which the hen is less likely to leave the nest, possibly if predation of the eggs is high, in which case the embryos may receive insufficient light stimulation to develop the light-dependent lateralities. As shown in the tests in which chicks had to search for grains of food while a model predator was presented overhead, such dark-incubated chicks would be expected to be more disturbed by novel stimuli that might be perceived as potential predators and this could well be an advantage if predation is high. There is also evidence that dark-incubated chicks in the lower ranks of the social hierarchy are more successful in competing with other chicks to gain access to a limited food source than are low-ranking chicks hatched from eggs exposed to light (Rogers & Workman, 1989).

Hence, it seems that exposure of the eggs to light may have benefits in some habitats and lack of this experience may be advantageous in other habitats. Whether these potential effects of light exposure, or its absence, before hatching persist past the first week or two of life has yet to be determined. Nevertheless, light experience would seem to have an important role in early survival of chicks, and likely also for other precocial, avian species.

We already know that filial imprinting is essential to survival of precocial species and now it seems that the experience of going out of sight of the imprinting stimulus on about day 11, in the chick at least, may be an important modification of attention to spatial cues. The long-term consequences of inattention to distal spatial cues that result from the absence of the out-of-sight- experience may well be important to animals that need to navigate in rather large territories but this remains to be investigated. Although the experiments conducted in laboratory conditions give us rather clear and testable predictions, the effects may be less obvious in animals developing in the more complex, natural habitat.

This point of caution is made since I agree with Gottlieb and Lickliter (2004), who drew attention to the fact that more than one pathway of development can lead to the same outcome. Moreover, as they also said, experiences that intervene between the sensitive period and the time of testing the animals when they are older may enhance or suppress the effects of the earlier experience. Gottlieb and Lickliter (2004) were particularly concerned that this be recognized when the results on animal models are extrapolated to human behavior. Nevertheless, they recognized the importance of animal models in discovering general principles of development that would apply to humans as well as other species (e.g., see Thomas & Johnson, 2008).

Some Final Remarks

The importance of specific experiences during sensitive periods has been discussed. Learning during these periods is crucial for survival. Three main sensitive periods have been considered in detail but other sensitive periods with differing outcomes

are certainly present. Some of these sensitive periods may be relatively independent of each other, whereas other sensitive periods may be entirely dependent on learning that has taken place during a previous sensitive period, as Schneirla and Rosenblatt (1963) showed for the development of social behavior in kittens. The dependency of sexual imprinting on prior filial imprinting is such a case, and so is the second phase of song learning, which depends on learning that has occurred during the earlier sensitive period when the bird established a template of its tutor's song (Bischof, 2007). An earlier sensitive period can canalize or constrain the learning that will take place in a later sensitive period (Gottlieb, 1991; 2002). Learning during sensitive periods can also be constrained by genetic predispositions (e.g., chicks imprint on a hen more readily that any other conspicuous object) but these too are not simply expressed without the need for environmental stimulation. For example, although chicks show a predisposition to imprint on their own species, prior experience of viewing a structured environment is necessary before the predisposition is expressed (Bolhuis, Johnson, & Horn, 1985).

Amongst other discussion, I have attempted to make connections between the sensitive periods occurring in precocial species before hatching and during the first two weeks of life after hatching by drawing on a common theme of lateralization. In doing so, the dynamic aspects of lateralization have been discussed with particular attention to the influences of experience on lateralization and to age-dependent shifts in which one of the hemispheres assumes a dominant role in processing inputs and controlling behavior.

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References

Adam, J., & Dimond, S. J. (1971). The effect of visual stimulation at different stages of embryonic development on approach behaviour. *Animal Behaviour*, **19**, 51–54.

Aizawa, H., Bianco, I. H., Hamaoka, T., Myyashita, T., Uemura, O., Concha, M. L., et al. (2005). Laterotopic representation of left-right information onto the dorsoventral axis of a zebrafish midbrain target nucleus. *Current Biology*, **15**, 238–243.

Andrew, R. J. (1988). The development of visual lateralization in the domestic chick. *Behavioural Brain Research*, **29**, 201–209.

Andrew, R. J. (2002). The earliest origins and subsequent evolution of lateralization. In L. J. Rogers & R.J. Andrew (Eds.), *Comparative vertebrate lateralization*, (pp. 70–93). New York: Cambridge University Press.

Andrew, R. J., & Brennan, A. (1983). The lateralization of fear behaviour in the male domestic chick: A developmental study. *Animal Behaviour*, **31**, 1166–1176.

Andrew, R. J., & Brennan, A. (1984). Sex differences in lateralization in the domestic chick. *Neuropsychologia*, **33**, 503–509.

Andrew, R. J., & Dharmaretnam, M. (1991). A timetable of development. In R. J. Andrew (Ed.), *Neural and behavioural plasticity: The use of the domestic chicken as a model* (pp. 166–176). Oxford: Oxford University Press.

Andrew, R. J., Dharmaretnam, M., Györi, B., Mikløsi, A., Watkins, J. A. S., & Sovrano, V. A. (2009). Precise endogenous control of involvement of right and left visual structures in assessment by zebrafish. *Behavioural Brain Research*, **196**(1), 99–105.

Andrew, R. J., Osorio, D., & Budaev, S. (2009). Light during embryonic development modulates patterns of lateralisation strongly and similarly in both zebrafish and chick. *Philosophical Transactions of the Royal Society London B*, **364**, 983–989.

Andrew, R. J., & Rogers, L. J. (1972). Testosterone, search behaviour and persistence. *Nature*, **237**, 343–346.

Andrew, R. J., & Rogers, L. J. (2002). The nature of lateralization in tetrapods. In L. J. Rogers & R.J. Andrew (Eds.), *Comparative vertebrate lateralization* (pp. 94–125). New York: Cambridge University Press.

Barth, K. A., Miklosi, A., Watkins, J., Bianco, I. H., Wilson, S. W., & Andrew, R. J. (2005). *fsi* zebrafish show concordant reversal of laterality of viscera, neuroanatomy, and a subset of behavioural response. *Current Biology*, **15**, 844–850.

Bateson, P. P. G. (1979). How do sensitive periods arise and what are they for? *Animal Behaviour*, **27**, 470–486.

Bateson, P. P. G. (1987). Imprinting as a process of competitive exclusion. In J. P. Rauschecker & P. Marler (Eds.), *Imprinting and cortical plasticity: Comparative aspects of sensitive periods* (pp. 151–168). New York: John Wiley.

Bateson, P. P. G. (2000). What must be known in order to understand imprinting? In C. Heyes & L. Huber (Eds.), *The evolution of cognition* (pp. 85–102). Cambridge, MA: The MIT Press.

Bateson, P. P. G. (2005). The return of the whole organism. *Journal of Bioscience*, **30**, 31–39.

Bateson, P., & Martin, P. (1999). *Design for a life: How behaviour develops*. London: Jonathan Cape.

Bateson, P. P. G., & Seaburne-May, G. (1973). Effects of prior exposure to light on chicks' behaviour in the imprinting situation. *Animal Behaviour*, **21**, 720–725.

Bauer, R. H. (1993). Lateralization of neural control of vocalization by the frog (*Rana pipiens*). *Psychobiology*, **21**, 243–248.

Bertin, A., Hausberger, M., Henry, L., & Richard-Yris, M.-A. (2007). Adult and peer influences on starling song development. *Developmental Psychobiology*, **49**(4), 362–374.

Bischof, H.-J. (2003). Neural mechanisms of sexual imprinting. *Animal Biology*, **53**, 89–112.

Bischof, H.-J. (2007). Behavioral and neuronal aspects of developmental sensitive periods. *NeuroReport*, **18**, 461–465.

Bischof, H.-J., Geissler, E., & Rollenhagen, A. (2002). Limitations of the sensitive period for sexual imprinting: Neuroanatomical and behavioural experiments in the zebra finch (*Taeniopygia guttata*). *Behavioural Brain Research*, **133**, 317–322.

Boakes, R., & Panter, D. (1985). Secondary imprinting in the domestic chick blocked by previous exposure to a live hen. *Animal Behaviour*, **33**, 353–365.

Bolhuis, J.-J. (1991). Mechanisms of avian imprinting: A review. *Biological Reviews of the Cambridge Philosophical Society*, **66**, 303–345.

Bolhuis, J.-J., Johnson, M. H., & Horn, G. (1985). Effects of early experience on the development of filial preferences in the domestic chick. *Developmental Psychobiology*, **18**, 299–308.

Bowlby, J. (1973). *Attachment and Loss, Vol. 2, Separation: Anxiety & Anger*. New York: Basic Books.

Bowlby, J. (1980). *Attachment & Loss, Vol. 3, Loss*. New York: Basic Books.

Braitenberg, V., & Kemali, M. (1970). Exceptions to bilateral symmetry in the epithalamus of lower vertebrates. *Journal of Comparative Neurology*, **138**, 137–146.

Budaev, S., & Andrew, R. J. (2009). Shyness and behavioural asymmetries in larval zebrafish (*Brachydanio rerio*) incubated in the dark. *Behavior*, **146**(8), 1037–1052.

Burne, T. H. J., & Rogers, L. J. (1995). Odors, volatiles and approach-avoidance behavior of the domestic chick (*Gallus gallus domesticus*). *International Journal of Comparative Psychology*, **8**(3), 99–114.

Burne, T. H. J., & Rogers, L. J. (2002). Chemosensory input and lateralization of brain function in the domestic chick. *Behavioural Brain Research*, **133**, 293–300.

Chiandetti, C., Regolin, L., Rogers, L. J., & Vallortigara, G. (2005). Effects of light stimulation in embryo on the use of position-specific & object-specific cues in binocular and monocular chicks (*Gallus gallus*). *Behavioural Brain Research*, **163**, 10–17. Erratum to this paper published in 2007 *Behavioural Brain Research*, *177*, 175.

Chiandetti, C., & Vallortigara, G. (2009). Effects of embryonic light stimulation on the ability to discriminate left from right in the domestic chick. *Behavioural Brain Research*, **198**(1), 240–246.

Cipolla-Neto, J., Horn, G., & McCabe, B. J. (1982). Hemispheric asymmetry and imprinting: The effect of sequential lesions to the hyperstriatum ventrale. *Experimental Brain Research*, **48**, 22–27.

Colombo, M., & Broadbent, N. (2000). Is the avian hippocampus a functional homologue of the mammalian hippocampus? *Neuroscience & Biobehavioral Reviews*, **24**, 465–484.

Concha, M. L., & Wilson, S. W. (2001). Asymmetry in the epithalamus of vertebrates. *Journal of Anatomy*, **199**, 63–84.

Cowell, P. E., & Denenberg, V. H. (2002). Development of laterality and the role of the corpus callosum in rodents and humans. In L. J. Rogers & R. J. Andrew (Eds.), *Comparative vertebrate lateralization*. (pp. 274–305). Cambridge: Cambridge University Press.

Crick, F. (1970). Central dogma of molecular biology. *Nature*, **227**, 561–563.

Cynx, J., Williams, H., & Nottebohm, F. (1992). Hemisphere differences in avian song discrimination. *Proceedings of the National Academy of Sciences of USA*, **89**, 1372–1375.

Dehaene-Lambertz, G., Hertz-Pannier, L., & Dubois, J. (2006). Nature and nurture in language acquisition: Anatomical and functional brain-imaging studies in infants. *Trends in Neurosciences*, **29**, 367–381.

Denenberg, V. H. (1984). Behavioural asymmetry. In N. Geschwind & A.M. Galaburda (Eds.) *Cerebral dominance: The biological foundations*. (pp. 114–133). Cambridge, MA: Harvard University Press.

Denenberg, V. H. (2005). Behavioral asymmetry and reverse asymmetry in the chick and rat. *Behavioral Brain Sciences*, **28**, 597.

Deng, C., & Rogers, L. J. (1997). Differential contributions of the two visual pathways to functional lateralization in chicks. *Behavioural Brain Research*, **87**, 173–182.

Deng, C., & Rogers, L. J. (2002a). Factors affecting the development of lateralization in chicks. In L. J. Rogers & R.J. Andrew (Eds.), *Comparative vertebrate lateralization* (pp. 206–246). New York: Cambridge University Press.

Deng, C., & Rogers, L. J. (2002b). Social recognition and approach in the chick: Lateralization and effect of visual experience. *Animal Behaviour*, **63**, 697–706.

Dharmaretnam, M., & Rogers, L. J. (2005). Hemispheric specialization and dual processing in strongly versus weakly lateralized chicks. *Behavioural Brain Research*, **162**, 62–70.

Dyer, A. B., Lickliter, R., & Gottlieb, G. (1989). Maternal and peer imprinting in mallard ducklings under experimentally simulated natural social conditions. *Developmental Psychobiology*, **22**, 463–475.

Ehret, G. (1987). Left hemisphere advantage in the mouse brain for recognizing ultrasonic communication calls. *Nature*, **325**, 249–251.

Evans, C. S., Evans, L., & Marler, P. (1993). On the meaning of alarm calls: Functional references in an avian vocal system. *Animal Behaviour*, **46**, 23–28.

Freire, R., & Cheng, H.-W. (2004). Experience-dependent changes in the hippocampus of domestic chicks: A model for spatial memory. *European Journal of Neuroscience*, **20**, 1065–1068.

Freire, R., Cheng, H.-W., & Nicol, C. J. (2004). Development of spatial memory in occlusion-experienced domestic chicks. *Animal Behaviour*, **67**, 141–150.

Freire, R., & Rogers, L. J. (2005). Experience-induced modulation of the use of spatial information in the domestic chick. *Animal Behaviour*, **69**, 1093–1100.

Freire, R., & Rogers, L. J. (2007). Experience during a period of right hemispheric dominance alters attention to spatial information in the domestic chick. *Animal Behaviour*, **74**, 413–418.

Galaburda, A. M., Corsiglia, J., Rogers, G. D., & Sherman, G. F. (1987). Planum temporale asymmetry, reappraisal since Geschwind and Levitsky. *Neuropsychologia*, **25**, 853–868.

Freud, A. (1974). *Infants without families and reports of the Hampstead nurseries*. London: Hogarth.

George, I., Hara, E., & Hessler, N. A. (2006). Behavioral and neural lateralization of vision in courtship singing of the zebra finch. *Journal of Neurobiology*, **66**, 1164–1173.

Gerlach, G., Hodgins-Davis, A., Avolio, C., & Schunter, C. (2008). Kin recognition in zebrafish: A 24-hour window for olfactory imprinting. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 2165–2170.

Gottlieb, G. (1971). *Development of species identification in birds: An inquiry into the prenatal determinants of perception*. Chicago: University of Chicago Press.

Gottlieb, G. (1991). Experiental canalisation of behavioural development: Results. *Developmental Psychobiology*, **27**, 35–39.

Gottlieb, G. (1993). Social induction of malleability in ducklings: Sensory bias and psychological mechanism. *Animal Behaviour*, **45**, 707–719.

Gottlieb, G. (1998). Normally occurring environmental and behavioural influences on gene activity: From central dogma to probabilistic epigenesis. *Psychological Review*, **105**, 792–802.

Gottlieb, G. (2000). Environmental and behavioral influences on gene activity. *Current Directions in Psychological Science*, **9**, 93–97.

Gottlieb, G. (2002). On the epigenetic evolution of species-specific perception: The developmental manifold concept. *Cognitive Development*, **17**, 1287–1300.

Gottlieb, G. (2007). Probabilistic epigenesis. *Developmental Science*, **10**, 1–11.

Gottlieb, G., & Klopfer, P. H. (1962). The relation of developmental age to auditory and visual imprinting. *Journal of Comparative and Physiological Psychology*, **55**, 821–826.

Gottlieb, G., & Lickliter, R. (2004). The various roles of animal models in understanding human development. *Social Development*, **13**, 311–325.

Gottlieb, G., & Lickliter, R. (2007). Probabilistic epigenesis. *Developmental Science*, **10**, 1–11.

Güntürkün, O. (1993). The ontogeny of visual lateralization in pigeons. *German Journal of Psychology*, **17**, 276–287.

Güntürkün, O. (2002). Ontogeny of visual asymmetry in pigeons. In L. J. Rogers & R.J. Andrew (Eds.), *Comparative vertebrate lateralization* (pp. 247–273). Cambridge: Cambridge University Press.

Halpern, M. E., Guntürkun, O., Hopkins, W. D., & Rogers, L. J. (2005). Lateralization of the vertebrate brain: Taking the side of model systems. *The Journal of Neuroscience*, **9**, 10351–10357.

Hambley, J. W., & Rogers, L. J. (1979). Retarded learning induced by intracranial administration of amino acids in the neonatal chick. *Neuroscience*, **4**, 677–684.

Hasler, A. D., & Schols, A. T. (1988). *Olfactory imprinting in salmon*. New York: Springer-Verlag.

Hauser, M. D. (1993). Right hemisphere dominance in the production of facial expression in monkeys. *Science*, **261**, 475–477.

Hauser, M. D., & Andersson, K. (1994). Left hemisphere dominance for processing vocalizations in adult, but not infant rhesus monkeys: Field experiments. *Proceedings of the National Academy of Sciences, U.S.A.*, **91**, 3946–3948.

Hess, E. H. (1973). *Imprinting: Early experience and developmental psychobiology of attachment*. New York: Van Nostrand Reinhold.

Hook-Costigan, M. A., & Rogers, L. J. (1998). Lateralized use of the mouth in production of vocalizations by marmosets. *Neuropsychologia*, **36**, 1265–1273.

Horn, G. (1985). *Memory, imprinting and the brain*. Oxford: Clarendon Press.

Horn, G., & McCabe, B. J. (1984). Changes in the structure of synapses associated with learning. *Journal of Neuroscience*, **5**, 3161–3168.

Johnston, A. N. B., Bourne, R. C., Stewart, M. G., Rogers, L. J., & Rose, S. P. R. (1997). Exposure to light prior to hatching induces asymmetry of receptor binding in specific regions of the chick forebrain. *Brain Research*, **103**, 83–90.

Johnston, A. N. B., & Rogers, L. J. (1998). Right hemisphere involvement in imprinting memory revealed by glutamate treatment. *Pharmacology, Biochemistry and Behaviour*, **60**, 863–871.

Johnston, A. N., Rogers, L. J., & Dodd, P. R. (1995). [^{3}H]MK-801 binding asymmetry in the IMHV region of dark-reared chicks is reversed by imprinting. *Brain Research Bulletin*, **37**, 5–8.

Johnston, A. N., Rogers, L. J., & Johnston, G. A. R. (1993). Glutamate and imprinting memory: The role of glutamate receptors in the encoding of imprinting memory. *Behavioural Brain Research*, **54**, 137–143.

Kaplan, G., & Rogers, L. J. (2003). *Gene worship: Moving beyond the nature/nurture debate over genes, brain and gender*. New York: Other Press.

Koboroff, A., Kaplan, G., & Rogers, L. J. (2008). Lateralized anti-predator behaviour in Australian magpies (*Gymnorhina tibicen*). *Brain Research Bulletin*, **76**, 304–306.

Kovach, J. K. (1968). Spatial orientation of the chick embryo during the last five days of incubation. *Journal of Comparative & Physiological Psychology*, **66**, 283–288.

Levin, M., Johnson, R. L., Sten, C. D., Kuehn, M., & Tabin, C. (1995). A molecular pathway determining left-right asymmetry in chick embryogenesis. *Cell*, **82**, 803–814.

Lewontin, R. C. (1991). *The doctrine of DNA*. London: Penguin.

Lickliter, R. (1990). Premature visual stimulation accelerates intersensory functioning in bobwhite quail neonates. *Developmental Psychobiology*, **22**, 651–667.

Lickliter, R., Dyer, A. B., & McBride, T. (1993). Perceptual consequences of early social experience in precocial birds. *Behavioural Processes*, **30**, 185–200.

Lickliter, R., & Gottlieb, G. (1986). Training ducklings in broods interferes with maternal imprinting. *Developmental Psychobiology*, **19**, 555–566.

Lorenz, K. (1935). Der Kumpan in der Umwelt des Vogels. *Journal für Ornithologie*, **83**, 137–413.

MacKenzie, R., Andrew, R. J., & Jones, R. B. (1998). Lateralization in chicks and hens: New evidence for control of response by the right eye system. *Neuropsychologia*, **36**, 51–58.

MacNeilage, P., Rogers, L. J., & Vallortigara, G. (2009). A new view of evolution of the two brain hemispheres. *Scientific American*, July.

Mascetti, G. G., & Vallortigara, G. (2001). Why do birds sleep with one eye open? Light exposure of the chick embryo as a determinant of monocular sleep. *Current Biology*, **11**, 971–974.

Maurer, D.(Ed.) (2005) Critical periods re-examined: Evidence from human sensory development. Special issue of *Developmental Psychobiology*, **46**(3).

McCabe, B. J., Davey, J. E., & Horn, G. (1992). Impairment of learning by localized injection of an N-methyl-D-aspartate receptor antagonist into the hyperstriatum ventrale of the domestic chick. *Behavioral Neuorscience*, **106**, 947–953.

Mench, J. A., & Andrew, R. J. (1986). Lateralization of a food search task in the domestic chick. *Behavioral Neural Biology*, **46**, 107–114.

Meyers, E. N., & Martin, G. R. (1999). Differences in left-right axis pathways in mouse and chick: Functions of *FGF8* & *Shh*. *Nature*, **285**, 403–406.

Michel, G. F. (1981). Right handedness: A consequence of infant supine head orientation preference? *Science*, **212**, 685–687.

Michel, G. F., & Goodwin, R. (1979). Intrauterine birth position predicts newborn supine head position preference. *Infant Behavior & Development*, **2**, 29–38.

Michel, G. F., & Tyler, A. N. (2005). Critical period: A history of the transition from questions of when, to what, to how. *Developmental Psychobiology*, **46**, 156–162.

Miller, D. B. (1981). Conceptual strategies in behavioural development: Normal development and plasticity. In K. Immelmann, G. W. Barlow, L. Petrinovich, & M. Main (Eds.), *Behavioral development*. (pp. 58–82). Cambridge: Cambridge University Press.

Miller, S. S., & Spear, N. E. (2008). Olfactory learning in the rat neonate soon after birth. *Developmental Psychobiology*, **50**, 554–565.

Moltz, H., & Stettner, L. J. (1961). The influence of patterned light deprivation on the critical period for imprinting. *Journal of Comparative & Physiological Psychology*, **54**, 279–283.

Nevitt, G., & Dittman, A. (1999). A new model for olfactory imprinting in salmon. *Integrative Biology*, **1**, 215–223.

Nottebohm, F. (1977). Asymmetries in neural control of vocalization in the canary. In S. Harnard, R.W. Doty, L. Goldstein, J. Jaynes, & G. Krauthamer (Eds.), *Lateralization in the nervous system*. (pp. 23–44). New York: Academic Press.

Nottebohm, F. (2000). The anatomy and timing of vocal learning in birds. In M. D. Hauser & M. Konishi (Eds.), *The design of animal communication*, (pp. 63–110). Cambridge, MA: MIT Press.

Oyama, S. (1985). *The ontogeny of information: Developmental systems and evolution*. Cambridge: Cambridge University Press.

Parsons, C. H. (1994). Neurochemical and behavioural factors affecting the sensitive period for imprinting. PhD Thesis, University of New England, Australia.

Parsons, C. H., & Rogers, L. J. (1997). Pharmacological extension of the sensitive period for imprinting in *Gallus domesticus*. *Physiology and Behavior*, **62**, 1303–1310.

Parsons, C. H., & Rogers, L. J. (2000). NMDA receptor antagonists extend the sensitive period for imprinting. *Physiology and Behaviour*, **68**, 749–753.

Patris, B., Ferrier, G., Schaal, B., & Coureaud, G. (2008). Early development of filial preferences in the rabbit: Implications of nursing- & pheromone-induced odour learning. *Animal Behaviour*, **76**, 305–314.

Previc, F. H. (1991). A general theory concerning the prenatal origins of cerebral lateralization in humans. *Psychological Review*, **98**, 299–334.

Ramsay, A. O., & Hess, E. H. (1954). A laboratory approach to the study of imprinting. *Wilson Bulletin*, **66**, 196–206.

Regolin, L., Garzotti, B., Rugani, R., Pagni, P., & Vallortigara, G. (2005). Working memory in the chick: Parallel and lateralized mechanisms for encoding object- and position-specific information. *Behavioural Brain Research*, **157**, 1–9.

Regolin, L., & Vallortigara, G. (1995). Perception of partly occluded objects by young chicks. *Perception and Psychophysics*, **57**, 971–976.

Rogers, L. J. (1982). Light experience and asymmetry of brain function in chickens. *Nature*, **297**, 223–225.

Rogers, L. J. (1986). Lateralisation of learning in chicks: A review. *Advances in the Study of Behaviour*, **16**, 147–189.

Rogers, L. J. (1990). Light input and the reversal of functional lateralization in the chicken brain. *Behavioural Brain Research*, **38**, 211–221.

Rogers, L. J. (1991). Development of lateralisation. In R. J. Andrew (Ed.) *Neural and behavioural plasticity: The use of the domestic chicken as a model*. (pp. 507–535). Oxford: Oxford University Press.

Rogers, L. J. (1995). *The development of brain and behaviour in the chicken*. Wallingford; CABI.

Rogers, L. J. (1997a). Early experiential effects on laterality: Research on chicks has relevance to other species. *Laterality*, **2**, 199–219.

Rogers, L. J. (1997b). *Minds of their own: Thinking and awareness in animals*. Boulder, CO: Westview,

Rogers, L. J. (1999a). *Sexing the brain*. London: Weidenfeld & Nicolson. [Also, Columbia University Press, 2001].

Rogers, L. J. (1999b). Effect of light exposure of eggs on posthatching behaviour of chickens. In N. Adams & R. Slotow (Eds.), *Making rain for African ornithology. Proceedings of the 22nd International Ornithological Congress* 16-22 August 1998, Durban, Birdlife South Africa, Johannesburg, S46. 2, 19 pp.

Rogers, L. J. (2000). Evolution of hemispheric specialisation: Advantages and disadvantages. *Brain & Language*, **73**, 236–253.

Rogers, L. J. (2002). Advantages and disadvantages of lateralization. In L. J. Rogers & R. J. Andrew (Eds.), *Comparative vertebrate lateralization*. (pp. 126–153). New York: Cambridge University Press.

Rogers, L. J. (2009). Hand and paw preferences in relation to the lateralized brain. *Philosophical Transactions of the Royal Society London B*, **364**, 943–954.

Rogers, L. J., & Andrew, R. J. (2002). *Comparative vertebrate lateralization*. New York: Cambridge University Press.

Rogers, L. J., & Anson, J. M. (1978). Cycloheximide produces attentional persistence and slowed learning in chickens. *Pharmacology, Biochemistry and Behavior*, **9**, 734–740.

Rogers, L. J., & Anson, J. M. (1979). Lateralisation of function in the chicken fore-brain. *Pharmacology, Biochemistry and Behavior*, **10**, 679–686.

Rogers, L. J., & Deng, C. (1999). Light experience and lateralization of the two visual pathways in the chick. *Behavioural Brain Research*, **98**, 277–287.

Rogers, L. J., & Ehrlich, D. (1983). Asymmetry in the chicken fore-brain during development and a possible involvement of the supraoptic decussation. *Neuroscience. Letters*, **37**, 123–127.

Rogers, L. J., & Sink, H. S. (1988). Transient asymmetry in the projections of the rostral thalamus to the visual hyperstriatum of the chicken, and reversal of its direction by light exposure. *Experimental Brain Research*, **70**, 378–384.

Rogers, L. J., & Workman, L. (1989). Light exposure during incubation affects competitive in domestic chicks. *Applied Animal Behaviour Science*, **23**, 187–198.

Rogers, L. J., Zappia, J. V., & Bullock, S. P. (1985). Testosterone & eye-brain asymmetry for copulation in chickens. *Experientia*, **41**, 1447–1449.

Rogers, L. J., Zucca, P., & Vallortigara, G. (2004). Advantage of having a lateralized brain. *Proceedings of the Royal Society London B–Biological Sciences*, 271, S420–S422. Also FirstCite, *Biology Letters*, May 2004 (DOI 10. 1098/rsbl. 2004. 0200).

Rose, S. (1997). *Lifelines: Biology, freedom, determinism*. London: Allen Lane.

Schaal, B., Hummel, T., & Soussignan, R. (2004). Olfaction in the fetal and premature infant: Functional status and clinical implications. *Clinics in Perinatology*, **31**, 261–285.

Schneirla, T. C., & Rosenblatt, J. S. (1963). "Critical periods" in the development of behavior. *Science*, **139**, 1110–1115.

Serra, J., & Nowak, R. (2008). Olfactory preference for own mother & litter in 1-day-old rabbits and its impairment by thermotaxis. *Developmental Psychobiology*, **50**, 542–553.

Siniscalchi, M., Quaranta, A., & Rogers, L. J. (2008). Hemispheric specialization in dogs for processing different acoustic stimuli. *PloS ONE*, **3**(10).: e3349. doi: 10. 1371/journal. pone.0003349.

Sluckin, W. S. (1962). Perceptual and associative learning. *Symposia of the Zoological Society of London*, **8**, 193–198.

Sluckin, W. S. (1966). *Imprinting and early learning*. London: Methuen, (First published 1964.)

Sun, T., Patione, C., Abu-Khalil, A., Visvader, J., Sum, E., Cherry, T. J., et al. (2005). Early asymmetry of gene transcription in embryonic human left and right cerebral cortex. *Science*, **308**, 1794–1798.

Thatcher, W. W., Walker, R. A., & Giudice, S. (1987). Human cerebral hemispheres develop at different rates and ages. *Science*, **236**, 1110–1113.

Thomas, M. S. C., & Johnson, M. H. (2008). New advances in understanding sensitive periods in brain development. *Current Directions in Psychological Science*, **17**, 1–5.

Tommasi, L., Gagliardo, A., Andrew, R. J., & Vallortigara, G. (2003). Separate processing mechanisms for encoding of geometric and landmark information in the avian hippocampus. *European Journal of Neuroscience*, **17**, 1695–1702.

Tommasi, L., & Vallortigara, G. (2004). Hemispheric processing of landmark and geometric information in male and female domestic chicks (*Gallus gallus*). *Behavioural Brain Research*, **155**, 85–96.

Vallortigara, G., & Andrew, R. J. (1991). Lateralization of response by chicks to change in a model partner. *Animal Behaviour*, **41**, 187–194.

Vallortigara, G., & Andrew, R. J. (1994a). Differential involvement of right and left hemisphere in individual recognition in the domestic chick. *Behavioural Processes*, **33**, 41–58.

Vallortigara, G., & Andrew, R. J. (1994b). Olfactory lateralization in the chick. *Neuropsychologia*, **32**, 417–423.

Vallortigara, G., Andrew, R. J., Sertori, L., & Regolin, L. (1997). Sharply timed behavioural changes during the first 5 weeks of life in the domestic chick (*Gallus gallus*). *Bird Behavior*, **12**, 29–40.

Vallortigara, G., Cozzutti, C., Tommasi, L., & Rogers, L. J. (2001). How birds use their eyes: Opposite left-right specialisation for the lateral and frontal visual hemifield in the domestic chick. *Current Biology*, **11**, 29–33.

Vallortigara, G., Pagni, P., & Sovrano, V. A. (2004). Separate geometric and non-geometric modules for spatial reorientation: Evidence from a lop-sided animal brain. *Journal of Cognitive Neuroscience*, **16**, 390–401.

Vallortigara, G., & Regolin, L. (2002). Facing and obstacle: Lateralization of object and spatial cognition. In L. J. Rogers & R. J. Andrew (Eds.), *Comparative vertebrate lateralization*. (pp. 383–444). Cambridge: Cambridge University Press.

Vallortigara, G., Regolin, L., Rigoni, M., & Zanforlin, M. (1998). Delayed search for a concealed imprinted object in the domestic chick. *Animal Cognition*, **1**, 17–24.

Voss, J., & Bischof, H.-J. (2009). Eye movements of laterally eyed birds are not independent. *Journal of Experimental Biology*, **212**, 1568–1575.

Wichman, A., Freire, R., & Rogers, L. J. (2009). Social behaviour in chicks is influenced by light exposure during incubation. *Laterality*, **14**, 381–394.

Wichman, A., Rogers, L. J., & Freire, R. (2009). Visual lateralization & development of spatial & social spacing behaviour of chicks (*Gallus gallus domesticus*). *Behavioural Processes*, **81**(1), 14–19.

Wismer Fries, A. B., Shirtcliff, E. A., & Pollak, S. D. (2008). Neuroendocrine dysregulation following early social deprivation in children. *Developmental Psychobiology*, **50**, 588–599.

Workman, L., & Andrew, R. J. (1989). Simultaneous changes in behaviour and in lateralization during the development of male and female domestic chicks. *Animal Behaviour*, **38**, 596–605.

Zappia, J. V., & Rogers, L. J. (1983). Light experience during development affects asymmetry of fore-brain function in chickens. *Developmental Brain Research*, **11**, 93–106.

14

Trans-Generational Epigenetic Inheritance

Lawrence V. Harper

This chapter addresses the possibility that experientially-induced phenotypic adjustments made by one generation could directly influence the phenotypes of succeeding generations. An overview of what is known about gene regulation and development is presented followed by evidence for trans-generational, epigenetic inheritance in animals, how it might be accomplished, under what conditions, and the implications of these phenomena for our understanding of behavioral development.

Background

The inheritance of acquired characteristics is an idea that has been raised repeatedly over the years, and in psychology, was most persuasively articulated by Baldwin (e.g., 1902). With the rediscovery of Mendel's work and its incorporation into evolutionary theory, the "modern synthesis" of then-current conceptions of inheritance made the idea seem implausible (e.g., Dobzhansky, 1937). Still, all organisms must adjust to environmental fluctuations, and experiments with insects have shown that, as a result of selection, a morphological response to extreme conditions (temperature) can become the norm in a population (Waddington, 1952) and that individuals can be bred to respond to different temperature levels with different larval pigmentation phenotypes (Suzuki & Nijhout, 2006).

Thus natural selection can act on environmentally induced modifications of the developmental process, a concept that is gaining wide recognition as fundamental for understanding speciation itself (e.g., Holland, 1999; West-Eberhard, 2003).

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Furthermore, as Waddington pointed out in 1942, development in metazoa can be considered an example of the inheritance of acquired characteristics because when cells begin to differentiate, that is, selectively express specific sets of genes that define different tissues, the pattern of gene expression specifying each tissue type is "canalized" and is transmitted from progenitor to daughter cells within the organism. Thus, it is plausible to assume that, under some conditions, selection could favor the transmission to offspring of adjustments made by the parent generation.

However, given clear evidence from cloning experiments that essentially all tissues in mammals share the same DNA (Eggan et al., 2004; Wilmut, Schneike, McWhir, Kind, & Campbell, 1997), the latter possibility has posed a conceptual problem: If development, tissue differentiation, results from selective and progressively differential expression of a subset of genes from the inherited corpus, the genomes of the germ cells in the parental generation must have been be purged of developmental modifications to confer totipotency (e.g., Chong & Whitelaw, 2004). That is, the zygote must be able to generate all the tissue variants and retain the potential to make appropriate adjustments to the conditions it may meet during its lifetime.

Although continuity across generations in a number of plastic, behavioral traits has been documented in vertebrates, most seemed explicable in terms of effects mediated by the parent either pre- or post-natally. For example, in hyenas, the mother's androgen level, which varies as a function of her social rank, affects her offsprings' postnatal androgen levels and dominance-related behavior (Dloniak, French, & Holekamp, 2006). In a viviparous lizard, the mother's exposure to a predator, a snake, before her offspring are born, results in the young showing both anatomical (larger size and long tail) and behavioral (enhanced sensitivity to the snake's odor) changes (Shine & Downes, 1999), and exposing pregnant female rats to complex environments (relative to standard laboratory conditions) accelerates the retinal development of their fetuses, apparently mediated by the levels of insulin-like growth hormone in the mothers blood and milk (Sale et al., 2007). In rodents, there are numerous additional examples of alterations in offspring behavior resulting from conditions impacting the mother during pregnancy: Restricting the rat mother's diet during gestation alters offspring body and brain size (Zamenhof, van Marthens, & Grauel, 1971) and maze learning performance (Cowley & Griesel, 1966). While the effects of prenatal dietary restriction in rodents were shown to affect subsequent generations, such trans-generational inheritance seemed explicable in terms of alterations in the female reproductive tract in response to nutrient deprivation that, in turn, affected offspring, and grand-offspring growth (see also Fazeli & Pewsey, 2008; Huck, Labov, & Lisk, 1986; Lee & Yeung, 2006).

A more recent report of the effects of environmental enrichment in laboratory-reared mice suggests that some experiential modifications transmitted via the maternal environment may be temporally limited. Arai, Li, Hartley, and Feig (2009) reported that environmental enrichment of females during the post-weaning,

juvenile period not only enhanced long-term memory in genetically wild-type animals but also facilitated memory formation in mutant juvenile animals carrying a genetic defect in a neuronal signaling pathway. Environmental enrichment of these females as juveniles also facilitated the memory formation of their typically-reared offspring through the preadolescent period and this occurred in animals of both genotypes. This effect waned as the animals aged in both generations (i.e., was not apparent in the offspring as young adults). Insofar as cross-fostering did not alter the facilitating effects of maternal experience it seemed that the effect was mediated via a transient alteration in maternal physiology that influenced the next generation by way of the uterine environment. The effect apparently influenced the transcription of genes that activated an otherwise latent neuronal signaling pathway involved in long-term potentiation of neurons in the hippocampus (Arai et al., 2009).

Postnatally, variations exploratory and "emotional" behaviors have been induced as a result of manipulations of early rearing conditions in rodents (e.g., Ader & Conklin, 1963; Denenberg & Whimbey, 1963) and rhesus monkeys (see Suomi & Levine, 1998 for review), and these effects could be seen in subsequent generations. The persistence of the phenotype in the absence of the initial, inducing conditions could be explained in terms of alterations in maternal behavior insofar as cross-fostering newborn young of the exposed generation to unexposed mothers broke the cycle of transmission (Denenberg & Rosenberg, 1967).

While the results of the foregoing studies with animals could be explained in terms of either the prenatal parental environment or postnatal caregiving, as early as the 1950s, experimental work with plants indicated patterns of inheritance across generations that seemed inexplicable in terms of known mediating factors. One of the first such reports documented phenotypic persistence of alterations in pigment in maize that resulted from crosses between the wild type and a mutant variety. The mutant phenotype was observed even after subsequent breeding yielded genetically homozygous wild type plants (Brink, 1956). Since then, a growing body of evidence in forms ranging from single celled organisms to mammals indicates that the transmission from one generation to another of alterations in the phenotype can occur in the absence of direct parent-to-offspring mediation via such pathways as prenatal hormones or postnatal parenting (see Jablonca & Lamb, 1995, 2005 for reviews). The issue, then, is to understand how, and under what conditions, such "trans-generational epigenetic inheritance" might be accomplished.

Gene Regulation and Epigenetics

Insofar as the range of possible phenotypic variation within an individual is constrained by genetic inheritance, an understanding of how phenotypic

modifications could be transmitted across generations requires an examination of gene regulation or epigenetics. The term "epigenetic," introduced by Waddington, refers to the processes by which expression of inherited genetic potential was regulated in metazoa during development, or as he described it in his *Principles of Embryology* (1956, p. 13), the "causal interactions" leading to the "bringing into operation one or another set of gene-activities."

As epigenetic processes become better understood, it is clear that all development is the result of dynamic transactions between the organism and its surroundings. As Gottlieb (1992) argued, one must examine the effects of these exchanges at all "levels," and it is becoming ever more apparent that the factors controlling gene expression cannot be appreciated without considering the multiple contexts in which they operate. Indeed, even at the cellular level, there exist vivid examples of a complex interplay between "heredity" and "the environment."

For example, experiments with mice have shown that when the nucleus of a just-fertilized egg from one inbred strain is transferred into the cytoplasm of a denucleated egg from a different strain, the developing young will show different levels of gene expression in certain tissues as compared with young who resulted from nuclear transfers to same-strain denucleated eggs (Reik et al., 1993). Thus, within the cell itself, there is a dynamic interplay between the nuclear material and its cytoplasmic environment. This work also shows that what is transmitted across generations is more than DNA; just as individuals "inherit" the modifications made to their environments by their ancestors (cf. Oyama, 1985), the nuclear material in the gametes is situated in a cellular context provided by the parents (see e.g., Tam et al., 2008; Tang et al., 2007) – and suggests a possible avenue for epigenetic inheritance.

This is not to minimize the significance of DNA – it clearly delimits what can develop – but it provides a good illustration of the fact that processes underlying development are much more complex and dynamic than once believed. As the following overview of gene regulation and the developmental process will make clear, even the definition of a "gene" itself is undergoing something of a revolution as the mechanisms underlying the regulation of genomic expression become better understood.

Gene Regulation

The effects of experience, responses to conditions that impact an organism, whether lifelong alterations in liver function in rodents as induced by prenatal nutrient levels (Lillycrop et al., 2007, 2008), or short-term reactions, such a bird's habituating to the song of a conspecific, (Kruse, Stripling, & Clayton, 2004), involve changes in at least the level of gene expression. Thus, an appreciation of gene regulation and how it impacts the developmental process is necessary for conceptualizing how the effects of parental experience could be transmitted directly to offspring.

Gene regulation is a dynamic process; beyond a certain level of stochastic events, (e.g., Newman et al., 2006; Pedraza & van Oudenaarden, 2005), in all life forms, genes are "turned on" and "off" depending on conditions (e.g., Harbison et al., 2004). For example metabolic processes involve selective activation of specific pathways in response to external conditions such as temperature (Tagkopoulos, Liu, & Tavazoie, 2008) or nutrient levels (Hap et al., 2005; Li, Tsang, Watkins, Bertram, & Zheng, 2006) and fluctuations in such conditions can alter the "behavior" – even of unicellular organisms. For example, bacteria, *b. subtilis*, when in a nutrient-poor substrate, "swim" by rotating the flagella on their outer membrane; when they sense a nutrient-rich substrate, they produce a protein that acts as a "clutch" inhibiting flagellar rotation, thereby permitting them to congregate and feed (K. Blair, Turner, Winkelman, Berg, & Kearns, 2008). That is, signals from within the cytoplasm representing the current state of the cell, that is, nutritional status, and from outside, representing conditions to which the cell must respond, that is, the presence of nutrients, are transmitted to the nucleus thereby activating the processes whereby certain segments of the DNA are "transcribed" into RNA and ultimately "translated" into protein, in this case, the flagellar clutch.

As currently understood, within the cell nucleus, the transcription process is activated when, in conjunction with additional protein complexes, DNA polymerase enzymes separate the DNA strands at a particular point to permit the formation of RNAs some of which (the "messenger" RNAs) will provide the substrates for the production of proteins (e.g., Cochella & Green, 2005). This is accomplished by attachment of the polymerase complex at segments of the strands called "promoters." "Transcription factors," (proteins modified or generated in response to signals emanating from within or from the exterior of the cell) bind to segments of the chromosome, the "enhancer" regions of the DNA, to facilitate polymerase enzyme access to the promoter region. This may be further modulated by additional forms of RNA, or modifications thereof, that are produced in the process (Grewal & Elgin, 2007; Morris, Chan, Jacobsen, & Looney, 2004; Sanchez-Elsner, Gou, Kremmer, & Sauer, 2006; Segal et al., 2008; Yi, Poy, Stoffel, & Fuchs, 2008 – see below). Once transcribed, the products of this gene-activation, the "pre-messenger" RNAs are "edited" in the nucleus (e.g., Ule et al., 2006) and those RNAs that are destined to become enzymes or other proteins, messenger RNAs, are transported to the cytoplasm with the assistance of "transfer" RNAs. There, the messenger RNAs are processed by ribosomes, organelles that mediate the biochemical interactions whereby the messenger RNAs are assembled (translated) into proteins (Daviter, Murphy, & Ramakrishnan, 2005; Young et al., 2007).

The foregoing processes are further regulated by the architecture of the nuclear materials. DNA strands in the nuclei of eukaryote cells are "packaged" among "nucleosomes," clusters of eight "histone" proteins – two each of four major types, H2A, H2B, H3, and H4, each of which has several variants (Bernstein & Hake, 2006). Sections of about 150 base pairs of the DNA strands are "wrapped" around each nucleosome (Grewal & Jia, 2007) and the nucleosome proteins have

varying chemical affinities to certain segments of the DNA strands (Kaplan et al., 2009; Segal et al., 2006). It has been proposed that there exists a "code" by which different combinations of histone variants are produced so that some cluster in combinations that are more or less easily displaced from the DNA to permit access to the gene promoter regions for transcription (Bernstein & Hake, 2006).

Moreover, the DNA-binding affinities of these clusters further vary as a function of enzymatically-mediated modifications of the component histone proteins, such as the attachment of acetyl or methyl hydrocarbon molecules to the "tails" of specific amino acids that make up the histone protein (Henikoff, 2008). These modifications change the conformation of the histone proteins and alter their binding affinities to the DNA and/or to other regulatory factors thereby constituting yet another "code" for regulating gene expression (Jenuwein & Allis, 2001). These histone modifications are reversible, dynamically mediated by enzymes that are activated by cellular state, and targeted to specific histone sites (Agger et al., 2007; Cloos et al., 2006; Klose et al., 2006), thereby permitting the cell to respond to current conditions, for example, in neurons, by the formation of synaptic connections (Guan et al., 2009).

In many life forms, DNA methylating enzymes, themselves products of the transcription process, can deposit methyl groups that adhere to a particular sequence of the DNA, so-called CpG islands, in which there are repeats of the sequences of cytosine and guanine bases. Although reversible via demethylases (Bhattacharya, Ramchandani, Cervoni, & Szyf, 1999), these methyl "marks," particularly when on the promoter segments of a gene sequence, are at least transiently (cf. Metivier et al., 2008) associated with silenced regions (Fan, Hagan, Kozlov, Stewart, & Muegge, 2005). Other regions of the DNA strands, "transposable elements" (McClintock, 1984) are also subject to methylation and may help to separate active from inactive regions (Fazzari & Greally, 2004). These methylated sequences themselves, or as a result of interactions with histone variants and their modifications, further modulate the accessibility of DNA promoter sequences to the factors necessary to activate transcription (Grewal & Jia, 2007; Kouzarides, 2007; Viré et al., 2006).

In addition, depending on the histone variants and their tail modifications, other proteins including "linker" histones attach to the nucleosomes and/or the DNA strands to form the "chromatin" in the nucleus (Lee, Habas, & Ababe-Shen, 2004). The densely packed areas, the "heterochromatin," are relatively inaccessible – until the cell replicates (Djupedal & Ekwall, 2008) – to the DNA polymerases. Other, less densely packed segments, the "euchromatin" leave regions of the DNA accessible for factors facilitating transcription.

In response to the metabolic state of the cell and/or to external signals, and variation in histone identity (Mizuguchi et al., 2004), nucleosome location along the DNA strands may be actively altered by energy (ATP) driven processes (Flanagan et al., 2005; Goldberg, Allis, & Bernstein, 2007). Presumably, as a function of intra- and inter-cellular chemical signaling, the sites of heterochromatin and euchromatin

on chromosomes vary both as a function of the dynamics of cell metabolism and, in metazoa, tissue identity (e.g., Cave, Loh, Surpris, Xia, & Caudy, 2005).

There is yet an additional aspect of control of chromatin within the nucleus. The above-mentioned modifications to the nucleosomes and the attachment of methyl groups to the DNA, in conjunction with additional energy-dependent enzymatic activities driven by cell state, can further alter the three-dimensional configuration of the nuclear materials. This causes different nucleosomes to cluster with one another within the nucleus leading to chromosomal "loops" (Ling et al., 2006; Misteli, 2007) where active sequences – even of different chromosomes – colocalize to facilitate transcription in response to such extracellular signals as hormones (e.g., Perillo et al., 2008). Moreover, contrary to prior conceptions of what constituted "a gene," perhaps as a result of such clustering, at least in human cells, functional proteins may be synthesized from RNAs produced by DNA sequences from entirely different chromosomes (Li, Wang, Mor, & Sklar, 2008), suggesting interactions at the level of promoter activation resulting from alterations in the locations of chromosomes in the nucleus (cf. Meaburn & Misteli, 2007).

As indicated above, silencing also can be accomplished by altering the three dimensional chromosomal landscape. This involves the formation of compacted areas in which transcription is inhibited such as in proximity to the nuclear membrane, where, at least in mammals, proteins associated with that membrane area, and with the nuclear membrane itself, may act to repress gene transcription (Reddy, Zullo, Bertolino, & Singh, 2008). The enzymes mediating compacting are presumably activated by signals relevant to the metabolic and tissue-specific functions of the cell – including its replicative status (Black, Brock, Bedard, Woods, & Cleveland, 2007).

Finally, it now seems that most of the accessible DNA, including the so-called non-(protein) coding regions, is transcribed into RNA and that this non-coding RNA may be intimately involved in regulating both the transcription process itself and translation of the messenger RNA into protein (Morris et al., 2004; Singh, Kagalwala, Parker-Thornburg, Adams, & Majumder, 2008; Zamore & Haley, 2005). In the last few years, it has become increasingly apparent that RNA molecules, including short segments of RNA, that are the products of longer sequences which are "trimmed" by enzymes, for example, "Dicer" (MacRae et al., 2006; Vagin et al., 2006; Wassenegger, 2005) play a central role in helping to regulate gene expression (e.g., Hobart, 2008). In addition, longer, non-coding RNA sequences themselves, that is, without being trimmed, may also have pivotal roles in gene regulation (Mercer, Dinger, & Mattick, 2009). These RNAs work in various ways, mostly by virtue of their complementarity either to DNA or to other RNAs, to inhibit translation, to guide enzymes or proteins that lead to the methylation of DNA sequences (Wilkins, 2005), or to facilitate alterations of the conformation of the proteins making up chromatin, for example, methylation or acetylation of histones (Yang & Kuroda, 2007).

Differentiation

These dynamics also provide the mechanisms by which tissues specialize during metazoan development, a process which begins with the fusion of two highly specialized, haploid cells, the sperm and the egg, to form a single, diploid cell, the zygote. Multiple divisions give rise to different tissues and organs and, ultimately, yet another generation more or less faithfully displaying the fundamental characteristics of the species. As indicated above, cloning experiments have proven that the nuclei of essentially all cells in the adult animal contain the same genetic information. As Waddington (1956) argued, in development the genome is not itself altered. Rather, with differentiation, subsets of the genetic potentials are expressed selectively, giving rise to specialized tissues whose fates become canalized so that the organs of the developing animal maintain their identities while still able to react appropriately to variations in the environment.

As indicated above, the cytoplasm of the zygote provides a context for regulating the activities of the nuclear material (cf. Reik et al., 1993). At some point after fertilization, as cells proliferate in metazoa, there will be differentiation of the contents of the cytoplasm in the daughter cells. The egg cytoplasm is not uniform or homogeneous (Zernicka-Goetz, 2002) and, even if it were, presumably, stochastic events would lead to unequal distribution of its contents (Deb, Sivaguru, Yong, & Roberts, 2006; Torres-Padilla, Parfitt, Kouzarides, & Zernicka-Goetz, 2007). Moreover, as a result of proliferation, cells' surroundings (i.e., location relative to other cells, etc.) become different (Gros, Feistel, Viebahn, Blum, & Tabin, 2009). In addition, in mammals, it is also likely that the point of entry of the sperm itself influences to some degree the division of the cytoplasm contents to daughter cells (Jenkins, Saam, & Mango, 2006; Piotrowska & Zernicka-Goetz, 2001). In any case, as cells replicate, variations in their surroundings set the stage for cell-specific patterns of transcription of DNA sequences and, ultimately, tissue-specific translation of proteins, including the ribosomal proteins (Young et al., 2007), which underlie tissue-specific functions, such as neurotransmitter reception (Ule et al., 2006).

Often, a commitment to a given cell fate is maintained by a positive feedback loop from the transcription factors specifying the tissue-specific products to the nuclear machinery (Tyson, Chen, & Novak, 2003). Other, evolutionarily conserved proteins help to regulate cellular commitment, such as the "trithorax" (facilitating) and "polycomb" (repressive) group proteins. The former are more readily displaceable from their chromatin locations thereby permitting certain sequences to "open" to respond to transcription factors. The latter attach to chromatin and/or DNA sites more tenaciously as cells differentiate to repress expression of products specifying alternate tissue types (Tanay, O'Donnell, Damelin, & Bestor, 2007). In conjunction with other proteins, the polycomb group proteins are involved in methylating histone tails and facilitating DNA methylation and heterochromatin formation, restricting DNA access for transcription and thereby

maintaining tissue identity (Goldberg et al., 2007; Reik, 2007; Viré et al., 2006). At least in human tissue, differentiation is characterized by patterns of histone modifications at the enhancer regions that are unique to the cell type (Heintzman et al., 2009).

The traditional view was that a gene constituted a sequence along a single chromosomal strand that, past the promoter, was comprised of "exons" (elements that were transcribed into mRNAs), with intervening "introns," DNA sequences that might occasionally be transcribed, but not translated into protein. The latter once were considered to play little or no role in development. However, it now seems that many of them play essential roles in tissue differentiation. For example, in mice, Ule et al., (2006) showed that there were certain untranslated DNA nucleotide clusters that provided sites for the attachment of a protein that acted to regulate splicing of the transcribed products. Depending on their locations along the DNA strand, these sites either enhanced or inhibited neuronal protein splice variants. Moreover, as mentioned above, it is now recognized that the greater part of the DNA is transcribed and apparently, both long and short, non-coding segments play important roles in regulating the production of proteins governing cellular differentiation.

For example, "micro" RNAs apparently play a central role in regulating the transcription factors that maintain pluripotency of stem cells (Tay, Zhang, Thomson, Lim, & Rigoutsos, 2008; Xu, Papagiannakopoulos, Pan, Thomson, & Kosik, 2009) and tissue differentiation, including mediating cell-specific protein splice-variants (Kishore & Stamm, 2006; Matlin, Clark, & Smith, 2005), and in maintaining cell commitment to a specific, differentiated state (Singh et al., 2008; Tay et al., 2008; Vasudevan, Tong, & Seitz, 2007). Indeed, the DNA binding sites for the above-mentioned polycomb and trithorax proteins, may themselves be transcribed into RNAs that help to maintain tissue identity (Hekimoglu & Ringrose, 2009). Moreover, at least some of these RNAs have their own promoter regions and their expression may be regulated by other proteins attaching to these sites (Singh et al., 2008). This, and their centrality in the regulatory process, has led some to refer to "RNA genes" (e.g., Ross et al., 2005).

In sum, along with the enhancer and promoter regions of the DNA strands, the introns, and other non-translated sequences of chromosomes that were once thought to be mostly parasitic elements or "junk," may not only provide docking sites for histones and other regulatory proteins and thereby control transcription (Bernstein et al., 2006) but also, as a result of their own transcription into RNA, help to regulate histone modifications and the selective translation (Selbach et al., 2008; Ule et al., 2006; Yi et al., 2008) of transcribed products. Thus, the DNA sequence codes for much more than just proteins; different segments serve as variable anchors for proteins that regulate transcription, provide the substrates for the attachment of methyl molecules that alter the probability of protein/enzyme attachment, and/or are transcribed into RNAs that play vital roles in both the transcription and translation processes.

RNA-mediated, regulatory processes are significant for the purposes of this volume in that they play key roles in mediating expression of the substrates for behavioral development. For example, Schratt et al. (2006) used hippocampal cell cultures from mouse brain to investigate the role of micro RNAs in the development of dendritic spines. They found that micro RNAs were localized in dendrites in regions where there were accumulations of mRNAs that, when translated, regulated spine growth. Apparently, the mico RNAs suppressed messenger RNA translation until the synapses were stimulated by neurotransmitters. In addition, Kishore and Stamm (2006) showed that a nucleolar RNA that is expressed selectively from a paternally-inherited chromosome was involved in editing pre-messenger RNAs involved in the formation of serotonin receptors and Mercer et al. (2008) have proposed that non-coding RNAs may be intimately involved in neural gene regulation and memory formation. In short, non-coding RNAs play major roles in both neural development and function.

Development thus involves elaborations of the basic transcription processes, resulting in relatively stable balances between facilitation and inhibition of the expression of tissue-specific genes in response to the interplay of intra- and extracellular conditions. For example, the production and localization of transcription factors during growth is regulated in part by signals that emanate from within the cell nucleus, such as non coding RNAs (Singh et al., 2008), physical forces such as tension on the cell membrane exerted by neighboring cells (Rosenberg, Keland, Tokar, Torre, & Chan, 2008), and/or signals from extracellular sources such as nutrients (Waterland & Jirtle, 2004), neurotransmitters (Kishore & Stamm, 2006), or hormones (Perillo et al., 2008).

For the purposes of this chapter, it is critical to note that during growth, cell replication in differentiated tissues involves duplication of the nuclear DNA strands, the histone variants, and other proteins with which they are associated, as well as partitioning the cytoplasmic contents. This means that the replication process itself must provide signals that facilitate maintenance of the modifications specifying tissue identity, such as histone and DNA methylation (Martin & Zhang, 2007). The latter processes may be facilitated by RNAs transcribed from docking sites for regulatory proteins (Hekimoglu & Ringrose, 2009) or by cytoplasmic RNAs gaining access to the DNA and thereby blocking expression of other genes during cell division, when the nuclear membrane is dissolved (Kawasaki & Taira, 2004).

At the same time, specialized tissues must also have the flexibility to meet the particular demands of the species' niche: for example, muscles must adjust for primarily aerobic or primarily anaerobic work (Salmons & Streter, 1976), and neural cells must produce and retract dendritic spines as memories are formed and replaced (e.g., Kruse et al., 2004; Schratt et al., 2006). This has to be accomplished without compromising the basic identity of these tissues, an issue particularly important where new cells are being generated as in the brain (e.g., Schmidt-Hieber et al., 2004). This means that, in metazoa, there are at least two levels of "cellular

memory" – tissue identity and tissue-specific adjustments to prevailing conditions – and both of these "acquired characteristics" (cf. Waddington, 1942) must be transmitted across cellular generations within the organism's lifetime.

Trans-Generational Epigenetic Transmission

Despite the fact that the genome of the gamete must be able to transmit to the next generation the potential to develop, relatively unfettered by all the regulatory modifications made by cells in the parental generation, there also is clear evidence for the inheritance of trans-generational "memories."

X-Chromosome Inactivation and Genetic Imprinting

In addition to the maintenance of tissue type and tissue-specific adjustments, there exists a trans-generational level of metazoan cellular memory as exemplified by the phenomena of selective X chromosome inactivation and "genetic imprinting." In forms that have differentiated sex chromosomes, such as the mammalian X and Y, it is generally held that there must be a mechanism to "balance" the "dosage" in the heterogametic sex (i.e., the male, XY, with only one X chromosome) so that neither sex has an "overdose" of sex-chromosome gene expression (Nguyen & Disteche, 2006). The process of marking one X chromosome for silencing in the female mammal involves a brief pairing of the two Xs at a sequence on the chromosome that is transcribed into an RNA, *Xist*, that mediates the selective silencing of the chromosome by facilitating the deposition of heterochromatin protein complexes (Augui et al., 2007; Xu, Tsai, & Lee, 2006). This pairing apparently permits a "count" of the number of Xs in the cell, and in mice, leads the other X chromosome to expresses an antisense sequence (*Tsix*) which inhibits widespread heterochromatin formation. (In humans, only the expression of *Xist* is involved in silencing (Morison, Ramsay, & Spencer, 2005).) Although, at least in the human case, the female's "extra" X is not entirely silent, typically, depending on cell type, the alleles from only one X chromosome are expressed (Filippova et al., 2005; Ross et al., 2005). Aside from helping to account for the mechanism(s) underlying X-inactivation, the discovery of *Xist* not only further demonstrates the widespread significance of RNAs in the process of regulating gene expression, but also suggests that RNA might have the potential to mediate the inheritance of trans-generational "memories."

The behavioral relevance of these phenomena is documented by the fact that, in certain tissues, such as the brain, at least some of this silencing is imprinted, that is, selectively expressed according to the parent of origin. For example, an examination of the behavior of girls with Turner's syndrome – the inheritance of a single

X chromosome – indicated that those girls whose X was inherited from their fathers behaved as expected for girls whereas those who inherited only a maternal X acted more impulsively and "boy-like" (Skuse et al., 1997). This finding suggested that, in typical female children with two X chromosomes, at least in the brain, there was selective inactivation of specific alleles inherited via the maternal X. This may be a general mammalian phenomenon insofar as somewhat similar patterns of selective, X-related gene inactivation were found to have comparable effects on the behavior of rodents. (See Isles, Davies, and Wilkinson (2006) for a review and discussion of the conditions that might have favored the evolution of imprinted genes involved in the development of social behavior.)

In the case of imprinted autosomal alleles, in contrast to chromatin on the silenced X, silencing often is evidenced by methylated DNA regions on or near the promoter of the gene in question (e.g., Bourc'his, Xu, Lin, Bollman, & Bestor, 2001). Moreover, there is evidence that RNAs may be involved in the process (Sleutels, Zwart, & Barlow, 2002), although the origin and nature of the autosomal "mark" signaling where on the DNA these modifications are to be located has yet to be identified. In any case, the fact that alleles are expressed according to the parent from whom they were inherited indicates that an additional level of "information" can be transmitted across generations.

Trans-Generational Epigenetic Inheritance

As mentioned in the introductory comments, there exists a well-documented continuum of transmission of behavioral adjustments across generations: from postnatal effects of parenting through prenatal influences mediated by the reproductive tract. With respect to prenatal influences, the fact that some birds can prepare their offspring to cope with current conditions such as the presence of nest ectoparasites prior to laying (Badayev, Hamstra, Oh, & Acevedo Seaman, 2006) is relevant to the question of epigenetic inheritance. Although in birds, to the extent that the maternal response – in this example, egg-provisioning – is evoked at or about the time the egg is developing prior to laying (e.g., Gil et al., 2006), and can be explained in terms of direct chemical signaling from mother to offspring via the reproductive tract (see also Fazeli & Pewsey, 2008; Lee & Yeung, 2006), it also indicates that information can be transmitted via the gamete – the egg.

A large number of observations indicate that adjustments / reactions made by the parental generation can directly influence the phenotypes of their progeny and that such transmission may play an important role in evolution (Jablonca & Lamb 1995; 2005). Evolutionary ecologists have documented a wide range of such "maternal effects," examples of transmission via the gametes that vary as a function of conditions impacting the female parent (Mousseau & Fox, 1998). While many of these examples are intuitively obvious adaptations, experimental demonstrations of their adaptiveness have been presented only recently. Among the first

of these, Agrawal, Laforsch, and Tollrian (1999) showed that, upon exposure to chemical cues indicating the presence of a predator, water fleas (*daphnia*) would develop thickened cuticles about the head region that reduced the flea's risk of being eaten. Similarly, when insects fed on the leaves of a radish, the plant would grow spines on its leaves that seemed to reduce the damage caused by insects. Once having been exposed to cues related to predation, the water fleas, even in the absence of predators, laid eggs that, upon hatching, displayed phenotypes more like those of their exposed parents as compared to the young of unexposed individuals. The radish also exhibited this trait. Not only did the initial phenotypic defenses protect the young relative to controls when they were subject to predation, but the experimental young mounted more pronounced defenses upon exposure.

Similar anticipatory adjustments have been documented for other insects: The desert locust displays a broad spectrum of phenotypic traits that vary depending on population density, ranging from anatomy to food selection and pheromone production. Females transmit these traits through their eggs and males that have experienced "crowding" also can transmit the behavioral characteristics to the young of solitary-reared females. Reversion to the "solitary" phenotype is less marked within a single generation; there is a tendency for the induced phenotype to be more stable across generations (Simpson & Miller, 2007). In the cricket, *Allonemobutus sactus*, on average, eggs will develop more or less immediately if laid under warm conditions; if it is cold, development will be delayed. However, the thermal conditions under which the mother herself developed bias the rate of development of her eggs; if she developed under cold conditions, regardless of prevailing temperature, her offspring's development is likely to be delayed (Huestis & Marshall, 2006). In addition, there is evidence in insects for trans-generational preparation to anticipate disease. Water fleas, as adults, were exposed or not to a bacterial parasite after having been housed either under good (uncrowded, with ample food) or stressful (crowded with low levels of food) conditions as juveniles or as reproductive adults. Offspring of exposed mothers were less susceptible to parasites (Mitchell & Read, 2005).

Similar trans-generational transmission has been demonstrated in vertebrates. In birds, a mother's experience before reaching maturity can affect her investment in her offspring. Under stressful conditions, brood size often is smaller. Naguib and Gil (2005) manipulated brood size in zebra finches and controlled for maternal and genetic effects by cross fostering. They showed that offspring condition and body size decreased with increasing brood size and that the effect was carried over to the next generation. That is, the body size of the second generation young as fledglings was a function of the mothers' brood size. This effect was more marked for female offspring.

The foregoing examples provide evidence of direct transmission of the effects of parental experience to offspring in ways that cannot easily be explained in terms of mediation by the condition of the mother's reproductive tract or her behavior. Rather, they indicate that the "message" can be passed via the gametes.

In mammals, a number of studies have examined the effects of early malnutrition on later generations (e.g., Huck et al.,1986; Mech, Nelson, & McRoberts, 1991; Stein et al., 2003). To the extent that these influences were transmitted via the female lineage, they might be explained in terms of alterations in the maternal (uterine) environment, as noted above. However, this explanation may not be adequate to account for all such cases. In rats, early diet-induced metabolic alterations could be transmitted across generations even when embryos were transferred to normally-fed, control mothers within a day or two of conception (Wu & Suzuki, 2006). In humans, studies in Sweden indicated that the effects of early malnutrition impacted at least two subsequent generations' risks for diabetes and heart disease and that these patterns of vulnerability were transmitted bi-parentally (Kaati Bygren & Edvinsson, 2002; Pembrey et al., 2006). Paternal transmission clearly rules out explanations in terms of alterations in the maternal environment.

The foregoing studies of humans also revealed patterns of inheritance of risk that differed according to the gender of the (grand) offspring and whether the male or the female (grand) parent was subject to malnutrition. Furthermore, whether the effect of exposure to malnutrition was passed on to subsequent generations depended on the age at which nutrient restriction was experienced by the (grand) parent. For grand mothers, sensitivity was greatest during the period from the end of gestation to the third postnatal year; for grandfathers, the effects of nutrient-restriction were most likely to be transmitted if they were experienced during the "slow growth period" from about 6 to 11 years of age (Kaati et al., 2002; Pembrey et al., 2006). Complex, gender-specific patterns of temporal sensitivity and transmission argue against some kind of cultural explanation for these cross-generation effects.

In addition, several recent studies with rats have shown that compounds that interfere with typical responses to hormonal signals, "endocrine disruptors," during prenatal sex differentiation may not only negatively impact the germ cell production and later physical health of the offspring of males so exposed (Anway et al., 2005), but, in addition, lead to changes in "anxiety-like" behaviors that also are manifest in their offspring and linger for several generations (Skinner, Anway, Savenkova, Gore, Crews et al., 2008). Of particular significance are the facts that these effects were transmitted by exposed males even when they mated with unexposed females and that the offspring of the exposed males showed distinctive DNA methylation patterns in a number of tissues (Chang, Anway, Rekow, & Skinner, 2006) and alterations in hippocampal gene expression (Skinner, et al., 2008) despite the fact that their mothers were unexposed. While these latter findings cannot be taken as examples of "adaptive" adjustments to environmental conditions, they demonstrate experimentally that phenotypic responses can be transmitted across generations by the male gametes.

In sum, then, there is clear evidence that the gametes of both male and female mammals have the potential to transmit epigenetic information across generations. And this raises the question of how that information might be conveyed.

Paramutation

Paramutation, trans-generational persistence of a phenotypic trait in the absence of inheritance of the allele responsible for the initial expression of that trait – such as the pigmentation of corn kernels, mentioned earlier – is an example of trans-generational epigenetic inheritance. Persistence of the mutant phenotype, even in backcrossed plants that were homozygous for the wild-type allele (Brink, 1956) suggested that a DNA-induced signal activating the trait, but not the DNA sequence of the affected parent, was transmitted to the offspring. Recently, an analysis of the processes underlying this phenomenon indicated that transmission was mediated by RNA (Alleman et al., 2006); apparently, RNA transcripts from the mutant allele were transmitted to the next generation, via the gametes, in the absence of the mutant DNA.

In mammals, an analysis, also of a pigment pattern, has yielded comparable results. Rassoulzadegan et al. (2006) examined the effects of an allele in mice (*Kit*) that plays a role in the synthesis of a tyrosine kinase receptor which is involved in melanogenesis, among other things. The heterozygous phenotype is distinguished by white patches of fur on the extremities, especially the paws and the tip of the tail. When heterozygous mutants – male or female – were crossed with homozygous, wild-type males or females, the offspring, even those inheriting the wild type alleles, showed the white paws and tail tip. In this case as well, what seemed to be happening was that some product, or by-product, of the *Kit* allele of the affected parent was transmitted via the gametes even when the mutated allele itself was not inherited by the offspring. There was reduced receptor expression in both the heterozygotes and the "paramutated," wild-type animals and this phenotype was associated with the presence of an atypical *Kit* RNA which could be detected in the sperm of both the heterozygotes and paramutated, wild-type males. When RNA from the *Kit* heterozygote sperm was injected into control, wild-type one-cell embryos, the white tail was expressed by about half of the treated animals. This provides experimental evidence that it is possible for the RNA in mammalian sperm to transmit a signal that alters the phenotype of the offspring – even in the absence of the allele responsible for the production of that RNA. The altered phenotype was not stably inherited indefinitely and the frequency of its expression diminished after the second generation.

In conjunction with evidence reviewed above for the importance of non-translated RNA in the regulation of development and the maintenance of tissue identity, the finding that the *Kit* paramutation could be transmitted by RNA in sperm (Rassoulzadegan et al., 2006) and evidence for the critical role of (maternal) RNAs in early zygotic development (e.g., Tang et al., 2007), indicate that RNAs are likely candidates for mediating some kinds of trans-generational transmission of epigenetic information in animals (see also Benneke et al., 2008; Cuzin, Grandjean, & Rassoulzadegan, 2008). Given the important roles of non-coding RNAs in

neuronal differentiation and function (Choi et al., 2008; Mercer et al., 2008) such findings suggest that behavior also could be so influenced. Moreover, although sperm are much smaller than eggs, there is now evidence that, in mice at least, nucleosome histone modifications in sperm affect zygotic chromatin (van der Heijden et al., 2006) and mammalian sperm transmits unique proteins to the zygote – including histone variants (Krawetz, 2005) – as well as functional RNAs (Ostermeier, Miller, Huntriss, Diamond, & Krawetz, 2004). Thus, it is possible that both gametes could transmit epigenetic information via proteins as well (cf. Ashe & Whitelaw, 2007; Chong et al., 2007).

Indeed, given Pembrey et al.'s (2006) finding of gender differences in the manifestation and sources of metabolic risks in humans, and examples of uni-parental transmission as well as gender differences in offspring phenotypic expression of various traits in animals (e.g., Bonduranski & Head, 2007; McMillen & Robinson, 2005; Naguib & Gil, 2005), it would be inadvisable to expect that a single pathway could explain all instances of trans-generational epigenetic inheritance. Insofar as the transcriptional machinery is involved in cell replication, there is the possibility that, where longer strands of RNA might be involved, even "reverse transcription," from RNA to DNA could occur. Although first reported as a possibility only in plants (Lolle, Victor, Young, & Pruitt, 2005), it cannot be entirely ruled out as another factor moderating the dynamics of trans-generational epigenetic inheritance in animals insofar as *in vitro* work with sperm suggests that reverse translation could occur in mammalian cells (Spadafora, 2008). In any event, at least one pathway for trans-generational transmission of epigenetic information – RNA – has been experimentally identified.

Contexts Favoring Trans-Generational Epigenetic Inheritance

If there exist examples of trans-generational epigenetic inheritance and demonstrable pathways for such transmission by the gametes, the question then becomes when and where such transmission might be expected.

The trans-generational transmission in humans of risks for diabetes and cardiovascular disease as a result of malnutrition during relatively brief developmental periods prior to sexual maturity (cf. Kaati et al., 2002; Pembrey et al., 2006) might seem at first glance to be patently maladaptive. However, periods of drought can last for decades and may account for the periodic abandonment of regions in Africa (Kuper & Kropelin, 2006), the collapse of entire civilizations in the Middle East (Lawler, 2007), and the disappearance of indigenous cultures practicing irrigation and water-storage in the American northwest (Kloor, 2007). Thus, the ability to anticipate long-term fluctuations in nutrient supplies may well have been – and still be – adaptive for humans. The costs of stored fat and altered insulin sensitivity (Gluckman, Hanson, & Beedle, 2007) may be/have been outweighed by the benefits of being able to endure extended periods of restricted energy supplies.

In the field of evolutionary ecology, such cost-benefit considerations have led to predictions regarding the conditions under which trans-generational, epigenetic inheritance might be expected. According to Harvell and Tollrian (1999; see also Rossiter, 1998), to the extent that environmental conditions remain relatively stable across generations, selection pressures would be expected to favor adaptations to mild fluctuations as they occurred within an individual's lifetime. In contrast, when environmental fluctuations are severe, and the costs of being unprepared are high, and if such conditions – for example, periods of famine – endure for extended periods of time and are unpredictable, selection would favor anticipating them. That is, when conditions fluctuate significantly and have major impacts on individual viability, are predictable only over the long term, and may endure across generations (such as long-term drought), then selection is likely to favor epigenetic inheritance, given that, in this example, the costs of smaller size and fat deposition are less than the risk of starvation.

The critical features for such trade-offs can be modulated by a number of conditions. As indicated above, one is variability in conditions in the species' niche, for example, consistency over time in the quantity and quality of resources such as food, resource-distribution in space, and the likelihood of predation. Another factor would be life history – whether or not there are reliable changes in habitat conditions with development. For example, where the species' niche is relatively constant, but conditions differ for juveniles and adults, the degree of maternal (metabolic) investment in offspring would be best determined by the mother's own juvenile experience (cf. Taborsky, 2006). Lifespan is another variable that would influence for what, and whether, there might be anticipatory adjustments. For example, in the human case, given a relatively long life span, and the fact that, in at least some locations, drought and famine endure for decades (Kloor, 2007;Kuper & Kropelin, 2006; Lawler, 2007), anticipatory adjustments would often be beneficial. Finally, another variable that could impact the adaptiveness of epigenetic inheritance across generations would be the species' facultative range of social systems (cf. Lott, 1984). For example, where patterns of social organization vary with conditions and extend over a lifespan or more, and these factors impact access to resources and/or vulnerability to predation, anticipatory transmission of adjustments could be beneficial (e.g., Simpson & Miller, 2007).

The cues that trigger such adjustments need not be as clearly related to the outcome as in the case of nutrient levels. What is critical is that they are reliable correlates or predictors of a yet-to-be encountered condition. For example, in short-lived forms such as the water flea, when living in variable climates, the next generation must be prepared for the conditions to which they will have to adjust. The pattern of development of eggs depends on temperature and nutrient availability, both of which vary over the course of the year. The female water flea adjusts the condition of her eggs to meet future conditions on the basis of the photoperiod which is a reliable correlate of temperature and nutrient availability (Alekseev & Lampert, 2001). This stimulus, the duration of daylight, is what I have

called an "environmental sign" (Harper, 1989) – a reliable indicator of events-to-come (see also Mclinn & Stephens, 2006).

In mammals, examples of such signs have been documented, typically relating to postnatal events. In rats, the mother's pattern of licking and grooming of her pups – which often ends before they exit the nest – sends a message about the environment that they are likely to encounter (Cameron et al., 2005). It can be mimicked by stroking pups with a paintbrush and influences a suite of responses including regulation of the HPA via expression of hippocampal corticosterone receptors (Gonzalez, Lovic, Ward, Wainwright, & Fleming, 2001). Likewise, in humans, certain reactions such as sexual attraction towards others rely at least as much on indirect cues. Lieberman, Tooby, & Cosmides (2007) found that, among college students, an older sibling's experience of the mother coming home with an infant, and for the younger sibling, the continuous co-residence with another child were as potent in predicting inhibition of incestual desires as declarative knowledge of kinship.

In short, to the extent that adversities often cannot be predicted on the basis of immediately obvious cues, we should expect that more indirect, but reliably predictable, signals might govern trans-generational epigenetic inheritance.

Possible Behavioral Domains

With respect to the adversities for which advance preparation behaviorally would be advantageous, thereby favoring trans-generational epigenetic inheritance, one likely case in humans would relate to social standing – slavery or other forms of oppression (Harper, 2005). In terms of phenotypic response, it would involve temperamental adjustment, such as caution in conditions of social uncertainty. Insofar as there is ample evidence that, in mammals, the HPA axis and neuromodulator function such as serotonin and dopamine receptor expression can be adjusted by experience (e.g., Berton et al., 2006; Cameron et al., 2005; Kofman, 2002; Vasquez, Lopez, Van Hoers, Watson, & Levine, 2000), candidate pathways are available for such an anticipatory modification. Indeed, C. Blair et al. (2008, p. 1106) mention the possibility that low-income African-American infants' relatively high cortisol levels could represent "an intergenerational adaptation to historical conditions of stress associated with racism and social injustice."

Timing

As suggested above, the experiences leading to adjustments propagated through trans-generational epigenetic inheritance in mammals are likely to be restricted to certain periods in development. At present, it seems that sensitivity would be greatest during the development of the egg or sperm themselves and/or of the

tissues that support their development. Unfortunately, relatively little is known about when the critical events occur in many species. Work on the effects of endocrine disruptors on rodents' sperm suggests a prenatal sensitive period when the gonads are developing (e.g., Chang et al., 2006). But the precise time window for the transmission of the metabolic impact of early dietary restriction to later generations in animals has not been determined. In humans, the apparent sensitive periods for transmitting adjustments to nutrient availability vary according to gender and seem to occur after the differentiation of the gonads and gametes has occurred. For females, the most sensitive period was in the latter part of gestation through the first two or three years; for males, it was during the postnatal "slow growth" period (Kaati et al., 2002; Pembrey et al., 2006), the time when spermatogonia were undergoing final development (Chaudhary, Sadler-Riggleman, Ague, & Skinner, 2005). At present, at least for humans, it would seem that a likely window for transmission is the period during which the gonadal "nurse cells" i.e., the Sertoli cells in the testes and the granulosa cells in the ovaries (e.g., Buccione, Schroeder, & Eppig, 1990; Chaudhary et al., 2005;Petersen & Soder, 2006), are undergoing maturation, but before they respond to gonadotrophic hormones.

Moderating Variables

Given indications that certain trans-generational adjustments are transmitted by one parent only, there remains the problem of determining the conditions that would favor maternal- or paternal-only transmission. For mammals, at least, one could speculate that, where variable, social organization (matrilocal residence, etc,), resource-acquisition strategies (e.g., gathering, food storage), and gender differences in dispersal patterns as individuals mature would be conditions that might be predictive of gender differences in the nature of the adjustments that would be advantageous to transmit across generations.

As implied above, to the extent that a species' habitat is not uniform across its range and at least some subpopulations remain in the same location for dozens of generations, it is also possible that, where a challenge is locally unlikely and an anticipatory response does impose a substantial cost, selection can favor those who do not transmit and/or respond to the relevant epigenetic messages. Thus, in generalist species that inhabit a wide range of environments, to the extent that some stable sub-populations have been relatively isolated from conditions favoring even moderately costly anticipatory adjustments, there may be intra-species heterogeneity in the likelihood of transmitting or inheriting an epigenetic modification. Indeed, in rodents, there is evidence for strain differences in the likelihood of trans-generational transmission of certain epigenetic traits (e.g., Rakyan et al., 2003). Therefore, genetic background (epistasis) may be a modifier and will need to be taken into account as well when examining possible instances of trans-generational epigenetic inheritance (see also Chong, Youngson, & Whitelaw, 2007).

Additional variables may influence the degree to which such inheritance is phenotypically expressed. Work with a color-coat (Avy) paramutation in mice that yields yellow pelage has indicated that the effect of the Avy allele can be modified by diet. When heterozygotes were reared with diets enriched with methyl donors, (folic acid, vitamin B12, and pyridoxal phosphate) the trans-generational persistence of the yellow phenotype was reduced (Waterland & Jirtle, 2004). Thus, additional conditions that could in some way impact the processes underlying gene expression might influence the penetrance of an epigenetically inherited trait.

Distinguishing Epigenetic from other Forms of Inheritance

Essentially by definition, trans-generational epigenetic inheritance differs from Mendelian inheritance in that trait variation can be related to the experiences of prior generations *per se*. A second characteristic distinguishing these two modes of inheritance, at least for traits whose expression can be costly under some conditions, is that the degree of expression of an epigenetically transmitted characteristic is likely to diminish over successive generations in the absence of the precipitating cause. Although current evidence suggests that trans-generational, gamete-transmitted, adaptive epigenetic adjustments typically begin to reverse themselves within three or more generations in the absence of the precipitating conditions (e.g., Agrawal et al., 1999), it is possible that, where an exigency endures for several generations, some anticipatory adjustments might endure for more than three generations, and where the precipitating condition lasts for a more brief period, the adjustment may endure for only a single generation.

Insofar as the results of the Swedish studies of nutrient availability in humans found a pattern of transmission which possibly involved the expression of imprinted genes – that is, that the specific patterns of inheritance depended on the gender of both the grand parent and the grand-offspring – another way to distinguish imprinting and Mendelian from epigenetic inheritance is evidence of gender-related differences in the temporal correlates and pathways of transmission (Kaati et al., 2002; Pembrey et al., 2006). That is, within a lineage, there would be gender-related differences in the pathways of inheritance as a function of (grand) parental experience and the age at which the grandparent was exposed to the precipitating event.

In addition to the foregoing, beyond the influence of ancestral gender, trans-generational epigenetic inheritance often is also associated with a distribution of phenotypes within a lineage that does not conform to a Mendelian model. However, in heterogeneous populations, epistatic interactions could lead to similar patterns. Therefore, when working with animals, the use of inbred strains can rule out the possible effects of epistasis that otherwise might be expected in outbred populations (cf. Chong et al., 2007). Similarly, given the possibility that in mammals, there could be interactions between the mother and the fetus while

it is in utero, for example, that insulin insensitivity in the mother might interact with female fetuses' demands for nutrients to establish a "vicious cycle" of risk for type 2 diabetes and other diseases across generations (Petry, Ong, & Dunger, 2007), in experimental animal models, postnatal cross-fostering in conjunction with prenatal zygote transfer would be optimal for determining whether or not a trait is influenced by epigenetic inheritance.

Implications and Conclusions

The facts that the gametes can transmit information above and beyond that which is carried by the chromosomes, and that this can alter the phenotype in ways that might mimic the effects of allelic variation, have implications for current models for assessing "what is inherited." For example, traditional twin study designs may actually yield reduced estimates of genetic concordance according to zygosity where there is an "overlay" of epigenetic adjustment that could increase the similarities among same-sex, dizygotic twin pairs (see also Rossiter, 1998; Wade, 1998). On the other hand, to the extent that epigenetic processes modulate the levels of gene expression and thus impact the magnitude of individual differences, the range of phenotypic variation may lead to overestimates of allelic heterogeneity within a population. Thus, more effort will be needed to obtain detailed assessment of environmental conditions, when they were experienced, and their nature, with additional attention to more indirect environmental signs relevant to long-term fluctuations in high-impact conditions in a species' habitat (Harper, 1989; Mclinn & Stephens, 2006). In addition, there will be a need to document potential mediating factors such as the details of diet, for example, the quantity of protein (cf. Zamenhof et al., 1971) and methyl donors (cf. Waterland & Jirtle, 2004). Moreover, such data will be needed for at least two generations.

Finally, on a more practical level, given the fact that some inherited epigenetic adjustments take several generations to wane and that they are biased toward anticipating hardship, when attempting to overcome the effects of environmental adversities in a population, one must face the possibility that the results of amelioration may not be either as great or as rapidly apparent as one would expect if they were simply reactions to immediate conditions. That is, it may take two or more generations to overcome the effects of deprivation or other environmental exigencies.

In summary, it is now clear that much more than just the protein-coding sequences of DNA must be appreciated in order to understand inheritance. Metazoan gametes transmit much more than just DNA; their nuclei contain proteins and non-coding RNAs whose distribution defines a state of pluripotency – the potential to differentiate into all cells of the organism. Moreover, the nucleus of the gamete is surrounded by a cytoplasm that contains the nutrients and metabolic

machinery required for its viability. The interplay between the DNA and its environment is complex and multi-layered, involving dynamics within the nucleus, and nuclear-cytoplasmic exchanges as influenced by the intercellular matrix which, in turn, are altered according to external conditions such as nutrient availability, temperature, and (other) sensory inputs. All these factors are equally important in understanding how the process of development is regulated and how an organism can adjust to external conditions. In addition, there is evidence that some of the epigenetic alterations in the transcription and/or translation of the DNA that are made in response to conditions as an organism develops can be transmitted across generations via the gametes, without alteration in the DNA itself. In short, just as development, inheritance itself must be examined at many levels and across a wide time frame.

References

Ader, R., & Conklin, P. M. (1963). Handling of pregnant rats: Effects on emotionality of their offspring. *Science*, **142**, 411–412.

Agger, K., Kloos, P. A., Christensen, J., Pasini, D., Rose, S., Rappsilber, J., et al. (2007). UTX and JMJD3 are histone H3K27 demethylases involved in *Hox* gene regulation and development. *Nature*, **449**, 731–734.

Agrawal, A. A., Laforsch, C., & Tollrian, R. (1999). Transgenerational induction of defenses in animals and plants. *Nature*, **401**, 60–63.

Alekseev, V., & Lampert, W. (2001). Maternal control of resting-egg production in *Daphnia*. *Nature*, **414**, 899–901.

Alleman, M., Sidorenko, L., McGinnis, K., Seshadri, V., Dorweiler, J. E., & White, J., et al. (2006). An RNA-dependent RNA polymerase is required for paramutation in maize. *Nature*, **442**, 295–298.

Anway, M. S., Cupp, A. S., Uzumcu, M., & Skinner, M. K. (2005). Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science*, **308**, 1466–1469.

Arai, J. A., Li, S., Hartley, D. M., & Feig, L. A. (2009). Transgenerational rescue of a genetic defect in long-term potentiation and memory formation by juvenile enrichment. *Journal of Neuroscience*, **29**, 1496–1502.

Ashe, A., & Whitelaw, E. (2007). Another role for RNA: A messenger across generations. *Trends in Genetics*, **23**, 8–10.

Augui, S., Filion, G. J., Huart, S., Nora, E., Guggiari, M., Maresca, M., et al. (2007). Sensing X chromosome pairs before X inactivation via a novel X-pairing region of the *Xic*. *Science*, **318**, 1632–1636.

Badayev, A. V., Hamstra, T. L., Oh, K. P., & Acevedo Seaman, D. (2006). Sex-biased maternal effects reduce ectoparasite-induced mortality in a passerine bird. *Proceedings of the National Academy of Sciences, USA*, **103**, 14406–14411.

Baldwin, J. M. (1902). *Development and evolution*. New York: Macmillan.

Benneke, J., Malone, C. D., Aravin, A. A., Sachidanandam, R., Stark, A., & Hannon, G. J. (2008). An epigenetic role for maternally inherited piRNAs in transposon silencing. *Science*, **322**, 1387–1392.

Bernstein, E., & Hake, S. B. (2006). The nucleosome: A little variation goes a long way. *Biochemistry and Cell Biology*, **84**, 505–517.

Bernstein, B. E., Mikkelsen, T. S., Xie, X., Kamal, M., Huebert, D. J., & Cuff, J., et al. (2006). A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell*, **125**, 315–326.

Berton, O., McClung, C. A., DiLeone, R. J., Krishnan, V., Renthal, W., Russo, S. J., et al. (2006). Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science*, **311**, 864–868.

Bhattacharya, S. K., Ramchandani, S., Cervoni, N., & Szyf, M. (1999). A mammalian protein with specific demethylase activity for CpG DNA. *Nature*, **397**, 579–583.

Black, B. E., Brock, M. A., Bedard, S., Woods, V. L., & Cleveland, D. W. (2007). An epigenetic mark generated by the incorporation of CENP-A into centromeric nucleosomes. *Proceedings of the National Academy of Sciences, USA*, **104**, 5008–5013.

Blair, C., Granger, D. A., Mills-Koonce, R., Willoughby, M., Greenberg, M. T., Hibel, L. C., et al. (2008). Maternal and child contributions to cortisol response to emotional arousal in young children from low-income rural communities. *Developmental Psychology*, **44**, 1095–1109.

Blair, K. M., Turner, L., Winkelman, J. T., Berg, H. C., & Kearns, D. B. (2008). A molecular clutch disables flagella in the *Bacillus subtilis* biofilm. *Science*, **320**, 1636–1638.

Bonduranski, R., & Head, M. (2007). Maternal and paternal condition affects offspring phenotype in *Telostylinus angusticollis* (Diptera: Neriidae). *Journal of Evolutionary Biology*, **20**, 2379–2388.

Bourc'his, D., Xu, D. L., Lin, C. S., Bollman, B., & Bestor, T. H. (2001). Dnmt3L and the establishment of maternal genomic imprints. *Science*, **294**, 2536–2539.

Brink, R. A. (1956). A genetic change associated with the *R* locus in maize which is directed and potentially reversible. *Genetics*, **41**, 872–889.

Buccione, R., Schroeder, A. C., & Eppig, J. J. (1990). Interactions between somatic cells and germ cells throughout mammalian oogenesis. *Biology of Reproduction*, **43**, 543–547.

Cameron, N. M., Champagne, F. A., Parent, C., Fish, E. W., Ozaki-Kuroda, K., & Meaney, M. J. (2005). The programming of individual differences in defensive responses and reproductive strategies in the rat through variations in maternal care. *Neuroscience and Biobehavioral Reviews*, **29**, 843–865.

Cave, J. W., Loh, F., Surpris, J. W., Xia, L., & Caudy, M. A. (2005). A DNA transcription code for cell-specific gene activation by *Notch* signaling. *Current Biology*, **15**, 94–104.

Chang, H.-S., Anway, M. D., Rekow, S. S., & Skinner, M. K. (2006). Transgenerational epigenetic imprinting of the male germline by endocrine disruptor exposure during gonadal sex determination. *Endocrinology*, **147**, 5524–5541.

Chaudhary, J., Sadler-Riggleman, I., Ague, J. M., & Skinner, M. K. (2005). The helix-loop-helix inhibitor of differentiation (ID) proteins induce post-mitotic terminally differentiated Sertoli cells to re-enter the cell cycle and proliferate. *Biology of Reproduction*, **72**, 1205–1217.

Choi, P. S., Zakhari, L., Chio, W.-Y., Caron, S., Alvarez-Saavedra, E., Miska, E. A., et al. (2008). Members of the miRNA-200 family regulate olfactory neurogenesis. *Neuron*, **57**, 41–55.

Chong, S., Vickaryous, N., Ashe, A., Zamudio, N., Youngson, N., Hemley, S., et al. (2007). Modifiers of epigenetic reprogramming show paternal effects in the mouse. *Nature, Genetics*, **39**, 614–622.

Chong, S., & Whitelaw, E. (2004). Epigenetic germline inheritance. *Current Opinion in Genetics and Development*, **14**, 692–696.

Chong, S., Youngson, N. A., & Whitelaw, E. (2007). Heritable germline epimutation is not the same as transgenerational epigenetic inheritance. *Nature, Genetics*, **39**, 574–575.

Cloos, P. S., Christensen, J., Agger, K., Maiolica, A., Rappsilber, J., Antal, T., et al. (2006). The putative oncogene, GASC1 demethylates tri- and dimethylated lysine 9 on histone H3. *Nature*, **442**, 307–311.

Cochella, L., & Green, R. (2005). Fidelity in protein synthesis. *Current Biology*, **15**, R36–R40.

Cowley, J. J., & Griesel, R. D. (1966). The effect on growth and behaviour of rehabilitating first and second generation low protein rats. *Animal Behaviour*, **14**, 506–517.

Cuzin, F., Grandjean, V., & Rassoulzadegan, M. (2008). Inherited variation at the epigenetic level: paramutation from the plant to the mouse. *Current Opinion in Genetics and Development*, **18**, 1–4.

Daviter, T., Murphy, F. V., IV., & Ramakrishnan, V. (2005). A renewed focus on transfer RNA. *Science*, **308**, 1123–1124.

Deb, K., Sivaguru, M., Yong, H. Y., & Roberts, R. M. (2006). *Cdx2* gene expression and trophectoderm lineage specification in mouse embryos. *Science*, **311**, 992–996.

Denenberg, V. H., & Rosenberg, K. M. (1967). Nongenetic transmission of information. *Nature*, **216**, 549–550.

Denenberg, V. H., & Whimbey, A. E. (1963). Behavior of adult rats is modified by the experiences their mothers had as infants. *Science*, **142**, 1192–1193.

Djupedal, I., & Ekwall, K. (2008). The paradox of silent heterochromatin. *Science*, **320**, 624–625.

Dloniak, S. M., French, J. A., & Holekamp, K. E. (2006). Rank-related maternal effects of anhydrogens on behaviour in wild spotted hyenas. *Nature*, **440**, 1190–1193.

Dobzhansky, T. (1937). *Genetics and the origin of species*. New York: Columbia University Press.

Eggan, K., Baldwin, K., Tackett, M., Osborne, J., Gogos, J., Chess, A., et al. (2004). Mice cloned from olfactory sensory neurons. *Nature*, **428**, 44–49.

Fan, T., Hagan, J. P., Kozlov, S. V., Stewart, C. L., & Muegge, K. (2005). Lsh controls silencing of the imprinted *Cdkn1c* gene. *Development*, **132**, 635–644.

Fazeli, A., & Pewsey, E. (2008). Maternal communication with gametes and embryos: A complex interactome. *Briefings in Functional Genomics and Proteomics*, **7**, 111–118.

Fazzari, M. J., & Greally, J. M. (2004). Epigenomics: Beyond CpG islands. *Nature Reviews Genetics*, **5**, 446–455.

Filippova, G. N., Cheng, M. K., Moore, J. M., Truong, J.-P., Hu, Y. J., Nguyen, D. K., et al. (2005). Boundaries between chromosomal domains of X inactivation and escape bind

CTCF and lack CpG methylation during early development. *Developmental Cell*, **8**, 31–42.

Flanagan, J. F., Mi, L.-Z., Chruszcz, M., Cymborowski, M., Clines, K. L., Kim, Y., et al. (2005). Double chromodomains cooperate to recognize the methylated histone H3 tail. *Nature*, **438**, 1181–1185.

Gil, D., Ninni, P., Lacroix, A., De Lope, F., Tirard, C., Marzal, A., et al. (2006). Yolk androgens in the barn swallow. (*Hirundo rustica*): A test of some adaptive hypotheses. *Journal of Evolutionary Biology*, **19**, 123–131.

Gluckman, P. D., Hanson, M. A., & Beedle, A. S. (2007). Non-genetic transgenerational inheritance of disease risk. *BioEssays*, **29**, 145–154.

Goldberg, A. D., Allis, C. D., & Bernstein, E. (2007). Epigenetics: A landscape takes shape. *Cell*, **128**, 635–638.

Gonzalez, A., Lovic, V., Ward, G. R., Wainwright, P. E., & Fleming, A. S. (2001). Intergenerational effects of complete maternal deprivation and replacement stimulation on maternal behavior and emotionality in female rats. *Developmental Psychobiology*, **38**, 11–32.

Gottlieb, G. (1992). *Individual, development and evolution*. New York: Oxford University Press.

Grewal, S. I. S., & Elgin, S. C. R. (2007). Transcription and RNA interference in the formation of heterochromatin. *Nature*, **447**, 399–406.

Grewal, S. I. S., & Jia, S. (2007). Heterochromatin revisited. *Nature Reviews, Genetics*, **8**, 35–46.

Gros, J., Feistel, K., Viebahn, C., Blum, M., & Tabin, C. J. (2009). Cell movements at Hansen's node establish left/right asymmetric gene expression in the chick. *Science*, **324**, 941–944.

Guan, J.-S., Haggarty, S. J., Giacometti, E., Dannenberg, J.-H., Joseph, N., Gao, J., et al. (2009). HDAC2 negatively regulates memory formation and synaptic plasticity. *Nature*, **459**, 55–60.

Hap, S., Sharp, J. W., Ross-Inta, K. W., McDaniel, B. J., Anthony, T. G., Wek, R. C., et al. (2005). Uncharged tRNA and sensing of amino acid deficiency in mammalian piriform cortex. *Science*, **307**, 1776–1778.

Harbison, C. T., Gordon, D. B., Lee, T. I., Rinaldi, N., MacIssac, K. D., Danford, T. W., et al. (2004). Transcriptional regulatory code of a eukaryotic genome. *Nature*, **431**, 99–104.

Harper, L. V. (1989). *The nurture of human behavior*. Norwood, NJ: Ablex.

Harper, L. V. (2005). Epigenetic inheritance and the intergenerational transfer of experience. *Psychological Bulletin*, **131**, 340–360.

Harvell, C. D., & Tollrian, R. (1999). Why inducible defenses? In R. Tollrian & C.D. Harvell (Eds.), *The ecology and evolution of inducible defense* (pp. 3–9) Princeton, NJ: Princeton University Press.

Heintzman, N. D., Hon, G. C., Hawkins, R. D., Kheradpour, P., Stark, A., Harp, L. F., et al. (2009). Histone modifications at human enhancers reflect global cell-type-specific gene expression. *Nature*, **459**, 108–112.

Hekimoglu, B., & Ringrose, L. (2009). Non-coding RNAs in polycomb/trithorax regulation. *RNA Biology*, **6**, 129–137.

Henikoff, S. (2008). Nucleosome destabilization in the epigenetic regulation of gene expression. *Nature Reviews Genetics*, **9**, 15–26.

Hobart, O. (2008). Gene regulation by transcription factors and microRNAs. *Science*, **319**, 1785–1786.

Holland, P. W. H. (1999). The future of evolutionary developmental biology. *Nature*, (Supp.) **402**, C41–C44.

Huck, U. W., Labov, J. B., & Lisk, R. D. (1986). Food restricting young hamsters affects sex ratio and growth of subsequent offspring. *Biology of Reproduction*, **35**, 592–598.

Huestis, D. L., & Marshall, J. L. (2006). Interaction between maternal effects and temperature affects diapause occurrence in the cricket *Allonemobius socius*. *Oecologia*, **146**, 513–520.

Isles, A. R., Davies, W., & Wilkinson, L. S. (2006). Genomic imprinting and the social brain. *Philosophical Transactions of the Royal Society, B*, **361**, 2229–2237.

Jablonca, E., & Lamb, M. J. (1995). *Epigenetic inheritance and evolution*. Oxford: Oxford University Press.

Jablonca, E., & Lamb, M. J. (2005). *Evolution in four dimensions: Genetic, epigenetic, behavioral, and symbolic variation in the history of life*. Cambridge, MA: MIT Press.

Jenkins, N., Saam, J. R., & Mango, S. E. (2006). CYK-4/GAP provides a localized cue to initiate anterioposterior polarity upon fertilization. *Science*, **313**, 1298–1301.

Jenuwein, T., & Allis, C. D. (2001). Translating the histone code. *Science*, **293**, 1074–1080.

Kaati, G., Bygren, L. O., & Edvinsson, S. (2002). Cardiovascular and diabetes mortality determined by nutrition during parents' and grandparents' slow growth period. *European Journal of Human Genetics*, **10**, 682–688.

Kaplan, N., Moore, I., Fondufe-Mittendorf, Y., Gossett, A. J., Tillo, D., Field, Y., et al. (2009). The DNA-encoded nucleosome organization of a eukaryotic genome. *Nature*, **458**, 362–366.

Kawasaki, H., & Taira, K. (2004). Induction of DNA methylation and gene silencing by short interfering RNAs in human cells. *Nature*, **431**, 211–216.

Kishore, S., & Stamm, S. (2006). The snoRNA HBII-52 regulates alternative splicing of the serotonin receptor 2C. *Science*, **311**, 230–232.

Kloor, K. (2007). The vanishing Fremont. *Science*, **318**, 1540–1543.

Klose, R. J., Yamane, K., Bae, Y., Zhang, D., Erdjuement-Bromage, H., Tempst, P., et al. (2006). The transcriptional repressor JHDM3A demethylates trimethyl histone H3 lysine 9 and lysine 36. *Nature*, **442**, 312–316.

Kofman, O. (2002). The role of prenatal stress in the etiology of developmental behavioural disorders. *Neuroscience and Biobehavioral Reviews*, **26**, 457–470.

Kouzarides, T. (2007). Chromatin modifications and their function. *Cell*, **128**, 693–705.

Krawetz, S. A. (2005). Paternal contribution: New insights and future challenges. *Nature Reviews, Genetics*, **6**, 633–642.

Kruse, A. A., Stripling, R. R., & Clayton, D. F. (2004). Context-specific habituation of the *zenk* gene response to song in adult finches. *Neurobiology of Learning and Memory*, **82**, 99–108.

Kuper, R., & Kropelin, S. (2006). Climate-controlled holocene occupation of the Sahara: Motor of Africa's evolution. *Science*, **313**, 803–807.

Lawler, A. (2007). Middle Asia takes center stage. *Science*, **317**, 586–590.

Lee, H., Habas, R., & Ababe-Shen, C. (2004). Msx1 cooperates with histone H1b for inhibition of transcription and myogenesis. *Science*, **304**, 1675–1678.

Lee, K.-F., & Yeung, W. S. B. (2006). Gamete/embryo-oviduct interactions: Implications for *in vitro* culture. *Human Fertility*, **9**, 137–143.

Li, H., Tsang, C. K., Watkins, M., Bertram, P. G., & Zheng, X. F. S. (2006). Nutrient regulates Tor1 nuclear localization and association with rDNA promoter. *Nature*, **442**, 1058–1061.

Li, H., Wang, J., Mor, G., & Sklar, J. (2008). A neoplastic gene fusion mimics trans-splicing of RNAs in normal human cells. *Science*, **321**, 1357–1361.

Lieberman, D., Tooby, J., & Cosmides, L. (2007). The architecture of human kin detection. *Nature*, **445**, 727–731.

Ling, J. Q., Li, T., Hu, J. F., Vu, T. H., Chen, H. L., Qiu, X. W., et al. (2006). CTCF mediates interchromosomal colocalization between *Igf2/H19* and *Wsb1/Nf1*. *Science*, **312**, 269–272.

Lillycrop, K. A., Phillips, E. S., Torrens, C., Hanson, M. A., Jackson, A. A., & Burdge, G. C. (2008). Feeding pregnant rats a protein-restricted diet persistently alters the methylation of specific cytosines in the hepatic PPARalpha promoter. *British Journal of Nutrition*, **100**, 278–282.

Lillycrop, K. A., Slater-Jefferies, J. L., Hanson, M. A., Godfrey, K. M., Jackson, A. A., & Burdge, G. C. (2007). Induction of altered epigenetic regulation of the hepatic glucocorticoid receptor in the offspring of rats fed a protein-restricted diet during pregnancy suggests that reduced DNA methyltransferase-1 expression is involved in impaired DNA methylation and changes in histone modifications. *British Journal of Nutrition*, **97**, 1064–1073.

Lolle, S. M., Victor, J. L., Young, J. M., & Pruitt, R. E. (2005). Genome-wide non-Mendelian inheritance of extra-genomic information in *Arabidopsis*. *Nature*, **434**, 505–509.

Lott, D. F. (1984). Intraspecific variation in the social systems of wild vertebrates. *Behaviour*, **88**, 266–325.

MacRae, I. J., Zhou, K., Li, F., Repic, A., Brooks, A., Cande, W. Z., et al. (2006). Structural basis for double-stranded RNA processing by dicer. *Science*, **311**, 195–198.

Martin, C., & Zhang, Y. (2007). Mechanisms of epigenetic inheritance. *Current Opinion in Cell Biology*, **19**, 266–272.

Matlin, C., Clark, F., & Smith, C. W. J. (2005). Understanding alternative splicing: Towards a cellular code. *Nature Reviews, Molecular and Cellular Biology*, **6**, 386–398.

McClintock, B. (1984). The significance of responses of the genome to challenge. *Science*, **226**, 792–801.

Mclinn, C., & Stephens, D. W. (2006). What makes information valuable: Signal reliability and environmental uncertainty. *Animal Behaviour*, **71**, 1119–1129.

McMillen, I. C., & Robinson, J. S. (2005). Developmental origins of the metabolic syndrome: Prediction, plasticity, and programming. *Physiological Review*, **85**, 571–633.

Meaburn, K. J., & Misteli, T. (2007). Chromosome territories. *Nature*, **445**, 379–381.

Mech, L. D., Nelson, M. E., & McRoberts, R. E. (1991). The effects of maternal and grandmaternal nutrition on deer mass and vulnerability to wolf predation. *Journal of Mammalogy*, **72**, 146–151.

Mercer, T. R., Dinger, M., & Mattick, J. S. (2009). Long non-coding RNAs: Insights into functions. *Nature Reviews, Genetics*, **10**, 155–159.

Mercer, T. R., Dinger, M. E., Mariani, J., Kosik, K. E., Mehler, M. F., & Mattick, J. S. (2008). Noncoding RNAs in long-term memory formation. *The Neuroscientist*, **14**, 434–445.

Metivier, R., Gallais, R., Tiffoche, C., Le Peron, C., Jurkowska, R. Z., Carmouche, R. P., et al. (2008). Cyclical DNA methylation of a transcriptionally active promoter. *Nature*, **452**, 45–50.

Misteli, T. (2007). Beyond the sequence: Cellular organization and genome function. *Cell*, **128**, 787–800.

Mitchell, S. E., & Read, A. F. (2005). Poor maternal environment enhances offspring disease resistance in an invertebrate. *Proceedings of the Royal Society B*, **272**, 2601–2607.

Mizuguchi, G., Shen, X., Landry, J., Wu, W.-H., Sen, S., & Wu, C. (2004). ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. *Science*, **303**, 343–348.

Morris, K. V., Chan, S. W.-L., Jacobsen, S. E., & Looney, D. J. (2004). Small interfering RNA- induced transcriptional gene silencing in human cells. *Science*, **305**, 1289–1292.

Morison, I. M., Ramsay, J. P., & Spencer, H. G. (2005). A census of mammalian imprinting. *Trends in Genetics*, **21**, 457–465.

Mousseau T., & Fox, C.W. (Eds.). (1998). *Maternal effects as adaptations*. New York: Oxford University Press.

Naguib, M., & Gil, D. (2005). Transgenerational effects on body size caused by early developmental stress in zebra finches. *Biology Letters*, **1**, 95–97.

Newman, J. R. S., Ghaemmaghami, S., Ihmels, J., Breslow, D. K., Noble, M., DeRisi, J. L., et al. (2006). Single-cell proteomic analysis of *S. cerevisiae* reveals the architecture of biological noise. *Nature*, **441**, 840–846.

Nguyen, D. K., & Disteche, C. M. (2006). Dosage compensation of the active X chromosome. *Nature Genetics*, **38**, 47–53.

Ostermeier, G. C., Miller, D., Huntriss, J. D., Diamond, M. P., & Krawetz, S. A. (2004). Delivering spermatozoan RNA to the oocyte. *Nature*, **429**, 154.

Oyama, S. (1985). *Ontogeny of information: Developmental systems and evolution*. Cambridge: Cambridge University Press.

Pedraza, J.M., & van Oudenaarden, A. (2005). Noise propagation in gene networks. *Science*, **307**, 1965–1969.

Pembrey, M., Bygren, L. O., Kaati, G., Edvinsson, S., Northstone, K., Sjostrom, M., et al. (2006). Sex-specific, male-line transgenerational responses in humans. *European Journal of Human Genetics*, **14**, 159–166.

Perillo, B., Ombra, M. N., Bertoni, A., Cuozzo, C., Sacchetti, S., Sasso, A., et al. (2008). DNA oxidation as triggered by H3K9me2 demethylation drives estrogen-induced gene expression. *Science*, **319**, 202–206.

Petersen, C., & Soder, O. (2006). The Sertoli cell – A hormonal target and "super" nurse for germ cells that determines testicular size. *Hormone Research*, **66**, 153–161.

Petry, C. J., Ong, K. K., & Dunger, D. B. (2007). Does the fetal genotype affect maternal physiology during pregnancy? *Trends in Molecular Medicine*, **13**, 414–421.

Piotrowska, K., & Zernicka-Goetz (2001). Role for sperm in spatial patterning of the early mouse embryo. *Nature*, **409**, 517–521.

Rakyan, V. K., Chong, S., Champ, M. E., Cuthbert, P. C., Morgan, H. D., Luu, K. V. K., et al. (2003). Transgenerational inheritance of epigenetic states at the murine *AxinFu* allele occurs after maternal and paternal transmission. *Proceedings of the National Academy of Sciences, USA*, **100**, 2538–2543.

Rassoulzadegan, M., Grandjean, V., Gounon, P., Vincent, S., Gillot, I., Cuzin, F. (2006). RNA-mediated non-Mendelian inheritance of an epigenetic change in the mouse. *Nature*, **441**, 469–474.

Reddy, K. L., Zullo, J. M., Bertolino, E., & Singh, H. (2008). Transcriptional repression mediated by repositioning of genes to the nuclear lamina. *Nature*, **452**, 243–247.

Reik, W. (2007). Stability and flexibility of epigenetic gene regulation in mammalian development. *Nature*, **447**, 425–432.

Reik, W., Romer, I., Barton, S. C., Surani, M. A., Howlett, S. K., & Close, J. (1993). Adult phenotype in the mouse can be affected by epigenetic events in the early embryo. *Development*, **119**, 933–942.

Rosenberg, S. S., Keland, E. E., Tokar, E., Torre, A. R., & Chan, J. R. (2008). Geometric and spatial constraints of the microenvironment induce oligodendrocyte differentiation. *Proceedings of the National Academy of Sciences, USA*, **105**, 14662–14667.

Ross, M. T., Grafham, D. V., Coffey, A. J., Scherer, S., McLay, K., Muzny, D., et al., (2005). The DNA sequence of the human X chromosome. *Nature*, **434**, 325–337.

Rossiter, M. (1998). The role of environmental variation in parental effects expression. In T. Mousseau & C.W. Fox (Eds.), *Maternal effects as adaptations* (pp. 112–134). New York: Oxford University Press.

Sale, A., Cenni, M. C., Ciucci, F., Putignano, E., Chierzi, S., & Maffei, L. (2007). Maternal enrichment during pregnancy accelerates retinal development of the fetus. *PLos ONE*, **2**(11), e1160. doi: 10.1371/journal.pone.0001160.

Salmons, S., & Streter, F. A. (1976). Significance of impulse activity in the transformation of skeletal muscle type. *Nature*, **263**, 30–34.

Sanchez-Elsner, T., Gou, D., Kremmer, E., & Sauer, F. (2006). Noncoding RNAs of trithorax response elements recruit *Drosophila* Ash1 to ultrabithorax. *Science*, **311**, 1118–1123.

Schmidt-Hieber, C., Jonas, P., & Bischofberger, J. (2004). Enhanced synaptic plasticity in newly generated granule cells in the adult hippocampus. *Nature*, **429**, 184–187.

Schratt, G. M., Tuebing, F., Nigh, E. A., Kane, C. G., Sabatini, M. E., Kieber, M., et al. (2006). A brain-specific microRNA regulates dendritic spine development. *Nature*, **439**, 283–289.

Segal, E., Fondufe-Mittendorf, Y., Chen, L., Thastrom, A., Field, Y., Moore, I. K., et al. (2006). A genomic code for nucleosome positioning. *Nature*, **442**, 772–778.

Segal, E., Raveh-Sadka, T., Schroeder, M., Unnerstaff, U., & Gaul, U. (2008). Predicting expression patterns from regulatory sequence in *Drosophila* segmentation. *Nature*, **451**, 535–540.

Selbach, M., Schwanhausser, B., Thierfelder, N., Fang, Z., Khanin, R., & Rajewsky, N. (2008). Widespread changes in protein synthesis induced by microRNAs. *Nature*, **455**, 58–63.

Shine, R., & Downes, S. J. (1999). Can pregnant lizards adjust their offspring phenotypes to environmental conditions? *Oecologia*, **119**, 1–8.

Simpson, S. J., & Miller, G. (2007). Maternal effects on phase characteristics in the desert locust, *Schistocerca gregaria:* A review of current understanding. *Journal of Insect Physiology*, **53**, 869–876.

Singh, S. K., Kagalwala, M. N., Parker-Thornburg, J., Adams, H., & Majumder, S. (2008). REST maintains self-renewal and pluripotency of embryonic stem cells. *Nature*, **453**, 223–227.

Skinner, M. K., Anway, M. D., Savenkova, M. I., Gore, A. C., & Crews, D. (2008). Transgenerational epigenetic programming of the brain transcriptome and anxiety behavior. *PLoS ONE*, **3**(11), e3745. doi: 10.1371/journal.pone.0003745.

Skuse, D. H., James, R. S., Bishop, D. V. M., Coppin, B., Dalton, P., Aanodt-Leeper, G., et al. (1997). Evidence from Turner's syndrome of an imprinted, X-linked locus affecting cognitive function. *Nature*, **387**, 705–708.

Sleutels, F., Zwart, R., & Barlow, D. P. (2002). The non-coding *Air* RNA is required for silencing autosomal imprinted genes. *Nature*, **415**, 810–813.

Spadafora, C. (2008). Sperm-mediated "reverse" gene transfer: A role of reverse transcriptase in the generation of new genetic information. *Human Reproduction*, **23**, 735–740.

Stein, A. D., Barnhart, H. X., Hickey, M., Ramakrishnan, U., Schroeder, D. G., & Martorell, R. (2003). Prospective study of protein-energy supplementation early in life and growth in the subsequent generation in Guatemala. *American Journal of Clinical Nutrition*, **78**, 162–167.

Suomi, S. J., & Levine, S. (1998). Psychobiology of intergenerational effects of trauma. Evidence from animal studies. In Y. Danilei (Ed.), *International handbook of multigenerational legacies of trauma*. (pp. 623–637). New York: Plenum Press.

Suzuki, Y., & Nijhout, H. F. (2006). Evolution of a polyphenism by genetic accommodation. *Science*, **311**, 650–652.

Taborsky, B. (2006). Mothers determine offspring size in response to own juvenile growth conditions. *Biology Letters*, **2**, 225–228.

Tagkopoulos, H., Liu, Y.-C., & Tavazoie, S. (2008). Predictive behavior within microbial genetic networks. *Science*, **320**, 1313–1317.

Tam, O. H., Aravin, A. A., Stein, P., Girard, A., Murchison, E. P., Cheloufi, S., et al. (2008). Pseudo-gene derived small interfering RNAs regulate gene expression in mouse oocytes. *Nature*, **453**, 354–358.

Tanay, A., O'Donnell, A. H., Damelin, M., & Bestor, T. (2007). Hyperconserved CpG domains underlie Polycomb-binding sites. *Proceedings of the National Academy of Sciences, USA*, **104**, 5521–5526.

Tang, F., Kaneda, M., O'Carroll, D., Hajkova, P., Barton, S. C., Sun, Y. A., et al. (2007). Maternal micro RNAs are essential for mouse zygotic development. *Genes & Development*, **21**, 644–648.

Tay, Y., Zhang, J., Thomson, A. M., Lim, B., & Rigoutsos, I. (2008). MicroRNAs to *Nanog*, *Oct4* and *Sox2* coding regions modulate embryonic stem cell differentiation. *Nature*, **455**, 1124–1128.

Torres-Padilla, M.-E., Parfitt, D.-E., Kouzarides, T., & Zernicka-Goetz, M. (2007). Histone arginine methylation regulates pluripotency in the early mouse embryo. *Nature*, **445**, 214–218.

Tyson, J. J., Chen, K. C., & Novak, B. (2003). Sniffers, buzzers, toggles and blinkers: Dynamics of regulatory and signaling pathways in the cell. *Current Opinion in Cell Biology*, **15**, 221–231.

Ule, J., Stefani, G., Mele, A., Ruggiu, M., Wang, X., Taneri, B., et al. (2006). An RNA map predicting Nova-dependent splicing regulation. *Nature*, **444**, 580–586.

Vagin, V. V., Sigova, A., Li, C., Seitz, H., Gvozdev, V., & Zamore, P. D. (2006). A distinct small RNA pathway silences selfish genetic elements in the germline. *Science*, **313**, 320–324.

van der Heijden, G. W., Derijk, A. A. H. A., Ramos, L., Giele M., van der Vlag, J., & de Boer, P. (2006). Transmission of modified nucleosomes from the male mouse germline to the zygote and subsequent remodeling of paternal chromatin. *Developmental Biology*, **298**, 458–469.

Vasquez, D. M., Lopez, J. F., Van Hoers, H., Watson, S. J., & Levine, S. (2000). Maternal deprivation regulates serotonin 1A and 2A receptors in the infant rat. *Brain Research*, **855**, 76–82.

Vasudevan, S., Tong, Y., & Seitz, J. A. (2007). Switching from repression to activation: MicroRNAs can up-regulate translation. *Science*, **318**, 1931–1934.

Viré, E., Brenner, C., Deplus, R., Blanchon, L., Fraga, M., Didelot, C., et al. (2006). The polycomb group protein EZH2 directly controls DNA methylation. *Nature*, **439**, 871–874.

Waddington, C. H. (1942). Canalization of development and the inheritance of acquired characteristics. *Nature*, **150**, 563–565.

Waddington, C. H. (1952). Selection of the genetic basis for an acquired character. *Nature*, **169**, 278.

Waddington, C. H. (1956). *Principles of embryology*. (4th ed.)London: George, Allen & Unwin.

Wade, M. J. (1998). The evolutionary genetics of maternal effects. In T. Mousseau & C.W. Fox (Eds.), *Maternal effects as adaptations*. (pp. 5–21). New York: Oxford University Press.

Wassenegger, M. (2005). The role of the RNAi machinery in heterochromatin formation. *Cell*, **122**, 13–16.

Waterland, R. A., & Jirtle, R. L. (2004). Early nutrition, epigenetic changes at transposons and imprinted genes, and enhanced susceptibility to adult chronic diseases. *Nutrition*, **20**, 63–68.

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. Oxford: Oxford University Press.

Wilkins, J. F. (2005). Genomic imprinting and methylation: Epigenetic canalization and conflict. *Trends in Genetics*, **21**, 356–365.

Wilmut, I., Schneike, A. E., McWhir, J., Kind, A. J., & Campbell, K. H. S. (1997). Viable offspring derived from fetal and adult mammalian tissues. *Nature*, **385**, 810–813.

Wu, Q., & Suzuki, M. (2006). Parental obesity and overweight affect the body-fat accumulation in offspring: The possible effect of a high-fat diet through epigenetic inheritance. *Obesity Reviews*, **7**, 201–208.

Xu, N., Tsai, C.-L., & Lee, J. T. (2006). Transient homologous chromosome paring marks the onset of X inactivation. *Science*, **311**, 1149–1152.

Xu, N., Papagiannakopoulos, T., Pan, G., Thomson, J. A., & Kosik, K. S. (2009). MicroRNA-145 regulates OCT4, SOX2, and KLF4 and rescues pluripotency in human embryonic stem cells. *Cell*, **137**, 647–658.

Yang, P. K., & Kuroda, M. I. (2007). Noncoding RNAs and intranuclear positioning in monoallelic gene expression. *Cell*, **128**, 777–786.

Yi, R., Poy, M. N., Stoffel, M., & Fuchs, E. (2008). A skin microRNA promotes differentiation by repressing "stemness." *Nature*, **452**, 225–229.

Young, D. W., Hassan, M. Q., Pratap, J., Galindo, M., Zaidi, S., Lee, S. et al. (2007). Mitotic occupancy and lineage-specific transcriptional control of *rRNA* genes by Runx2. *Nature*, **445**, 442–446.

Zamenhof, S., van Marthens, E., & Grauel, L. (1971). DNA (cell number) in neonatal brain: Second generation (F2) alteration by maternal (F0) dietary protein restriction. *Science* **172**, 850–851.

Zamore, P. D., & Haley, B. (2005). Ribo-gnome: The big world of small RNAs. *Science*, **309**, 1519–1524.

Zernicka-Goetz, M. (2002). Patterning of the embryo: The first spatial decisions in the life of a mouse. *Development*, **129**, 815–829.

15

The Significance of Non-Replication of Gene-Phenotype Associations

Carolyn Tucker Halpern

The absence of strict predictability is now recognized . . . as a defining feature of development. (Gottlieb, 2003, p. 341)

It is widely acknowledged in the molecular genetics and genetic epidemiology literatures that the majority of reported gene–phenotype associations are not replicated, or are erratically replicated (Hirschhorn, Lohmueller, Byrne, & Hirschhorn, 2002; Ioannidis, 2007). (As examples, see reviews related to the dopaminergic and serotonergic systems (e.g., Bobb, Castellanos, Addington, & Rapoport, 2006; D'Souza & Craig, 2006; Munafò, Durrant, Lewis, & Flint, 2009; Talkowski, Bamne, Mansour, & Nimgaonkar, 2007; Wong, Buckle, & Van Tol, 2000).) In fact, depending on how strictly one defines replication, even a biometric or population (i.e., variance partitioning) approach to behavioral genetics could be seen as exhibiting extensive non-replication as the heritability of a phenotype in one population does not necessarily predict the heritability of the same phenotype in another population (Visscher, Hill, & Wray, 2008). In reviews and evaluations of the state of our knowledge regarding a given gene–phenotype association, and reasons for non-replication, discussion primarily focuses on design and methodological issues that are central to *population analysis*. However, to further our understanding of this issue in relation to individual development, it is critical to consider the meaning of non-replication of gene – phenotype associations in the context of the nature of the causal processes that move development forward.

Population genetics centers on quantitative analysis or variance partitioning of genetic and environmental contributions to individual differences. Historically, specific genetic and environmental factors have not been explicitly identified in behavioral genetic analyses as derived from population genetics. Although it is

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increasingly appreciated that this nonspecificity is not enlightening or useful (cf., Rutter, Pickles, Murray, & Eaves, 2001), and that more detail about particular environmental factors operating in particular contexts is needed, the focus remains on between-individual variability. Technical developments in molecular genetics have allowed for more specificity about genetic factors (i.e., investigating specific variants or polymorphisms). Again, however, developmental associations are approached from the perspective of examining individual differences, with the general logic that a given genotype is associated with a particular phenotype and the variant genotype is associated with a different phenotype. In contrast, a developmental analysis of the individual requires a focus on the individual, not between-individual differences, and on the ongoing bidirectional processes that yield growth and change.

In two *Human Development* articles published almost 10 years apart (1995, 2003), Gilbert Gottlieb described the incongruity of a population approach to behavioral genetics with the goal of understanding individual development. Gottlieb noted that because population behavioral genetics is a statistical enterprise, assessment of heritability cannot enlighten us about the course of individual development. Variation between individuals, based on information derived from pooling across persons, does not explain developmental change (or variation) within individuals because typically conditions do not allow for application of population findings to individuals (Gottlieb, 1995; Molenaar & Campbell, 2009; Molenaar, Huizenga, & Nesselroade, 2003). Along with like-minded theorists of developmental science and biological systems (e.g., Dewey & Bentley, 1949; Lerner & Kaufman, 1985; Sameroff, 1983; Valsiner, 1987), Gottlieb argued that development reflects a process of probabilistic epigenesis, that is, development is the relationship between two (or more) components of a multi-level psychobiological developmental system (Gottlieb, 1991). Thinking of genetic contributions specifically, development reflects the "continued dependence on gene-environment coactions" (Gottlieb, 2003, p. 10). If we accept Gottlieb's (or a similar) meta-theory of development, then our efforts to describe and understand development require an investigatory approach that is longitudinal, bidirectional, focused on the individual, and includes both biological and experiential factors. Further, our expectations about the replicability of demonstrated gene–phenotype associations must accommodate the probabilistic nature of development.

In this chapter I briefly discuss various conceptualizations of replication and describe the high prevalence of non-replication in the gene–phenotype literature. I then enumerate frequently cited reasons for non-replication as seen from a population perspective, and explore the implications of expanding work in genome-wide association studies (GWAS) in relation to these methodological considerations. Finally I contrast the population perspective on replication with the perspective of Gottlieb's Developmental-Psychobiological Systems Model of

individual development, and discuss the implications of a probabilistic model of development for conceptualizations of replication.

The Concept of Replication

According to one estimate, 19 out of 20 reported marker-disease associations are false (Colhoun, McKeigue, & Davey Smith, 2003), with the judgment of "false" being primarily determined by empirical non-replication. Replication is a fundamental component of the scientific method and is the criterion that has assumed the most importance in inferring causality in the gene-phenotype literature (Campbell & Manolio, 2007). However, despite the *universally* professed importance of replication, there are probably more attempts at replication in the gene–phenotype association literature than in many other scientific topic areas. In fact, consensus among investigators in the field (Chanock et al., 2007), and the policy of some journals, preclude the publication of findings from genome-wide association studies without demonstrated replication in independent samples (Spencer, Su, Donnelly, & Marchini, 2009). Thus the many demonstrations of non-replication in the genetic literature may be partly a function of the larger number of attempts at replication.

At the most basic level, investigators expect that by repeating a well-designed experiment or observational analysis they can produce one or more additional demonstrations of a "significant effect," which in the gene–phenotype literature (as in most social science literature) has typically been defined in the framework of null-hypothesis significance testing. That is, wherein a statistic (e.g., an "effect" indicated by the absolute difference between means) can be deemed "significantly" different from zero with some degree of scientifically acceptable probability (e.g., $p < .05$; Killeen, 2005). The level of acceptable statistical probability indicates our cultural comfort in attributing differences or associations to the effects of systematic versus accidental factors. Null-hypothesis significance testing is based on hypothetical values for parameters and inferred sampling distributions, and reflects a frequentist account of probability, that is a real, physical (or objective) tendency of something to occur.

More broadly, however, the concept of replication can have different meanings. For example, it can refer to repeating a study, analysis or experimental procedure; it can also refer to achieving a similar result or finding, or it can refer to "reproducibility of the theoretical interpretation" (Schmidt, 2009 following Radder, 1992). Within the meaning of duplicating a finding, there is ambiguity about what "successful" replication is, and whether it is more useful to aim for direct or strict replication (i.e., an exact repetition of a study in terms of design, population, measures, research setting, etc. with the hope of demonstrating an association or effect of the same direction and similar size) or to aim for conceptual replication, wherein aspects of the earlier study are systematically varied. The two types of replication reflect differences in the scientific goal of the replication effort – that is,

whether the goal is to confirm "facts" with relatively little regard for external validity, or to extend the generality of the originally demonstrated association and thereby further develop models and theories. The latter goal typically is approached via multi-method, multi-variable replication efforts.

In reality, it may never be possible to implement a direct or strict replication because of ongoing change in multiple factors such as the experiential history of respondents or the social-historical-cultural context of the research, which preclude conducting *exactly the same* study (Rosenthal, 1990; van der Veer, van Ijzendoorn, & Valsiner, 1994). In this view, "strict" can only refer to an "acceptable degree of similarity" (Danziger & Shermer, 1994, p. 18). Further, what is deemed an "acceptable replication" will vary across time, disciplinary perspectives, and emerging fields. In multidisciplinary collaborations, such as many investigations of gene–phenotype associations, there may be disagreement in what collaborators deem to be "acceptable similarity." Disciplinary prescriptions about what needs to be replicated, and how it could be replicated, reflect differing opinions on the proper questions to be asked and fundamental conceptions of the subject matter. As will be discussed further, approaching development from the perspective of intra-individual variation versus inter-individual variation, as reflected in population analyses, is an example of such a fundamental difference.

The approach to integrating the findings of attempted replications also varies. It has not been unusual for reviews of gene–phenotype associations to simply count up the number of significant and non-significant findings and compare the totals (i.e., how often the null hypothesis was rejected versus not rejected). If studies conducted after the original report of a gene–phenotype association fail to consistently show statistically significant associations in the same direction (i.e., fail to reject the null hypothesis because of failing to get an acceptably small or "significant" p value), the association is deemed questionable or not replicated. However, even this simple, and many would say inadequate, approach to determining "replication" is subject to different schools of thought, not elaborated here, as to what constitutes "consistent" and what level of statistical significance (i.e., what is an acceptably small p value) is needed at different points in the discovery and replication phases of an investigation (Chanock et al., 2007).

More recently, empirical meta-analyses, which offer formal statistical methods to synthesize findings across studies, have become more common. However, these also entail a series of decisions about how to conduct the analysis, as will be described later in this chapter, and are controversial for observational studies because of the potential for confounding, selection effects, and publication bias. Further, methods for the meta-analysis of studies investigating interactions are not yet well developed. For example, Taylor and Kim-Cohen (2007) conducted a meta-analysis of an interaction effect (described in more detail later in this chapter) using differences in correlations between the environmental factor and the selected phenotype for each genotype group as the effect measure. Meta-analysis by genotype group determines the consistency of the associations across studies

by genotype (Taylor & Kim-Cohen, 2007). However, difficulties such as converting effect measures to a common metric, choice of method for meta-analysis of correlations, and especially choice of a genetic model can have an effect on comparability of findings across studies. Meta-analysis of correlations is limited to two genotype groupings (dominant or recessive); thus some genotype groups may need to be collapsed, thereby losing information.

An important concept related to non-replication in the gene–phenotype association literature is "inconsistency," which is the characteristic of large between-study heterogeneity in the *magnitude* of empirically demonstrated gene–phenotype associations (Ioannidis, 2007). Although cross-study variation in effect size is considered by some to detract from evidence of a "real" gene–phenotype association, the metric of comparing effect size (as opposed to exclusive reliance on null hypothesis testing) has been suggested as a more meaningful way to assess replication (e.g., Rosenthal, 1990). Because of the low statistical power of many studies, there is no reason to expect high proportions of significant results across studies even when there is a "real" effect, or to necessarily conclude that several failures to reject the null hypothesis demonstrate the absence of an association (Ioannidis, Ntzani, Trikalinos, & Contopoulos-Ioannidis, 2001; Rosenthal, 1990).

Putting the issue of probabilistic development aside for the moment, imprecision alone in measures of genetic variants, experience, context, and developmental history (e.g., variations in experience that may be labeled or grouped as the same) should lead us to expect variation in the degree of associations we demonstrate. Thus, some authors reject dichotomous decision making about replication (i.e., an association is "replicated" when several similar studies have rejected the null hypothesis) as untenable (Rosenthal, 1990), and advocate examination of the "inconsistency" in association size across studies as a better approach to the issue of replication. A focus on inconsistency would indicate framing assessments of replication in terms of effect sizes and confidence intervals (CIs) because they provide more relevant information. "On average, a 95% CI will capture about 83% of replication means" (Cumming & Fidler, 2009, p. 19). Thus a CI can be thought of as a prediction interval for future replication means (Cumming & Fidler, 2009). A focus on confidence intervals yields a continuous, more precise indicator of replication. Although the "continuous" approach is more suitable for a probabilistic model of development, as will be elaborated later, both it and the null hypothesis model have limitations in reference to replication of gene–phenotype associations in probabilistic approaches to individual development.

Reasons for Non-Replication from a Population Perspective

Explanations of non-replication and inconsistency in gene–phenotype associations are usually approached from a population perspective, and center on issues of study

design and methodology. This literature includes a long list of potential contributors to non-replication, treated only briefly here. One prominent, widely documented problem is that of inflated "first effects" or "winner's curse" (Kraft, 2008; Lin, Vance, Pericak-Vance, & Martin, 2007; Van den Oord, 2008; Zollner & Pritchard, 2007). That is, initial strong associations between gene and phenotype, often based on small samples, that are not replicated in later studies, or that are demonstrated but are much smaller in magnitude. Given the typically small contribution of any given genetic variant to a phenotype or phenotype precursor, inflated first effects often lead, in turn, to underpowered replication efforts and consequently to failure to find (replicate) a significant association. Changes in the strengths of associations across studies have been evident in a variety of topic areas, such as alcoholism (DRD2), Parkinson's disease (CYP2D6), and schizophrenia (DRD3). The evolution of demonstrated associations over time for these and other markers is nicely illustrated in a 2001 publication by Ioannidis and colleagues.

Among other frequently cited reasons for non-replication are population stratification (differences in allele frequency and phenotype of interest that are related to imperfect matching between case and control subjects, and that may therefore produce spurious gene–phenotype associations); failure to adjust p-values when multiple hypotheses are tested (and differences of opinion about how adjustment should be done), thereby increasing the likelihood of false positives (Type I error); various methodological deficiencies (e.g., faulty selection of subjects for comparisons, differences in storage and genetic analysis of samples collected from cases and controls); differences in analytical choices (e.g., logistic regression versus hazard models); differences in phenotype definition and measurement, or in exposure/control classification; genotyping errors; differences in statistical power across studies; the possibility of missing true "causal" variants because of selective genotyping of markers; and publication biases (Colhoun et al., 2003; Ioannidis, 2008; Khoury, Little, Gwinn, & Ioannidis, 2007; Van den Oord, 2008).

As an illustration of a population approach to assessment of replication, Munafò, Matheson, and Flint (2007) conducted a meta-analysis of 40 case-control studies investigating the association between the DRD2 Taq1A polymorphism and alcoholism. They were especially interested in evaluating publication bias as a source of inconsistency or between-study heterogeneity. They concluded there is a small but statistically significant association between the polymorphism and phenotype, and evidence of possible publication bias as well as an association between publication year and effect size (larger effect size in earlier studies as also demonstrated by Ioannidis et al., 2001). Munafò and collaborators estimated the DRD2 Taq1A polymorphism accounted for 0.2% of variance in alcoholism after adjustment for publication bias, clearly a modest contribution from a population perspective.

"Biological and phenotypic complexity" and "true differences" between study populations – sometimes defined as the result of environmental or genetic

modifiers – have also been cited as reasons for non-replication (Cardon & Palmer, 2003; Hirschhorn & Altshuler, 2002). Despite past demonstrations that the use of small sample sizes has precluded identification of significant interactions between context and genotype (Wahlsten, 1990), some authors have dismissed gene effect modification by other genetic or environmental factors (i.e., heterogeneity of effect size between studies) as unlikely or "to be established" (e.g., Todd, 2006). As will be described later, complexity and "true differences" are aspects of Gottlieb's argument about why gene–phenotype associations often fail to replicate. However in population oriented analyses, these "true differences" are not typically viewed as part of the phenomenon or development to be explained, but rather as something of a nuisance that obscures the "true effect" of genes. This assumption of a "true effect" of genes is akin to the past tendency, described by Kindermann & Valsiner (1995), of developmental researchers to try to measure "true developmental change" by controlling or assuming a static environment. Attempts to assume or impose a static context when investigating dynamic process are antithetical to a developmental systems perspective.

To address the pervasive problem of non-replication, the National Cancer Institute-National Human Genome Research Institute (NCI-NHGRI) Working Group on Replication in Association Studies has published a set of criteria for replication (Chanock et al., 2007). These criteria: 1) are directed at correcting for the methodological issues described above (e.g., sufficient sample size, independent data sets, same or similar phenotype measured, etc.); 2) incorporate the null-hypothesis significance testing model; and 3) assume that direct replication is possible. The criteria define replication essentially as "strict" homogeneity of findings ("similar magnitude of effect and significance should be demonstrated, in the same direction, with the same SNP [single nucleotide polymorphism] or a SNP in perfect or very high linkage disequilibrium with the prior SNP [r^2 close to 1.0];" Chanock et al., 2007, p. 658).

Genome-Wide Association Studies and Implications for a Population View of Replication

Traditional pedigree-based linkage studies and candidate gene approaches (the bulk of gene–phenotype association literature until recently) are by design directed more toward rare genetic variants that are thought to offer larger contributions to phenotypes. However, increasing acknowledgment of the likely minor contribution of any given polymorphism to a given phenotype has led many investigations to adopt the common disease–common variant (i.e., seen in 5% or more of the population) model, which stipulates that common diseases are caused by (hopefully relatively few) genetic variants that individually have small effects. Technological advances (e.g., micro-satellite panels and high density

genechip SNP arrays) and the genome data provided by the Human Genome Project, International HapMap Project and others have opened the door to genome-wide association studies, which are based on the common disease–common variant model.

The National Institutes of Health (NIH) define a genome-wide association study as a study of common genetic variation across the entire human genome designed to identify genetic associations with observable traits (NIH, 2007). The trait might be qualitative (e.g., having or not having a disease) or quantitative (e.g., height or personality score), and typically thousands of bivariate associations between genetic markers and the phenotype of interest are examined. Because of the haplotypic structure of the human genome, it is possible to identify common, low-risk variants using a subset of well-chosen markers. The association of SNPs that tend to be inherited together (linkage disequilibrium) allows for imputation of other SNPs (see Marchini, Howie, Myers, McVean, & Donnelly, 2007 for an example of one imputation method). That is, SNPs that are not directly genotyped are "tagged;" because of linkage disequilibrium tagged SNPs can be predicted by one or more SNPs that are directly genotyped. Although this approach allows for a reduction in the number of markers to be genotyped, it also clouds the picture in efforts to determine which variants may be more directly involved in contributing to a phenotype (Ku & Chia, 2008). Genome-wide association studies are becoming more common, although scientists suggest that "the definition of even what constitutes a genome-wide investigation is a moving target, given the continuous increase in the number of polymorphisms that can be covered by available genotyping platforms" (Ioannidis, 2007, p. 205).

Genome-wide association studies are atheoretical and are viewed as an important discovery tool. The extensive use of tests of bivariate associations between SNPs and complex phenotypes implicitly assumes the probable detection and priority of genetic "main effects." Genome-wide association studies vastly compound many of the methodological challenges described earlier. As in candidate gene studies, inflated first effects (winner's curse) are an issue in GWAS for similar reasons. The magnitude of the "winner's curse" phenomenon depends on the underlying distribution of effect sizes; the greater the number of variants with small effects, the more likely one or more of these variants will approach significance (Lettre et al., 2008). Further, variants conferring the highest relative risks will likely be identified first and therefore be overrepresented in the first wave of findings from genome-wide association studies (Kraft & Hunter, 2009). As noted earlier, there is a preference in the field for genome-wide studies to use a multi-stage design to control Type I error and maintain power. In such a design, discovery work is done in one sample, and promising SNPs are examined again in another sample (e.g., split-half of one original, large sample [internal replication] or a separate sample [external replication]). However, one possible disadvantage of this approach is that "promising SNPs" may only be those that have relatively large contributions to the phenotype of interest.

As one illustration of a multi-stage study directed at common variation across the human genome, O'Donovan et al. (2008) conducted a genome-wide association study based on two replication samples to identify genetic loci associated with schizophrenia. There were 362,532 SNPs that passed quality control. The GWAS yielded 12 loci (i.e., significant bivariate associations); six were replicated in the first sample. Of these six, three had strong support in replication sample number 2. Meta-analysis pointed to one locus in particular, however none of the loci "implicates clear functional candidates on the basis of current understanding of pathophysiology" (O'Donovan et al., 2008, p. 1055). The authors note that their analytical strategy fits within the (individual difference) paradigm of multiple loci with small effects, which it does. The investigators followed currently accepted GWAS methodology, reflecting the approach of many other genome-wide efforts. However, it is interesting to speculate about how such an approach would be received in other disciplines that prioritize theory driven hypothesis testing. For example, if a social scientist tested almost 400,000 bivariate associations, and replicated three of them two times, would peer reviewers be convinced that a meaningful contributor to the outcome of interest had been detected (even with controls for multiple testing), especially if there were no theoretical (or biological) reason to assume that these three variables are associated with the outcome?

As is evident from the above, GWAS have the clear disadvantage of wildly increasing the opportunity for false positives, given the number of possible statistical tests. "At the conventional $p < .05$ significance, an association study of 1 million SNPs will show 50,000 to be 'associated' with disease, almost all false positives and due to chance alone" (Pearson & Manolio, 2008, pp. 1341–1342). This consequence is even more pervasive with any attempts to investigate the many possible interactions. Although most GWAS assume a simple additive model, "for every n SNPs or genes there are $n(n-1)/2$ pairwise interactions. A genome-wide scan with 1 million SNPs (3 kb coverage) will afford 10^{12} possible pairwise tests of SNP by SNP interactions" (Clark, Boerwinkle, Hixson, & Sing, 2005, p. 1464). The Bonferroni correction is most commonly used to adjust for multiple tests, but it is quite conservative (Cardon & Bell, 2001) and demands a level of statistical significance (e.g., $p < 5 \times 10^{-8}$) necessitating extremely large sample sizes. As in candidate gene studies, initial estimates of odds ratios are usually biased upwards, and subsequent replication studies may not be adequately powered to detect what is actually a smaller association (e.g., odds ratios of 1.1–1.4; Ioannidis, 2008). Although theoretically addressable with the use of large samples, reliance on extremely large samples may preclude the collection of information about personal characteristics and physical/psychosocial context which, as will be argued in the next section, would allow for more meaningful examination of coactional processes. In fact when measures of environmental factors and phenotypes are continuous, smaller studies that have repeated and more precise measurements can achieve the

statistical power of much larger sample sizes (Wong, Day, Luan, Chan, & Wareham, 2003). In sum, many of the "population reasons" for non-replication are potentially compounded in GWAS.

Despite these concerns, the GWAS literature does include some notable consistencies for diverse common diseases, including age-related macular degeneration, prostate cancer, Crohn disease, and type I diabetes mellitus (Ennis et al., 2007; Gudmundsson et al., 2007; Libioulle et al., 2007; Saxena et al., 2007). Nevertheless, most genome-wide association work done to date, like candidate gene studies, suffers from extensive problems with non-replication, which is largely attributed to methodological issues. Consideration of the omission of information about context and life experience in the original studies and in discussions of why cross-study results are inconsistent is relatively rare. A series of articles reviewing the efforts to identify specific genetic loci that contribute to human height illustrates this omission well. Using biometric analyses, it is estimated that up to 90% of the variability in height within populations is "due to" genetic differences. However, studies of candidate genes have not explained familial height resemblance. Weedon et al. (2007) reported the first replicated results linking a SNP to height. Using genome-wide data from 4,921 individuals, they linked rs1042725 (a common variant in the HMGA2 oncogene) to height. They replicated the association in 19,064 adults drawn from four other studies, a study of 6,827 children, and a tall/short case-control study (N = 3,207). Weedon et al. (2007) estimated rs1042725 accounts for about 0.3% of the population variation in height. Later, three consortia of research groups using multi-stage designs reported "a total of 54 loci affecting height variation in the population, identified using genome-wide association studies of hundreds of thousands of genetic markers on 63,000 people" (Visscher, 2008, p. 489). Of the series of four articles discussing these efforts (Gudbjartsson et al., 2008; Lettre et al., 2008; Visscher, 2008; Weedon et al., 2008) and the failure to account for much of the variation in height, even with large samples and huge numbers of loci investigated, only one (Gudbjartsson et al., 2008) *even mentions* that environmental and nutritional factors also affect height.

Beyond these disappointing results, some investigators have questioned the field's current heavy reliance on the common disease–common variant model for other reasons. Currently genome-wide array-based genotyping technology only assesses a subset of genetic variants, and not all types of genetic variation. Further, the number of common genetic variants contributing to a given condition (e.g., Type II diabetes) may be unmanageably large. "If effect sizes were so small as to require a large chunk of the genome to explain the genetic component of a disorder, then no guidance would be provided: in pointing at everything, genetics would point at nothing" (Goldstein, 2009, p. 1696). Investigators with these concerns conclude that "attention should shift from genome scans of ever larger samples to studies of rarer variants of larger effect" (Goldstein, 2009, p. 1698).

Non-Replication from a Probabilistic Epigenetic Perspective

The methodological issues summarized in the previous sections are important for population analysis of individual differences, and clearly can contribute to non-replication and variations in magnitude of gene–phenotype associations across studies. But in contrast to a perspective that tends to see complexity, moderation, and "true differences" as essentially noise, Gottlieb contended that these complexities are the central subject matter of developmental analysis because they reflect the coactional, probabilistic nature of development. To understand individual development, we must look to the characteristics and life experiences of the individual (Gottlieb, 2003). Therefore, from a probabilistic systems perspective, the more fundamental reason for the failure to replicate gene–phenotype associations consistently is the failure to approach development as a probabilistic epigenetic process that must be investigated in terms of intra-individual variation (Gottlieb, 2007). That is, to see development as the result of constant coactions of at least two biological, contextual, or experiential factors within and across levels of a developmental-psychobiological system (Gottlieb & Halpern, 2002).

A developmental-psychobiological system is one that encompasses genetic and neural activity, as well as behavior and the physical, social, and cultural aspects of the environment (see Gottlieb, 2002 or chapter 2 this volume for one of several elaborations of this systems model). The system reflects time-based, bi-directional, probabilistic relations. There is no direct causality, no genetic "main effects" that "may or may not" be moderated by other genes, environment, or experience, because in a probabilistic system genes are *not* the primary driver of development. "Genes are not exempt from influences at other levels of analysis but are, in fact, dependent upon them for initiating and terminating their activity" (Gottlieb, 2007, p. 2). Development is not predetermined and fixed; any element of the system, and the relations among elements, can change at any time across time. Thus persons with the same genotype, but distinctive life experiences, may have quite dissimilar outcomes on any given phenotype. Gottlieb's reference to differences in body type evidenced by monozygotic twins reared apart in very different family environments from birth is a striking example of this point (Gottlieb, 1998).

Given acceptance of coactional, probabilistic, bidirectional change in a developmental-psychobiological system, a bivariate investigation of "x" causing "y" is essentially nonsensical. One element "x" (e.g., gene) does not independently cause outcome "y." By definition, there is only, at a minimum, "x_1" and "x_2" and "y." Gottlieb (2007) argued that the reason a gene–phenotype association is sometimes evident and sometimes not (even when "x_1" is truly involved in the emergence of "y" in some fashion) is that at least one other central piece, a "coactor," is missing from the analysis. The typical omission of biological sex in genetic analyses is a pervasive example. Despite the sexual dimorphism of the regulatory genome and

sex differences in epigenetic mechanisms (e.g., DNA methylation; Ober, Loisel, & Gilad, 2008; Weiss, Pan, Abney, & Ober, 2006), even the basic context of biological sex and genotype–sex interactional contributions to development are not systematically examined (Ober et al., 2008), and sex-specific gene–environment interactions are not widely studied (Hauber, Sewell, & Zuk, 2008). In Gottlieb's (2007) view the typical failure to consider bi-directional, coactional processes is the most fundamental reason for extensive non-replication in the gene–phenotype association literature.

This is not to say that bivariate investigations of "x_1" and "y" will never yield statistically significant associations – clearly they can and do. But, the absence of statistical "genetic main effects" cannot be used to guide the investigation of coactional processes (usually operationalized as statistical interactions) for both meta-theoretical (i.e., by definition there are no singular causal factors or "main effects") and statistical (main effects of factors are not a prerequisite for interactions between them) reasons. The importance of these points is being increasingly appreciated in quantitative genetics and other disciplines. For example, in reports based on studies of *Drosophila*, Mackay and Anholt (2006) note that genome scans for pairwise epistasis (nonadditive interaction between segregating alleles at two or more loci) affecting longevity "reveal more interactions than expected by chance, with most interactions being between markers that do not have significant main effects" (p. 346).

Although coaction is typically *assessed* via tests of statistical interaction (see Wahlsten, 1990 for a discussion of power issues in detecting statistical interactions) and tests are used to make inferences about biological and/or social processes, the developmental notion of "coaction" is not the same as the statistical concept of interaction, or departure from additivity in findings pooled across individuals. Therefore the absence of a significant statistical interaction does not necessarily have implications for the absence or presence of a given coactional process. It is possible to see evidence of coaction that is not manifested as a statistical interaction. See, for example, Gottlieb's description of rats' maze performance based on varying rearing conditions, as illustrated in Figure 4 of his 2003 publication (Gottlieb, 2003).

Gottlieb sought and accumulated a large number of empirical demonstrations of coactional process and probabilistic development (more recently see Gottlieb, 2007). Illustrations, albeit as derived from inter-individual analysis, are increasingly appearing in the literature. There are a number of examples related to serotonin transporter variants. The serotonin transporter is of interest because it is involved in the reuptake of serotonin at brain synapses. Reduced serotonergic transmission is hypothesized to contribute to decreased impulse control. The gene is diallelic, having a short and long length polymorphism in the promoter region, and yields three combinations (*ss*, *sl*, *ll*). In general, the presence of the *s* allele is associated with lower transcriptional efficiency of the promoter compared with the long (*l*) allele; thus the *ll* combination is thought to confer protective characteristics in the context of certain environments. For example, a study investigating serotonin

transporter variants in rhesus macaques using the separation–rearing experimental model found that macaques with the *sl* genotype had higher adrenocorticotrophic hormone levels during chronic separation than those with the *ll* genotype, and that cortisol levels increased in response to separation stress (Barr et al., 2004). Rat models offer other illustrations of probabilistic development. Maternal licking and grooming behavior, for example, has been demonstrated to influence hypothalamic-pituitary-gonadal function, which in turn is associated with altered pubertal timing and sexual behavior (Cameron et al., 2008). Such studies illustrate the contributions of life experience to biological processes, and as noted by D'Souza and Craig (2006), "underscore the importance of incorporating environmental measures," without which associations with genetic variants would be missed (p. 7). Other chapters in this volume (see for example Bennett & Pierre (chap. 12) and Propper, Moore, & Mills-Koonce (chap. 17) offer additional empirical illustrations of coactional processes based on human and animal models.

It is recognized in the genetic epidemiology literature that gene–gene and gene–environment interactions (or coactions) exist and clearly contribute to heterogeneity in effect sizes across studies. Quantitative geneticists are increasingly calling for attention to gene–environment interaction. Although not described in terms that Gottlieb would have used, quantitative geneticists report, for example, that in evaluations of the life span of recombinant inbred lines of Drosophila under standard culture conditions and four stressful environments, gene–environment interaction accounted for 79% of the total genetic variance (Mackay & Anholt, 2006). Mounting recognition of the importance of including life experience in investigations is encouraging, although Gottlieb would be dismayed by the field's ongoing lack of consensus about when in the investigatory process such coactions (i.e., interactions) should be examined. That is, whether gene–life experience interactions should be sought from the outset of the discovery process or after the "main effect" of a gene has been identified and replicated (Hunter, 2005). Such discussions suggest, as Gottlieb often said, that the authors don't really "get it." That is, there is acknowledgment of the importance of considering life experience, which suggests acceptance of a systems model wherein levels are linked via coactional process. However, the view that the main effect of a gene must first be identified to warrant further investigation indicates lack of true appreciation of the fundamental character of coaction. Gottlieb would say that there is no issue regarding whether to seek main effects versus gene–life experience interactions from the outset, as there are no meaningful main effects.

Implications of Probabilistic Epigenesis for Models of Replication

A meta-theory of probabilistic development complicates what we should expect in terms of replication, and how we should proceed to assess which findings are

robust. Two meta-analyses that assessed efforts to replicate two heavily cited findings about gene–environment interactions in their contribution to complex behavioral phenotypes illustrate the complications. The first interaction is that between childhood maltreatment and the variable number tandem repeat (VNTR) in the promoter region of the monoamine oxidase A (*MAOA*) gene. The *MAOA* gene encodes the *MAOA* enzyme, which metabolizes neurotransmitters such as norepinephrine, serotonin, and dopamine. Deficient *MAOA* activity may be linked to neural hyper-reactivity to stress or threat. Studies investigating the association were spawned by the breakthrough report (Caspi et al., 2002) of an interaction in which respondents with the "low activity" version of the *MAOA* enzyme who had been maltreated in childhood were more likely to display conduct disorder, antisocial personality, and violent criminality than maltreated respondents who had the "high activity" version of the *MAOA* enzyme.

Taylor and Kim-Cohen (2007) conducted a meta-analysis of eight studies that attempted to replicate this interactive association. The meta-analysis compared the correlations between maltreatment and antisocial behavior within each *MAOA* genotype group, and taking into account issues of heterogeneity, sensitivity, and publication bias, identified "a small but significant combined effect of .17" (Taylor & Kim-Cohen, p. 1035). Though seemingly small, this effect size is somewhat larger than is typically seen in gene–phenotype associations, and the authors concluded that the findings indicate a "robust" interaction that merited additional investigation.

In contrast, in a 2009 meta-analysis (Risch et al., 2009) of 14 studies that had attempted to replicate another highly cited finding from Caspi and colleagues (Caspi et al., 2003), an interaction between the serotonin transporter gene (the *ll* versus *ss*/*sl* variants described earlier) and stressful life events in predicting depression, the authors came to a different conclusion. Risch and collaborators selected studies of greatest comparability to the original 2003 publication, requested original data from investigators who had attempted to replicate, and examined the association for males and females both separately and combined. They also conducted analyses to examine gene-environment correlation; tested a dosage, recessive and dominant model for the polymorphism; examined heterogeneity; and conducted multiple stratified analyses (e.g., by number of stressful life events). Their conclusions, which were consistent across all models and also consistent with those of an earlier meta-analysis of a subset of these studies (Munafò et al., 2009), were that stressful life events greatly increase the odds of depression, but "addition of the serotonin transporter genotype did not improve the prediction of risk of depression beyond that associated with exposure to negative life events" (either as a main effect or interaction; Risch et al., 2009, p. 2468). Thus, the meta-analysis indicated no reliable support for the life event–serotonin promoter polymorphism interaction, even though the smallest sample used in the analysis contained more than 10,000 persons, indicating that statistical power was not the issue. One striking aspect of the 2009 paper is the authors' conclusion that these

results "should not deter investigators from including environmental risk factor information in their studies, once robust marginal gene associations have been identified" (Risch et al., 2009, p. 2469). Given the powerful associations demonstrated with life events, one might have expected that encouragement would be offered for the inclusion of *genetic* information in future studies of stress-related conditions!

Both the 2002 and 2003 publications by Caspi and colleagues were important because they included a known "key life event" and plausible biological linkages. That is, they approached the questions using a strategy that is more consistent with a probabilistic developmental model. However, the methodological differences across the studies and analytical limitations of the meta-analysis likely played a role in the findings of the 2009 meta-analysis. Further, despite the strong association between number of stressful life events and depression in the Risch et al. (2009) analysis, it is also likely that measurements of life stress in some or all of the component studies were inadequate. Evidence from animal models (rhesus monkeys) indicates that the coactional effect of genotype *ls* carriers versus *ll* alleles in the promoter region of the serotonin transporter gene (*SLC6A4*) and exposure to stressful challenges on intermediate brain phenotypes (e.g., regional brain activity, such as amygdala reactivity or metabolic activity in the bed nucleus of the stria terminalis, that is a precursor to anxiety or depression) varies according to the *specific characteristics of a stressor* (Kalin et al., 2008), suggesting a need for more precision and validity in experiential measures.

Human studies that have attempted to replicate the 2003 Caspi et al. findings reflect substantial diversity and levels of adequacy in measures of stressful life events (SLEs). Several authors (Monroe & Reid, 2008; Uher & McGuffin, 2008) have pointed out that SLE measurement differences and their "loose psychometrics" render the literature on the serotonin promoter–life stress interaction essentially uninterpretable. Data support interview-based stress assessment procedures that cover a range of experiences and offer clear operational definitions (Monroe & Reid, 2008). Uher and McGuffin (2008), in their review of the methodology employed by 17 studies examining the serotonin–environmental adversity interaction, point out that the five studies that used a structured interview to measure SLEs replicated the interaction. Other issues such as event time frames and temporal ordering are also often improperly addressed. For example, acute events that precipitate depression relatively quickly (i.e., one to three months) may be most compatible with psychobiological process underlying gene–environment interactions (Monroe & Reid, 2008), although this may vary by type of event (Uher & McGuffin, 2008). Greater theoretical coherence in the selection and measurement of "key life events" that are so central to a probabilistic developmental model might improve what has been labeled "replication drift" (Monroe & Reid, 2008, p. 953).

Amidst ongoing discussions of whether, when, and how to combine life experience with genetic analysis, researchers are increasingly calling for gene–phenotype

studies to broaden the array of investigated genetic measures and measures of the environment to include more social and contextual information (e.g., Chanock & Hunter, 2008; Manolio, 2009). Expanding the realm of *what* is studied through more thoughtful (i.e., theory-driven) and systematic incorporation of biological, psychosocial, and physical/contextual/cultural experience would provide opportunities to move the field closer to the investigatory requirements of a probabilistic epigenetic system, and to the type of multi-level analysis Gottlieb argued would improve the consistency of findings from replication efforts.

Escalating the use of whole genome technology could hypothetically facilitate this effort, given that a strength of genome-wide genotyping is the ability to capture information about *many* potential genetic contributors to a given phenotype, thereby opening the door to the incorporation of multiple genetic contributors examined in the context of multiple life experiences. However, the feasibility of successfully implementing such changes and their conceptual adequacy are unclear. First, considering feasibility, genome-wide association studies require large samples, especially when investigating interactions, and the larger the sample of participants, the harder it may be to collect diverse contextual and experiential information about each person, and, ideally, to collect such information over time.

There is discussion of these practical challenges in the scientific literature. In a recent commentary, Chanock and Hunter (2008) echoed Gottlieb's long standing call for consistent and systematic inclusion of life experiences in genetic analyses. Commenting on the significant disagreement in conclusions reached by three studies (Amos et al., 2008; Hung et al., 2008; Thorgeirsson et al., 2008) that examined the association between the SNP variation at 15q24/15q25.1 on the long arm of chromosome 15 and lung cancer, and how that association relates to nicotine dependence, Chanock and Hunter point to the absence of environmental data as a severe limitation in understanding these study differences, as well as differences seen in other sets of studies on other variants and phenotypes. They note the "flight to quantity" stimulated by genome-wide analysis, and how large sample sizes have typically been achieved by sacrificing contextual and experiential data (to the modest extent that such data had been incorporated in candidate gene studies anyway). They further point out that when environmental and lifestyle data are available for large samples they are, unfortunately, usually collected retrospectively. Chanock and Hunter call for inclusion of prospective contextual and lifestyle/experiential information in future work, hoping that this is "perhaps the end of the beginning." Further enhancements in the form of long-term longitudinal design (to better tap "historical" processes such as whether the timing of an experience contributes to the manifestation of gene–environment coactions) and inclusion of theoretically driven measures would also increase compatibility with a systems approach.

The notion of gene–environment coaction does not imply that every feature of experience or context applies to every genetic process. To progress in our understanding of individual development we must make decisions about *which*

genetic variants and *which* aspects of the environment are more important to pursue (Wahlsten, 2003). For example, Monroe and Reid (2008) suggest that investigations of the serotonin promoter–stressful life events interaction might benefit from designating methodologically sound "candidate stressors" in the same spirit of candidate genes. It is not obvious how to proceed with choosing the right questions and knowing how to evaluate our answers. For purposes of individual studies, Moffitt and colleagues (Moffitt, Caspi, & Rutter, 2005) suggest a strategy of identifying the theorized environmental factor (life experience, atmospheric contaminant, etc.), and recruiting samples with "known exposure" to the environmental factor of interest. The next step would then be to determine how genetic variation does or does not distinguish persons with and without the phenotype of interest (Moffitt et al., 2005). This could be implemented in genome-wide analysis based on cohort samples of diverse experience, although examination through tests of statistical interaction would still have the well-documented power issues. Such an approach, though helpful, does not address the need for biologically informed theory, data reduction, and the distinctions between questions being asked in population-based versus individual analysis. That is, once *what* to study is decided, there remain issues about *how* to study it.

There are multiple fundamental issues surrounding *how* to investigate developmental aspects of gene–phenotype associations. One issue again relates to feasibility, given the challenges presented by data quality, data quantity, the need for data reduction, and limitations in analytical techniques. The amount of genetic information produced by array technology is enormous; its integration with experiential and contextual information can be overwhelming both conceptually and analytically. Person-centered techniques, such as cluster analysis, could be employed to capture implicit interactions among these multiple elements of the developmental system, and perhaps address, to a modest extent, the issue of multiple testing. Because the bidirectional processes in a developmental system tend to be correlated and may limit or facilitate individual pathways (i.e., produce correlated constraints), cluster analysis also has the advantage of simultaneously considering multiple constraints (Cairns & Rodkin, 1997). However, use of many elements to construct clusters will still yield a large number of clusters if the goal is to adequately capture the variability in the original elements.

Suggestions to change the unit of genetic analysis also offer more compatibility with a systems approach. Most association mapping is indirect; SNPs can be tagged because of their linkage with other SNPs that are directly genotyped. Neale and Sham (2004) suggest that, as technology permits, investigations could move away from reliance on indirect associations, and from SNPs and haplotypes as the units of analysis, toward a gene-based approach in which the common variation within a candidate gene is considered jointly. In contrast to the NCI-NHGRI recommended criteria that call for strict replication with the same SNP, Neale and Sham note that replication attempts that are focused on precisely the same polymorphism cannot account for more complex patterns of associations with other polymorphisms in

the same gene, and that such attempts implicitly assume, sometimes incorrectly, that allele frequencies or linkage disequilibrium structure are the same across different populations. They argue that these circumstances may be central to failures to replicate in cases where there is little information about functional variation in the gene.

Efforts to cluster genes, even without inclusion of non-genetic factors however, have proven to be complex. Clustering is usually done with the goal of producing clusters that reflect genetic expression trajectories. However, because "most genes are not expressed under most conditions most of the time" (Bryan, 2004, p. 52), one has to assume "true" clusters would be context-specific. Further, even genes that are expressed in almost all cell types often have variants affecting gene regulation that act in cell-type specific ways (Dimas et al., 2009). Thus, as would be expected in a developmental systems perspective, there are no natural gene clusters that are independent in their contributions to a phenotype across biological and experiential contexts (Bryan, 2004).

A more fundamental issue in how to study gene–phenotype associations in a developmentally sensitive way is the issue that began this chapter, that is, the distinction between population-based assessments of differences across individuals at specified time points versus investigation of change within individuals over time. Gottlieb argued inter-individual differences, and by implication the replication of patterns of individual differences, do not inform our understanding of individual development. To understand individual development, we need to examine within-individual variation. As Molenaar and colleagues have compellingly argued (e.g., Molenaar & Campbell, 2009; Molenaar et al., 2003), examination of inter-individual and intra-individual variation will only lead to the same answers when variance structure is identical across individuals and when the process being examined does not change over time. Both of these conditions, by definition, are clearly at odds with developmental analysis. Thus, a major disciplinary shift toward analysis of within-individual variation over time will be essential to adequately address genetic contributions to development (Molenaar & Campbell, 2009). Such a shift will also require development and refinement of specialized analytical techniques.

With progress toward *what* is needed to address developmental questions about gene–phenotype associations, and *how* to approach such an analysis, we are left with the most vexing issue – how to evaluate our answers. If replication is to remain as the centerpiece of evaluation, we must determine how to reconcile replication with a probabilistic conceptualization of development. Within-individual analysis must allow ultimately for the identification of subsets of individuals who evidence similar developmental processes and trajectories, and for methods to demonstrate repeated identification of the same groups.

Given the notion of probabilistic epigenesis, direct or strict replication is, by definition, not possible. A more compatible goal may be conceptual replication, in which we attempt to identify certain regularities of development or norms of reaction, and thereby reproduce theoretical interpretation. Developmental scientists propose

that the correlated constraints of a developmental system mean that not all patterns of development have similar probabilities (Gest, Mahoney, & Cairns, 1999), suggesting the manageability of predicting probabilistic outcomes. However, Gottlieb points out that "while we continue to try to correlate genotypes with events at the neural and behavioral levels, we need to remind ourselves of the uncertainty involved . . . even with the inclusion of the 'crucial' life-experience factor, we are still talking about probabilities" (Gottlieb, 2003, p. 352). Thus, our expectations about conceptual replication of gene–phenotype associations appear to ultimately hinge on theoretical and operational elaboration of "probability" in Gottlieb's meta-theory.

Valsiner (2007) suggested that Gottlieb intentionally declined the pursuit of theoretical elaboration of the Developmental-Psychobiological Systems approach when such elaboration entered territory unsupported by empirical data, and for this reason did not pursue further development of the key notion of probability. Valsiner observes that Gottlieb's notion of "probabilistic" does not seem to quite fit any of three ways of looking at probability (frequentist, Bayesian, and propensity or structural), because each probability type is essentially fixed and non-historical. Bayesian or evidential probability, which requires specification of a prior probability that is revised in light of new information, may best approximate Gottlieb's conceptualization. However, the Bayesian approach to prediction is still lacking, because it does not take into account the full set of life events and experience that have come before – critical for developmental system and life course models.

Conclusion

Given a probabilistic system, whether and how we can apply the construct of conceptual replication to determine if we have a good account of developmental process is not clear. Addressing this pressing issue is critical to further our understanding of genetic contributions to both normal and pathological development, and deserves the attention of developmentalists in their empirical and theoretical work. If "the probability of any developmental event is constantly changing as the event unfolds" (Valsiner, 2007, p. 836), then our best predictions may be limited to just an outline of a field of possibilities, with some pathways being more likely than others (Beloussov, 1998). Moving away from dichotomous questions (e.g., is it a "real" association or not) toward relational questions about changing person-context associations (e.g., in the context of this life experience there is an association between gene x and phenotype y; Lerner, 1995) better reflects the precepts of a developmental system. However, as illustrated by the results of meta-analyses described in the previous section and as expected from the misapplication of inter-individual analysis to the investigation of individual development, inclusion of life experiences alone will not necessarily yield cross-study consistency in findings about individual differences in gene–phenotype associations.

If we accept that the norm of reaction is "essentially nonpredictive because . . . each new environment is expected to have a different influence on developmental outcomes that cannot be stated in advance of actual empirical investigation" (Gottlieb, 1991, p. 5), are we, as has been suggested (Goldstein, 2009), pointing at nothing because we are pointing at everything? Gottlieb would answer no. Not every element of a developmental system is key to every process. The organism and context constrain each other, and all outcomes do not have an equal probability of occurrence (Kindermann & Valsiner, 1995). The promise of this constraint points to the need to gather data about gene–phenotype associations within individuals over time and to develop further suitable methods of analysis and aggregation, in a context of ever increasing amounts of information, which can inform theoretical elaboration of "Gottlieb's probability." More methodical and widespread application of a developmental psychobiological systems approach to the investigation of gene–phenotype associations can provide the empirical foundation for theoretical elaboration. Such a foundation is vital for efforts to define and operationalize the construct of replication in the framework of probabilistic development.

References

Amos, C. I., Wu, X., Broderick, P., Gorlov, I. P., Gu, J., Eisen, T., et al. (2008). Genome-wide association scan of tag SNPs identifies a susceptibility locus for lung cancer at 15q25.1. *Nature Genetics*, **40**, 616–622.

Barr, C. S., Newman, T. K., Shannon, C., Parker, C., Dvoskin, R. L., Becker, M. L., et al. (2004). Rearing condition and the rh5-HTTLPR interact to influence limbic-hypthalamic-pituitary-adrenal axis response to stress in infant macaques. *Biological Psychiatry*, **55**, 733–738.

Beloussov, L. V. (1998). *The dynamic architecture of a developing organism: An interdisciplinary approach to the development of organisms*, Dordrecht: Kluwer.

Bobb, A. J., Castellanos, F. X., Addington, A. M., & Rapoport, J. L. (2006). Molecular genetic studies of ADHD: 1991 to 2004. *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics)*, **141ß**, 551–565.

Bryan, J. (2004). Problems in gene clustering based on gene expression data. *Journal of Multivariate Analysis*, **90**, 44–66.

Cairns, R. B., & Rodkin, P. C. (1997). Phenomena regained: From configuration to pathways. In R. B. Cairns, L. R. Bergman, & J. Kagan (Eds.), *Methods and models for studying the individual* (pp. 245–264). Thousand Oaks, CA: Sage.

Cameron, N., Del Corpo, A., Diorio, J., McAllister, K., Sharma, S., & Meaney, M. J. (2008). Maternal programming of sexual behavior and hypothalamic-pituitary-gonadal function in the female rat. PLoS ONE, **3**(5), e2210. doi: 10.1371/journal.pone.0002210.

Campbell, H., & Manolio, T. (2007). Commentary: Rare alleles, modest genetic effects and the need for collaboration. *International Journal of Epidemiology*, **36**, 445–448.

Cardon, L. R., & Bell, J. L. (2001). Association study designs for complex diseases. *Nature Reviews Genetics*, **2**, 91–99.

Cardon, L. R., & Palmer, L. J. (2003). Population stratification and spurious allelic association. *The Lancet*, **361**, 598–604.

Caspi, A., McClay, J., Moffitt, T. E., Mill, J., Martin, J., Craig, I. W., et al. (2002). Role of genotype in the cycle of violence in maltreated children. *Science*, **297**, 851–854.

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., et al. (2003). Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science*, **301**(5631), 386–389.

Chanock, S. J., & Hunter, D. J. (2008). When the smoke clears. *Nature*, **452**(3), 537–538.

Chanock, S. J., Manolio, T., Boehnke, M., Boerwinkle, E., Hunter, G. T., Thomas, G., et al. (2007). Replicating genotype–phenotype associations: What constitutes replication of a genotype–phenotype association, and how best can it be achieved? *Nature*, **447**, 655–660.

Clark, A. G., Boerwinkle, E., Hixson, J., & Sing, F. F. (2005). Determinants of the success of whole-genome association testing. *Genome Research*, **15**, 1463–1467.

Colhoun, H. M., McKeigue, P. M., & Davey Smith, G. (2003). Problems of reporting genetic associations with complex outcomes. *Lancet*, **361**, 865–872.

Cumming, G., & Fidler, F. (2009). Confidence intervals: Better answers to better questions. *Journal of Psychology*, **217**(1), 15–26.

Danziger, K., & Shermer, P. (1994). The varieties of replication: A historical introduction. In R. van der Veer, M. van Ijzendoorn., & J. Valsiner, J. (Eds.), *Reconstructing the mind: Replicability in research on human development* (pp. 17–36). Norwood, NJ: Ablex Publishing.

Dewey, J., & Bentley, A. F. (1949). *Knowing and the known*. Boston: Beacon.

Dimas, A. S., Deutsch, S., Stranger, B. E., Montgomery, S. B., Bore, C., Attar-Cohen, H., et al. (2009). Common regulator variation impacts gene expression in a cell type-dependent manner. *Science Express*, 30 July; 10.1126/science.1174148.

D'Souza, U. M., & Craig, I. W. (2006). Functional polymorphisms in dopamine and serotonin pathway genes. *Human Mutation*, **27**(1), 1–13.

Ennis, S., Goverdhan, S., Cree, A. J., Hoh, J., Collins, A., & Lotery, A. J. (2007). Fine scale linkage disequilibrium mapping of age-related macular degeneration in the complement factor H gene region. *British Journal of Ophthalmology*, **91**, 966–970.

Gest, S. D., Mahoney, J. L., & Cairns, R. B. (1999). A developmental approach to prevention research: Configural antecedents of early parenthood. *American Journal of Community Psychology*, **27**(4), 543–565.

Goldstein, D. B. (2009). Common genetic variation and human traits. *The New England Journal of Medicine*, **360**(17), 1696–1698.

Gottlieb, G. (1991). Experiential canalization of behavioral development: Theory. *Developmental Psychology*, **27**, 4–13.

Gottlieb, G. (1995). Some conceptual deficiencies in "developmental" behavioral genetics. *Human Development*, **38**, 131–141.

Gottlieb, G. (1998). Normally occurring environmental and behavioral influences on gene activity: From central dogma to probabilistic epigenesis. *Psychological Review*, **105**(4), 792–802.

Gottlieb, G. (2002). *Individual development and evolution: The genesis of novel behavior*. Mahwah, NJ: Erlbaum.

Gottlieb, G. (2003). On making behavioral genetics truly developmental. *Human Development*, **46**, 337–355.

Gottlieb, G. (2007). Developmental neurobehavioral genetics: Development as explanation. In B. C. Jones & P. Mormede (Eds.), *Neurobehavioral genetics: Methods and applications* (2nd ed., pp. 17–27). Boca Raton, FL: CRC/Taylor & Francis.

Gottlieb, G., & Halpern, C. T. (2002). A relational view of causality in normal and abnormal development. *Development and Psychopathology*, **46**, 421–435.

Gudbjartsson, D. F., Walters, G. B., Thorleifsson, G., Stefansson, H., Halldorsson, B. V., Zusmanovich, P., et al. (2008). Many sequence variants affecting diversity of adult human height. *Nature Genetics*, **40**(5), 609–615.

Gudmundsson, J., Sulem, P., Manolescu, A., Amundadottir, L. T., Gudbjarttsson, D., Helgaston, A., et al. (2007). Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24. *Nature Genetics*, **39**, 631–637.

Hauber, M. E., Sewell, M. A., & Zuk, M. (2008). Gender genomics and equality. *Heredity*, **101**, 395.

Hirschhorn, J. N., & Altshuler, D. (2002). Once and again – Issues surrounding replication in genetic association studies. *Journal of Clinical Endocrinology and Metabolism*, **87**, 4438–4441.

Hirschhorn, J. N., Lohmueller, K., Byrne, E., & Hirschhorn, K. (2002). A comprehensive review of genetic association studies. *Genetics in Medicine*, **4**, 45–61.

Hung, R. J., McKay, J. D., Gaborieau, V., Boffetta, P., Hashibe, M., Zaridze, D., et al. (2008). A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25. *Nature*, **452**(3), 633–637.

Hunter, D. J. (2005). Gene– environment interactions in human diseases. *Nature Reviews Genetics*, **6**(4), 287–298.

Ioannidis, J. P. A. (2007). Non-replication and inconsistency in the genome-wide association setting. *Human Heredity*, **64**, 203–213.

Ioannidis, J. P. A. (2008). Why most discovered true associations are inflated. *Epidemiology*, **19**(5), 640–648.

Ioannidis, J. P. A., Ntzani, E. E., Trikalinos, T. A., & Contopoulos-Ioannidis, D. G. (2001). Replication validity of genetic association studies. *Nature Genetics*, **29**, 306–309.

Kalin, N. H., Shelton, S. E., Fox, A. S., Rogers, J., Oakes, T. R., & Davidson, R. J. (2008). The serotonin transporter genotype is associated with intermediate brain phenotypes that depend on the context of an eliciting stressor. *Molecular Psychiatry*, **13**, 1021–1027.

Khoury, M. J., Little, J., Gwinn, M., & Ioannidis, J. P. A. (2007). On the synthesis and interpretation of consistent but weak gene–disease association in the era of genome-wide association studies. *International Journal of Epidemiology*, **36**, 439–45.

Killeen, P. R. (2005). An alternative to null-hypothesis significance tests. *Psychological Science*, **16**(5), 345–353.

Kindermann, T. A., & Valsiner, J. (1995). Directions for the study of developing person-context relations. In T. A. Kindermann & J. Valsiner, J. (Eds.), *Development of person-context relations* (pp 227–240). Hillsdale, NJ: Erlbaum.

Kraft, P. (2008). Curses – winner's and otherwise – in genetic epidemiology. *Epidemiology*, **19**(5), 649–651.

Kraft, P., & Hunter, D. J. (2009). Genetic risk prediction – Are we there yet? *The New England Journal of Medicine*, **360**(17), 1701–1703.

Ku, K. C., & Chia, C. K. (2008). The success of the genome-wide association approach: A brief story of a long struggle. *European Journal of Human Genetics*, **16**, 554–564.

Lerner, R. M. (1995). Developing individuals within changing contexts: Implications of developmental contextualism for human research, policy, and programs. In T. A. Kindermann & J. Valsiner, J. (Eds.), *Development of person-context relations* (pp 13–37). Hillsdale, NJ: Erlbaum.

Lerner, R. M., & Kaufman, M. B. (1985). The concept of development in contextualism. *Developmental Review*, **5**, 309–333.

Lettre, G., Jackson, A. U., Gieger, C., Schumacher, F. R., Berndt, S. I., Sanna, S., et al. (2008). Identification of ten loci associated with height highlights new biological pathways in human growth. *Nature Genetics*, **40**(5), 584–591.

Libioulle, C., Louis, E., Hansoul, S., Sandor, S., Farnir, F., Franchimont, D., et al. (2007). Novel Crohn disease locus identified by genome-wide association maps to a gene desert on 5p13.1 and modulates expression of PTGER4. *PLoS Genet.* **3**: e58.

Lin, P. I., Vance, J. M., Pericak-Vance, M. A., & Martin, E. R. (2007). No gene is an island: The flip-flop phenomenon. *The American Journal of Human Genetics*, **80**, 531–538.

Mackay, T. F. C., & Anholt, R. R. H. (2006). Of flies and man: *Drosophila* as a model for human complex traits. *Annual Review of Genomics and Human Genetics*, **7**, 339–367.

Manolio, T. A. (2009). Cohort studies and the genetics of complex disease. *Nature Genetics*, **41**(1), 5–6.

Marchini, J., Howie, B., Myers, S., McVean, G., & Donnelly, P. (2007). A new multipoint method for genome-wide association studies by imputation of genotypes. *Nature Genetics*, **39**(7), 906–913.

Moffitt, T. E., Caspi, A., & Rutter, M. (2005). Strategy for investigating interactions between measured genes and measured environments. *Archives of General Psychiatry*, **62**, 473–481.

Molenaar, P. C. M., & Campbell, C. G. (2009). The new person-specific paradigm in psychology. *Current Directions in Psychological Science*, **18**(2), 112–117.

Molenaar, P. C. M., Huizenga, H. M., & Nesselroade, J. R. (2003). The relationship between the structure of inter-individual and intra-individual variability: A theoretical and empirical vindication of developmental systems theory. In U. M. Staudinger & U. Lindenberger (Eds.), *Understanding Human Development* (pp. 339–360). Boston: Kluwer.

Monroe, S. M., & Reid, M. W. (2008). Gene–environment interactions in depression research: Genetic polymorphisms and life stress polyprocedures. *Psychological Science*, **19**(10), 947–956.

Munafò, M. R., Durrant, C., Lewis, G., & Flint, J. (2009). Gene-environment interactions at the serotonin transporter locus. *Biological Psychiatry*, **65**, 211–219.

Munafò, M. R., Matheson, I. J., & Flint, J. (2007). Association of the DRD2 gene Taq1A polymorphism and alcoholism: A meta-analysis of case-control studies and evidence of publication bias. *Molecular Psychiatry*, **12**, 454–461.

National Institutes of Health (NIH) (2007). Policy for sharing of data obtained in NIH supported or conducted genome-wide association studies (GWAS). *Federal Register*, **72**(166), 49290–49297.

Neale, B. M., & Sham, P. C. (2004). The future of association studies: Gene-based analysis and replication. *American Journal of Human Genetics*, **75**(3), 353–362.

Ober, C., Loisel, D. A., & Gilad, Y. (2008). Sex-specific genetic architecture of human disease. *Nature Reviews Genetics*, **9**, 911–922.

O'Donovan, M. C., Craddock, N. Norton, N., Williams, H., Peirce, T., et al., (2008). Identification of loci associated with schizophrenia by genome-wide association and follow-up. *Nature Genetics*, **40**(9) 1053–1055.

Pearson, T. A., & Manolio, T. A. (2008). How to interpret a genome-wide association study. *Journal of the American Medical Association*, **299**(11), 1335–1344.

Radder, H. (1992). Experimental reproducibility and the experimenters' regress. In D. Hull, M. Forbes, & K. Okruhlik (Eds.), *Proceedings of the 1992 biennial meeting of the philosophy of science association* (pp. 63–73). East Lansing, MI: Philosophy of Science Association.

Risch, N., Herrell, R., Lehner, T., Liang, K. -Y., Eaves, L., Hoh, J., et al. (2009). Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: A meta-analysis. *Journal of the American Medical Association*, **301**(23), 2462–2471.

Rosenthal, R. (1990). Replication in behavioral research. *Journal of Social Behavior and Personality*, **5**(4), 1–30.

Rutter, M., Pickles, A., Murray, R., & Eaves, L. (2001). Testing hypotheses on specific environmental causal effects on behavior. *Psychological Bulletin*, **127**, 291–324.

Sameroff, A. (1983). Developmental systems. In W. Kessen (Ed.), *Handbook of child psychology: History, theory, and methods: Vol. 1* (4th ed., pp. 237–294). New York: Wiley.

Saxena, R., Voight, B. F., Lyssenko, V., Burrt, N. P., de Bakker, P. I. W., Chen, H., et al. (2007). Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science*, **316**, 1331–1336.

Schmidt, S. (2009). Shall we really do it again? The powerful concept of replication is neglected in the social sciences. *Review of General Psychology*, **13**(2), 90–100.

Spencer, C. C. A., Su, Z., Donnelly, P., & Marchini, J. (2009). Designing genome-wide association studies: Sample size, power, imputation, and the choice of genotyping chip. *PLOS Genetics*, **5**(5), e1000477. 1–13.

Talkowski, M. E., Bamne, M., Mansour, H., & Nimgaonkar, V. L. (2007). Dopamine genes and schizophrenia: Case closed or evidence pending? *Schizophrenia Bulletin*, **33**(5), 1071–1081.

Taylor, A., & Kim-Cohen, J. (2007). Meta-analysis of gene–environment interactions in developmental psychopathology. *Development and Psychopathology*, **19**, 1029–1037.

Thorgeirsson, T. E., Geller, F., Sulem, P., Rafnar, T., Wiste, A., Magnusson, K. P., et al. (2008). A variant associated with nicotine dependence, lung cancer and peripheral arterial disease. *Nature*, **452**(*3*), 638–641.

Todd, J. A. (2006). Statistical false positive or true disease pathway? *Nature Genetics*, **38**(7), 731–733.

Uher, R., & McGuffin, P. (2008). The moderation by the serotonin transporter gene of environmental adversity in the aetiology of mental illness: Review and methodological analysis. *Molecular Psychiatry*, **13**, 131–146.

Valsiner, J. (1987). *Culture and the development of children's action*. New York: Wiley.

Valsiner, J. (2007). Gilbert Gottlieb's theory of probabilistic epigenesis: Probabilities and realities in development. *Developmental Psychobiology*, **49**(8), 832–840.

Van den Oord, E. J. C. G. (2008). Controlling false discoveries in genetic studies. *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics)*, **147**, 637–644.

van der Veer, R., van Ijzendoorn, M., & Valsiner, J. (1994). General introduction. In R. van der Veer, M. van Ijzendoorn & J. Valsiner (Eds.), *Reconstructing the mind: Replicability in research on human development* (pp. 1–9). Norwoord, NJ: Ablex Publishing.

Visscher, P. M. (2008). Sizing up human height variation. *Nature Genetics*, **40**(5), 489–490.

Visscher, P. M., Hill, W. G., & Wray, N. R. (2008). Heritability in the genomics era – concepts and misconceptions. *Nature Reviews*, **9**, 255–266.

Wahlsten, D. (1990). Insensitivity of the analysis of variance to heredity-environment interaction. *Behavioral and Brain Sciences*, **13**, 109–161.

Wahlsten, D. (2003). Genetics and the development of brain and behavior. In J. Valsiner and K. J. Connolly (Eds.), *Handbook of developmental psychology* (pp. 18–47). London: Sage.

Weedon, M. N., Lango, H., Lindgren, C. M., Wallace, C., Evans, D., Mangino, M., et al. (2008). Genome-wide association analysis identifies 20 loci that influence adult height. *Nature Genetics*, **40**(5), 575–583.

Weedon, M. N., Lettre, G., Freathy, R. M., Lindgren, C. M., Voight, B. F., Perry, J. R. B., et al. (2007). A common variant of *HMGA2* is associated with adult and childhood height in the general population. *Nature Genetics*, **39**(10), 1245–1250.

Weiss, L. A., Pan, L., Abney, M., & Ober, C. (2006). The sex-specific genetic architecture of quantitative traits in humans. *Nature Genetics*, **38**, 218–222.

Wong, A. H. C., Buckle, C. E., & Van Tol, H. H. M. (2000). Polymorphisms in dopamine receptors: What do they tell us? *European Journal of Pharmacology*, **410**, 183–203.

Wong, M. Y., Day, N. E., Luan, J. A., Chan, K. P., & Wareham, N. J. (2003). The detection of gene-environment interaction for continuous traits: Should we deal with measurement error by bigger studies or better measurement? *Internationl Journal of Epidemiology*, **32**, 51–57.

Zollner, S., & Pritchard, J. K. (2007). Overcoming the winner's curse: estimating penetrance parameters from case–control data. *American Journal of Human Genetics*, **80**, 605–615.

16

Canalization and Malleability Reconsidered

The developmental basis of phenotypic stability and variability

Robert Lickliter and Christopher Harshaw

Evolution is a striking tapestry in which conservation and innovation, permanence and change, and necessity and contingency are thoroughly intertwined. (Evan Thompson, 2007, p. 195)

Introduction

A long-standing problem for both developmental and evolutionary theory has been how to account for the stability *and* variability of phenotypes observed within and across generations of any given species. For most of the last century, genes were thought to be the answer to this problem. Genes were proposed to contain all the information necessary for the development of phenotypic traits, and moreover, circumstances during individual development were not thought to influence genes or directly influence the traits or characteristics of offspring (e.g., Ayala & Valentine, 1979; Mayr, 1982; Williams, 1966). As a result, most 20th-century biologists believed that the influence of development on evolution was minimal. The stability and variability of phenotypes within and across generations was assumed to be determined by an organism's genes, with minimal contribution from the physical, biological, or social features of its environment.

As we review in this chapter, the last several decades have seen a different account of phenotypic stability and variability take shape in developmental biology,

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evolutionary biology, and developmental psychology. This new account is based on a relatively simple but profound insight: given that all phenotypes arise during ontogeny as products of individual development, it follows that a primary basis for both phenotypic stability and variability must be the process of development itself. The thread of this insight can be traced back to several pioneering embryologists and developmental biologists, including Walter Garstang (1922), Edward Russell (1930), Gavin de Beer (1940), Richard Goldschmidt (1940), Conrad Waddington (1942), and Ivan Schmalhausen (1949). Although each of these biologists had a distinctive perspective on the links between development and evolution, they all promoted the notion that changes in individual development were an important basis for evolutionary change. This view was well outside mainstream 20th-century thinking about evolution, but is now being reconsidered across the life sciences.

As the morphologist Pere Alberch (1982) noted some 30 years ago, development contributes to the evolutionary process in at least two key ways, one regulatory and the other generative. First, the process of development generates the reliable reproduction of phenotypes across generations and constrains phenotypic diversity by limiting the "range of the possible" in terms of both form and function. This robustness of development, despite genetic or environmental perturbations, is the *regulatory* function of development (Maynard-Smith et al., 1985; Siegal & Bergman, 2002; Wimsatt, 1986). Since the work of Waddington (1942, 1975), constraints on phenotypic variation have typically been referred to as *canalization*. In the most general sense, canalization refers to the constancy or reliability of phenotypes across a normal range of developmental conditions (Ariew, 1999). Second, the process of development introduces phenotypic variation and novelties of potential evolutionary significance. This is the *generative* function of development and provides an important source of variation upon which natural selection can act (Gottlieb, 2002; Johnston & Gottlieb, 1990; West-Eberhard, 2003). For example, many phenotypes show graded responses to factors or events that occur along natural environmental gradients (e.g., temperature, pH levels) and apparently dichotomous responses (polyphenisms) to factors or events that occur in a dichotomous manner (e.g., the presence or absence of predators or particular food items, see Nijhout, 2003). This flexibility of phenotypic outcomes in response to variations in or modifications of genetic and environmental factors is usually referred to as *phenotypic plasticity* or *malleability*.

These regulative and generative roles of development challenge the notion that the natural selection of random genetic mutations (a cornerstone of the neo-Darwinian framework of evolution) can be sufficient to account for the ways and means of evolutionary change. A growing acknowledgement of this insight by biologists and psychologists over the last several decades has fueled a renewed interest in development within evolutionary biology (e.g., Arthur, 2002; Bjorklund, 2006; Gilbert, Opitz, & Raff, 1996; Gottlieb, 1992; Lickliter & Honeycutt, 2009; Pigliucci, 2007; Robert, 2004; West-Eberhard, 2003). Of particular importance in this concern is the recognition that variations in morphology,

physiology, and/or behavior arising from modifications to the developmental process can place organisms in different ecological or functional relationships with their environments. If these phenotypic variations provide even slight advantages in survival and reproduction, then competitors without the novel phenotype will eventually decrease in frequency in the population, a key factor contributing to evolutionary change.

For example, a European passerine bird, the blackcap, has recently shown changes in its migratory behavior which appear to result in changes in mating behavior, size of egg clutches, and success at fledging young (Bearhop et al., 2005). Many passerine birds are seasonal migrants and the timing of spring migration constrains when breeding starts each year. Until recently, all European blackcaps migrated back and forth together, spending summers in northern Europe and the British Isles and winters in Portugal, Spain, and North Africa before gathering in mating grounds in southern Germany and Austria to breed. Blackcaps were typically seen in the British Isles only during the summer months, but the number of them wintering in Britain and Ireland has increased dramatically over the last 40 years. This change is thought to be due to the increased availability of winter provisioning provided by bird feeders, landscapers, and other related human activities. The resulting shift in migratory patterns has allowed northern-wintering blackcaps to be exposed some 10 days earlier than their southern wintering counterparts to the critical photoperiods that contribute to the initiation of migration and the onset of gonadal development. Even though all blackcaps continue to gather each year at the same mating sites in Germany and Austria, isotopic data indicate that northern blackcaps arrive earlier at the breeding grounds and establish territories and mate with other earlier arriving birds; southern-wintering blackcaps arrive at the same mating sites some two weeks later and are more likely to mate with each other, serving to reproductively isolate northern-wintering birds from the later-arriving southern-wintering population. This shift in migratory patterns appears to confer an advantage to the northern blackcaps, who lay about one more egg per season than do their later arriving cohorts from the south (Bearhop et al., 2005).

To anyone unfamiliar with the history of evolutionary biology, it might seem obvious to assume that knowledge of developmental and behavioral processes like these would be necessary to understand evolutionary processes. This supposition was in fact widely held by many biologists working in the 19th century, only to be abandoned by the dominant school of evolutionary theory (the "Modern" or "neo-Darwinian" Synthesis) in the first half of the 20th century (Amundson, 2005; Sapp, 2003). Attempts to integrate Darwin's theory of evolution by natural selection with Mendel's theory of genetics during the first decades of the last century contributed to the rapid growth of the science of population genetics and a corresponding decline in concerns with development. Population genetics focused on how genetic mutation, recombination, and selection could lead to changes in gene frequencies found within a population of breeding organisms over generations. It assumed that modification and transmission of genes were the only

possible source of evolutionary change. As a result, it also assumed that knowledge of developmental processes was irrelevant to understanding the ways and means of evolution (see Dawkins, 1976 for a radical example of this approach).

This genocentric focus resulted in most evolutionary biologists embracing a very narrow view of evolution as "a change in gene frequencies in populations" (e.g., Ayala & Valentine, 1979; Dobzhansky, 1951). This definition of evolution was the established view for most of the last half of the 20th century and at least in some quarters of the biological sciences continues to be the dominant metric for what qualifies as evolution. The widespread use of this narrow definition of evolution within the life sciences effectively eliminated serious consideration of the possible importance of development to evolutionary issues for some 40 years (but see Gottlieb, 1987, 1992; Gould, 1977; Ho & Saunders, 1979; Matsuda, 1987; Reid, 1985; West-Eberhard, 1989 for notable exceptions). As various chapters of this volume make clear, the lack of interest in development on the part of evolutionary theorists is no longer the case.

The Rise of Evolutionary Developmental Biology

The last several decades have seen the established "gene-centered" view that dominated 20th century biology shift to gradually accommodate a much broader and more integrative perspective on the role of development in evolution. This shift has involved moving beyond the established notion of genes as the fundamental cause of phenotypic traits, thereby allowing for the consideration of a variety of extragenetic factors now known to contribute to the emergence, maintenance, and modification of phenotypes (e.g., Gilbert et al., 1996; Goodwin, 1994; Jablonka & Lamb, 1995, 2005; Newman & Müller, 2000; Rossiter, 1996). Recent advances in genetics and molecular and developmental biology have converged to demonstrate that the expression of genes is routinely affected or modified not only by other genes, but also by the local cellular as well as the extracellular environment of the developing organism, including cell cytoplasmic factors, hormones, and sensory, motor, and social stimulation provided by the external environment (reviewed in Davidson, 2001; Johnston & Edwards, 2002; Gerhart & Kirschner, 1997; Gilbert, 2000; Gottlieb, 1998; Weaver et al., 2004). A growing number of developmental biologists are thus expanding the focus of their research attention to not only the internal features of the developing organism (genes, cells, hormones), but also to the contributions of the physical, biological, and social resources available to the individual in its developmental context (e.g., diet, temperature, social interaction, see Gilbert, 2005).

This critical reassessment of the links between development and evolution has contributed to the coalescence of one of the most rapidly growing fields within contemporary biology, evolutionary developmental biology. Evolutionary

developmental biology (usually referred to as *evo-devo*) involves a partnership among evolutionary, developmental, and molecular biologists to integrate our understanding of developmental processes operating during ontogeny with those operating across generations (e.g., Arthur, 2004; Hall, 1999; Kirschner & Gerhart, 2005; Raff, 2000). In contrast to the reductionistic premises of the Modern Synthesis of evolutionary biology, evo-devo views evolution as changes in developmental processes rather than simply changes in gene frequencies. This agenda addresses a variety of concerns, including how modifications in developmental processes lead to the production of novel phenotypes, the role of developmental plasticity in evolution, and how ecology influences developmental and evolutionary change (Hall & Olson, 2003). These concerns are motivated in large part by the growing appreciation that a wide range of environmental factors are key participants in gene activity and expression, in some cases well beyond the timescale of individual development.

This represents a paradigmatic shift in thinking within the biological sciences and is requiring a reformulation of several established ways of thinking about development, heredity, and evolution. For example, the notion of heredity has been undergoing a significant transformation within the biological sciences over the last decade. It is now widely accepted that what is passed on from one generation to the next are genes *and* a host of other necessary internal and external factors (or resources) that contribute to the development of an organism's traits. As we review in later sections, this developmental manifold (Gottlieb, 1970) or developmental system (Oyama, 1985) is increasingly recognized to be the source of both the *stability* and the *variability* of development, eliminating the need for notions of preformed genetic programs or blueprints. This perspective emphasizes the dynamic and contingent nature of the development of phenotypic traits and recognizes that a focus on how phenotypes are generated during development is a critical feature of understanding how the process can be changed or modified. As Gottlieb (1991b; 1997) pointed out, the realization of new behavioral phenotypes typically requires a change in normal or usual rearing circumstances that ordinarily function to canalize development along species-typical trajectories.

A Reformulation of Species-Typical Behavior

If the normal or usual circumstances encountered by individuals of a species during the course of development remain reliable and repeatable over multiple generations, then the timing, range, and quantity of species' typical experiences can be said to be "inherited." In other words, individuals of that species will tend to have *normally occurring* experiences due to the reliable reoccurrence of the developmental resources typically present in their developmental context. Comparative research with birds and mammals has provided a number of examples of how such

normally occurring experience, including experience during the prenatal period, plays a key role in the development and maintenance of species-typical perception and behavior (e.g., Fifer & Moon, 1995; Gottlieb, 1997; Lickliter, 1996, 2005; Pedersen & Blass, 1982; Ronca & Alberts, 1994; Smotherman & Robinson, 1990; Wallace & Stein, 2007). It is important to note that we use the term "species-typical" to refer to those behaviors commonly observed across members of a population. It thus refers to behavioral phenotypes that are reliably found across individuals and across generations of a species; it does not imply or assume notions of innate, instinctive, or hard-wired behavior (see Schneirla, 1956). From this framework, species-typical behavioral development is a historical and contingent process, the result of reliable and repeatable transactions and relationships that take place within and between levels of integration both inside and outside the developing organism (Gottlieb, 1991a; Lickliter, 2000). Species-typical behavioral phenotypes are thus generated during individual ontogeny due to particular aspects of the temporal and spatial arrangements of organisms and their contexts reliably occurring at times when the organism is in particular developmental states, having had a particular developmental past (see Oyama, 1985, 1993 for discussion). As a result, the causes of species-typical behavior cannot be understood without an analysis of ontogeny.

For example, the bidirectional influence of organismic and environmental factors present in early development has been shown to induce patterns of species-typical behavioral lateralization and forebrain function in a number of precocial bird species. During the later stages of prenatal development the precocial avian embryo is oriented in the egg such that its left eye is occluded by the body and yolk sac, whereas the right eye is exposed to diffuse light passing through the egg shell when the brooding hen is intermittently off the nest during the incubation period. This differential prenatal visual stimulation resulting from the embryo's invariant postural orientation in the egg has been shown to facilitate the development of the left hemisphere of the brain in advance of the right hemisphere. Further, this light induced developmental advantage for the left hemisphere has been shown to influence the direction of hemispheric specialization for a variety of postnatal behaviors, including visual discrimination, spatial orientation, feeding behavior, and various visual and motor asymmetries (reviewed in Rogers, 1995). Experimentally altering the normal pattern of light stimulation available during prenatal development can modify this typical pattern of brain and behavioral development. For example, a left visual bias can be established by occluding the right eye and stimulating the left eye with light prior to hatching. Likewise, the induction of lateralization can be prevented by incubating eggs in darkness or by providing the same level of light stimulation to both eyes in the period prior to hatching (Casey & Karpinski, 1999; Casey & Lickliter 1998; Deng & Rogers, 2002).

Proponents of an innate or prespecified view of species-typical behavior often explain such instances of context-contingency in developmental outcomes by claims that environmental factors encountered during individual development

(such as light exposure in the egg prior to hatching) simply trigger or activate latent developmental programs, assumed to be contained in the genes. However, relying on explanations of the phenotype that refer to latent or hidden programs inside the organism effectively sidesteps the issue of development and minimizes the role of extragenetic factors involved in the achievement of phenotypic outcomes. The historical and contingent view of species-typical behavior we favor represents a radically different view from that assumed by traditional notions of innate, instinctive, or other internally determined characterizations of the regularities of species-typical behavior. In our view, species-typical behavior is best understood to be generated and maintained through the activities and experiences of a historical organism engaged with a structured developmental context.

The reliable and repeatable features of stimulation and experience occurring in an organism's developmental context have been termed the "ontogenetic niche" by West and King (1987), which they defined as the set of ecological and social circumstances typically inherited by members of a given species. This ontogenetic niche is available both prenatally and postnatally and provides diverse but dependable resources and influences for the developing individual. The ontogenetic niche can be described in terms of temperature, humidity, salinity, light level and cycle, energy sources and their distribution, patterns of social interaction, and so on (Alberts & Cramer, 1988). These extragenetic factors are part of each organism's inheritance and every ontogenetic cycle depends on the availability of a particular set of these developmental resources, reconstructed in each generation (see Avital & Jablonka, 2000; Oyama, 1985 for further discussion and examples). From a developmental perspective, the recurrence from generation to generation of the specific developmental resources and interactions that make up an organism's ontogenetic niche serves as a primary basis for the development and maintenance of its species-typical behavior (see Haraway & Maples, 1998; Kaufman, 1975; Miller, 1997; West, King, & White, 2003).

On the other side of the coin, significant alterations or modifications in the normally available resources and interactions of an organism's ontogenetic niche are a primary basis for the generation of *novel* behaviors (or "neophenotypes," see Kuo, 1967; Johnston & Gottlieb, 1990). New or novel behaviors brought on by alterations in normal prenatal and postnatal rearing environments can lead to new organism–environment relationships, including changes in diet, habitat use, and/or social and reproductive behavior. These behavioral shifts can be maintained across generations if such changes or alterations in the developmental rearing environment persist, promoting a cascade of possible changes in morphology and physiology over time (Gottlieb, 2002; Johnston & Gottlieb, 1990).

The phenomenon of domestication, the process by which organisms change in terms of morphology, physiology, or behavior as a result of the human control of their breeding, feeding, and care (Hale, 1969), provides a compelling example of the cascade of phenotypic changes that can result from altered rearing environments. The variance of phenotypes among wild and domestic strains of a single species has

long been appreciated. Darwin (1859, 1868), for example, documented the wide array of alterations in size, shape, coloration, productivity, and behavior evident in domesticated animals and speculated on their possible origin. Following the neo-Darwinian synthesis of the first half of the 20th century and its emphasis on population genetics (see Mayr & Provine, 1980 for an overview), most students of domestication assumed that the morphological, physiological, and behavioral differences between wild and domestic strains of animals could be explained by random and non-random genetic mechanisms associated with captive rearing. These genetic mechanisms include natural and artificial selection, inbreeding, genetic drift, and genetic mutation (Price & King, 1968).

Although the importance of genes as sources of phenotypic variation in both wild and domestic animals is indisputable, domestication is certainly not simply a matter of changing gene frequencies. The transition from free-living to captivity is accompanied by many and varied changes in an animal's physical, biological, and social environments and we know that these changes can bring about significant modifications in phenotypic development. For example, Clark and Galef (1977, 1979, 1980, 1981) have shown that specific differences in the morphology, physiology, and behavior of wild and domestic strains of gerbils can be traced to relatively minor changes in the developmental resources available in their early rearing experiences. Gerbils reared in standard laboratory cages without access to shelter show accelerated eye opening following birth, earlier sexual maturity, increased docility, and reduced reactivity to humans when compared to gerbils reared in laboratory conditions that allow free access to shelter, as would normally occur in the wild.

The change from free-living to captivity for most species is typically accompanied by changes in the availability of not only shelter, but also space, food and water, predation, and possibilities for social interaction (Price, 1999). The large majority of domesticated animals are reared under conditions that lack many of the physical, biological, and social sources of stimulation that would be routinely available under naturally occurring circumstances. The impact of such changes to the nature and availability of developmental resources cannot be underestimated in accounting for the domestic phenotype. From a developmental perspective, animals of a species are either similar or not in the expression of phenotypic traits (e.g., wild or domestic) not simply because they share or lack similar genes, but because they share or lack similar developmental systems (see Lickliter & Ness, 1990 for further discussion).

The Significance of Behavioral Malleability

Beyond concerns with the dynamics of domestication, researchers have long appreciated that substantially changing species-typical developmental circum-

stances (in particular the physical or social environment) can foster behavioral change even within a single generation. The results of early handling experiments with rodents (Denenberg & Rosenberg, 1967; Levine, 1956) and environmental enrichment studies with various laboratory animals (Greenough & Chang, 1989; Renner & Rosenzweig, 1987; Rosenzweig & Bennett, 1972) provided compelling evidence of the developmental induction of modified anatomical and behavioral phenotypes by changes in early rearing conditions. Research with avian brood parasites (species that lay their eggs in the nests of other species, thereby exploiting the other species' parental care) has recently provided an intriguing example of how changes in typical developmental circumstances during early development could rapidly lead to reproductive isolation, an important step in the process of speciation.

In laboratory studies, Payne, Payne, Woods, and Sorenson (2000) have found that if chicks of the village indigobird, a brood parasite of the firefinch, are raised by a novel host species (Bengalese finch), their behavior differs dramatically from that of their biological parents. Male indigobirds raised by the novel Bengalese finch host develop a different song repertoire, consisting of Bengalese finchlike songs (rather than the typical firefinch songs sung by males raised by firefinches). Female indigobirds raised by the novel Bengalese finch host also prefer the new Bengalese-like song over the typically preferred firefinch song and prefer to lay their eggs in the nests of Bengalese finch rather than in the nests of firefinches. These new behavioral phenotypes emerged within a single generation as a result of the fledglings' development in a modified social rearing environment. Such rapid induction of changes in host selection and mate preferences highlight how learning mechanisms and the behaviors they generate can contribute to the potential for evolutionary change, without any precipitating changes in gene frequencies within the population (ten Cate, 2000; see Freeberg & White, 2006 for a similar example from another brood parasite, the brown-headed cowbird).

Gottlieb (1991b, 1997) placed particular emphasis on the role that such modifications to prenatal and early postnatal experience can have on species-typical behavior and speculated on the long-term effects of such modifications on the individual, its offspring, and future descendents. In this light, findings from the study of birds and mammals have consistently demonstrated that features of available prenatal and early postnatal sensory stimulation (such as amount, intensity, or the timing of presentation of stimulation) can coact with specific organismic factors (such as the stage of organization of the sensory systems, previous history with the given properties of stimulation, and the current state of arousal of the young organism) to guide and constrain the developmental course of species-typical perceptual preferences, learning, and memory (e.g., Gottlieb, 1991a; Lickliter, 1995, 2005; Spear, 1984; Spear & McKinzie, 1994). Changes in these basic processes can in turn lead to modifications in typical patterns of species identification, habitat selection, diet preference, and other key aspects of the organism–environment system. These modifications can in turn lead to changes in patterns of gene activation, regulation, and selection.

Shifts in behavior brought about by alterations to the species-typical developmental system can arise at any stage of the life cycle, but are generally more likely to occur earlier than later in development. This point was highlighted by several developmental and evolutionary theorists over the last century (e.g., de Beer, 1940; Denenberg, 1969; Garstang, 1922; Goldschmidt, 1940; Kuo, 1967; Levine, 1956; Waddington, 1975), who despite their different backgrounds realized the significance of embryonic and neonatal periods of development for the generation of phenotypic novelties. These early periods of development are a time of rapid morphological, physiological, and behavioral change, and modifications to an individual's developmental system during this time can initiate a host of physical and behavioral novelties, and in some cases (given the availability of appropriate developmental conditions) persist across subsequent generations.

The insight that there is generally a higher degree of malleability during earlier as compared to later phases of development (explained by the individual's cumulative developmental history, see Kuo, 1967) has at least two important implications for understanding evolutionary processes. First, in addition to selection acting upon phenotypic outcomes as they are expressed in breeding age adults, selection can also occur at earlier stages of development, allowing for the potential for the rapid spread of novel phenotypes in response to modified developmental circumstances (Alberts, 1994). In addition, the young of a species frequently have more potential than older members of the species to facilitate or accelerate phenotypic change. Learning mechanisms and developmental plasticity allow the young of many organisms to readily establish novel relationships with their environments. Such novelties can arise in the absence of optimal morphology and/or physiology and, once established, can provide the basis for the eventual expression of a host of other novel phenotypic traits.

As a case in point, in the early 1980s Ran Aisner and Joseph Terkel observed that black rats had invaded recently planted forests of Jerusalem pine in Israel (Terkel, 1996). Not only had the rats colonized the forest, they had adopted an arboreal lifestyle, which included constructing nests woven out of and attached to tree limbs with pine needles and consuming a diet of pinecones that were stripped and consumed in a specific and highly efficient manner. Through an elegant series of experiments (summarized in Terkel, 1996), Terkel and his colleagues were able to show that young rats learned this efficient method of feeding largely via social learning, a key component of which was simply being exposed to partially eaten pinecones during early development. Although it is unknown how this novel behavioral tradition emerged in these rats, it is possible that the novel habit of pinecone eating emerged initially in only one or a few relatively juvenile rats and quickly spread throughout the local population, eventually leading to the occupation of a novel arboreal niche. As an alternative scenario, one can imagine a single female rat that was given to the habit of pinecone eating, who became pregnant and gave birth. Her pups would have thus been exposed to a novel developmental system–a system that would have included many of the opportunities for social

learning documented by Terkel and his colleagues. Given that none of the 47 non-pinecone eating adult rats of the same species tested by Aisner and Terkel (1992) ever acquired the habit of efficiently stripping pinecones (even when reared with pinecone stripping rats), a combination of these two scenarios may be more likely. Regardless of how it occurred, the adoption of a novel behavioral phenotype opened up the possibility for the rapid colonization of a new ecological niche, with modified developmental resources and species-atypical opportunities for learning and selection.

In light of these and similar findings from other avian and mammalian species (see Avital & Jablonka, 2000 for multiple examples), it seems to us that the underlying processes involved in phenotypic plasticity are not different in kind from those of stability. That is, the developmental processes involved in producing the reliable reoccurrence (*canalization*) of phenotypes under species-typical conditions are the same as those involved in producing novel phenotypic outcomes (*malleability*) under species-atypical circumstances. Canalization and malleability are not distinct developmental phenomena – both are products of the dynamics of the organism's entire developmental system.

A rich area of investigation supportive of our proposal that the regulative and generative aspects of development are deeply intertwined is filial imprinting, a topic that was a focus of Gottlieb's research attention for nearly 40 years. His body of work on species identification in ducklings demonstrated how the features and patterns of recurring prenatal sensory experience (including self-stimulation) guide and constrain young organisms' selective attention, perception, learning, and memory during early development. His work also called attention to the remarkable specificity of timing and stimulus parameters of prenatal experience that contribute to the emergence and maintenance of normal or species-typical behavior.

The Illustrative Case of Filial Imprinting

Imprinting has long been of interest to ethologists and psychologists, in part because of the high degree of malleability or plasticity displayed by maternally deprived hatchlings of precocial avian species (e.g., ducks, geese, chickens, quail). Following the pioneering work of Douglas Spalding (1873), who reported the effects of early experience in establishing filial preferences in newly hatched domestic chicks, the acquisition of filial preferences in precocial birds came to be portrayed as a unique type of learning that was part of the innate endowment of precocial birds (e.g., Heinroth, 1911; Lorenz, 1937). For example, the ethologist Konrad Lorenz argued that precocial avian hatchlings were adaptively predisposed to rapidly form preferences or "imprint" upon whatever mother or surrogate happened to be present at the time of hatch. Imprinting was thus viewed as a classic

example of innate or instinctive behavior, thought to be genetically determined and occurring relatively independent of the young bird's prior experience. This idea was later imported into human psychology by John Bowlby as the evolved proximity maintenance or "attachment" system thought to be innately present in all newborns (Bowlby, 1969; see Lickliter, 2008). Ironically, what should have been viewed as a paragon case of developmental plasticity or malleability during early development came to be widely viewed as a classic example of an innate or hard-wired behavioral capacity.

At the time that Gottlieb entered the field in the early 1960s, imprinting was a booming area of scientific investigation. Lorenz (1937) had observed that goslings and other waterfowl incubated and hatched in the absence of a mother would rapidly form an attachment to him (or to his boots) and would subsequently follow him wherever he went. He proposed that imprinting was a special type of learning, unique and highly adapted to the ecological requirements of precocial avian hatchlings, that it had a clearly defined "critical period" that lasted only a short time following hatch, and that it was irreversible, once established. Lorenz's papers on imprinting, combined with Bowlby's importation of the idea into human psychology, fueled a great amount of interest in the phenomenon in labs across the United States and Europe. Much of this work was aimed at delineating the actual extent and triggers of the onset and termination of the "critical period," the exact nature of the learning process involved (i.e. was it some form of classical and/or instrumental learning), and whether or not imprinting was indeed irreversible (Bateson, 1966).

Each of Lorenz's three characterizations of imprinting (a unique type of learning that had a narrow critical period and was irreversible) eventually succumbed to the weight of contrary experimental evidence (Bolhuis, 1991). In its current formulation, filial (as opposed to sexual) imprinting is most often characterized as a phenomenon that results from an interaction between two dissociable processes: the emergence of a predisposition to approach and prefer (i.e., show social behavior toward) certain classes of stimuli over others, in response to nonspecific experiential factors, and a learning process that is particular to the requirements of socially living precocial neonates (e.g., Bolhuis, 1999; Bolhuis & Honey, 1998). As we review below, this revised formulation does not, however, go far enough in emphasizing the highly contextual and contingent nature of the processes at play in the development of early filial preferences and species identification.

Beginning early in his career, Gottlieb emphasized the necessity of paying careful attention to the ecology of the species under study, as well as making direct behavioral observations of the species in its natural environment (Gottlieb, 1963a, 1963b). Despite a similar emphasis by ethologists on the importance of naturalistic observations, Lorenz's conception of imprinting was based largely upon observations of birds reared under highly artificial conditions (Gottlieb, 1963b). The laboratory study of imprinting was similarly dominated by the study of birds reared and imprinted under non-naturalistic conditions. For example, despite the highly social nature of most precocial avian species, imprint-

ing was largely studied using young chicks that were isolated before, during, and after the imprinting session (cf., Lickliter & Gottlieb, 1985). Further, most laboratory studies of imprinting prior to Gottlieb's work focused on imprinting as an exclusively visual phenomenon. Gottlieb observed that hens typically began vocalizing prior to hatch and increased the intensity and frequency of their vocalizations up to the time of leaving the nest site with their hatchlings (Gottlieb, 1963a, 1963b). Based on these observations, Gottlieb argued that such auditory exposure likely had a significant impact on the emergence of species-typical preferences in chicks, and that auditory imprinting likely takes precedence over and facilitates later visual imprinting to the mother hen.

In one of his earliest experiments, Gottlieb (1965) incubated and hatched groups of mallard ducklings and domestic chicks that were deprived of any prenatal or postnatal experience with the maternal calls of their own species. He found that maternally deprived hatchlings of both species showed a significant preference for their respective species-typical maternal call over the calls of other avian species, despite having never been exposed to these calls. This problem of how such an exquisitely adaptive behavioral phenotype could be expressed in the absence of any obvious stimulative or experiential input that was directly related to the behavior in question set the stage for much of Gottlieb's subsequent research career. Had Gottlieb taken the path favored by most nativists, that of proclaiming the behavior in question to be "instinctive" or the product of some "innate module" and then moving on to other topics, developmental science would have been deprived of one of its most interesting series of discoveries. As Gottlieb wrote at the time, "it seems worthwhile to try to find an empirical answer to this question rather than positing an unanalyzed factor of instinctive parental or species identification" (1965, p. 355).

One of the most important steps in this challenging quest involved devising a means of experimentally devocalizing avian embryos to allow complete experimental control of their pre- and post-natal auditory experience (Gottlieb & Vandenbergh, 1968). With this procedure in hand, Gottlieb was able to show that devocalized mallard ducklings that were incubated in isolation failed to prefer the mallard maternal call over a chicken maternal call in postnatal testing, whereas control and sham-operated hatchlings showed normal, species-typical preferences for the mallard maternal call (Gottlieb, 1971b). Ducklings that were devocalized and isolated but also exposed to playbacks of duckling embryonic contentment calls prenatally likewise showed normal, species-typical preferences for the maternal call of their own species (Gottlieb, 1975). Gottlieb went on to replicate these findings with wood ducklings, as well as show that it was prenatal exposure to very specific acoustic properties of their own embryonic vocalizations (but different properties in each species) that resulted in the species-typical auditory preferences displayed by mallard and wood duck hatchlings (see Gottlieb, 1971b).

These surprising findings contributed to the formulation of Gottlieb's concepts of *probabilistic epigenesis* and the *developmental manifold*. Development, in his view, could not be explained as the unidirectional flow of information from genes to

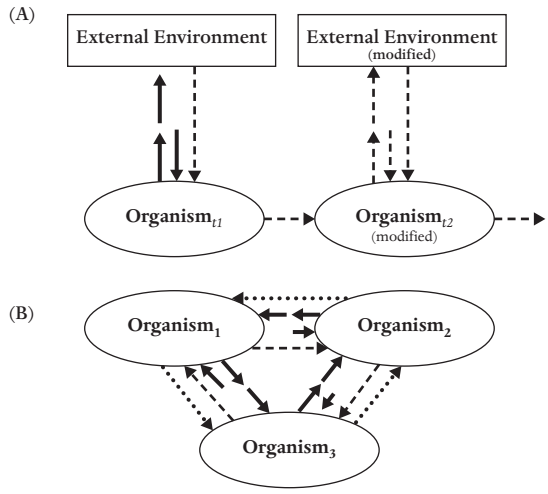

Figure 16.1. a. An illustration of the simple situation in which a developing organism produces behavioral (or other) output that: 1) modifies the immediate (internal and/or external) environment; 2) is directly sensed or experienced by the organism (interoceptively and/or exteroceptively); and 3) modifies the environment to influence the subsequent experience/development of the same organism. b. An illustration of a more complicated situation, in which a social organism produces behavior and/or other output that is directly sensed or experienced both by the organism itself and other conspecifics, in addition to potentially modifying the environment in such a way as to impact the subsequent experience/development of the entire group of organisms. In this scenario, the organism's own activity can potentially impact the organism via: 1) direct self-stimulation; 2) the elicitation of detectable responses from other organisms; and 3) by modifying the experience and/or activity of a second organism that modifies the experience and/or activity of a 3rd organism that feeds back to itself in a tertiary manner. All of this organismic activity can, in addition, modify the local environment in ways that will contribute to the dynamics of subsequent experience and development.

phenotypic outcomes. Instead, he proposed that all phenotypes were probabilistic outcomes of a host of recurrent interactions or coactions situated temporally and physically within complex developmental manifolds or systems (Gottlieb, 1971b). Gottlieb emphasized that the activity of the organism itself (and the feedback it provides) is a significant but often overlooked contributor to the process of development (e.g., Brainard & Doupe, 2000). Developing organisms, in this view, play an *active* role in their own development. The energetic production or output of the organism (whether motorical, acoustic, chemical or otherwise) frequently feeds back to or is experienced by the organism via a variety of channels. Such feedback, as illustrated by Gottlieb's devocalization studies, can result in perceptual and likely many other forms of learning and is an integral part of normally recurring, species-typical experience.

In addition to the organism's activities influencing its own development, they are also capable of influencing the development of conspecifics, particularly at phases of ontogeny when contact with conspecifics is a normally occurring feature of species-typical rearing conditions (see Figure 16.1). For example, most precocial birds are

highly social and are incubated and hatched in clutches of up to 10 or more birds. The embryos of such species typically begin vocalizing 24–36 hours prior to hatch, after the embryo has punctured the egg-shell membrane and entered the airspace of the egg. These vocalizations are experienced by their producers as well as by other embryos of the clutch and conspecifics in the vicinity of the nest. Gottlieb (1971b) demonstrated that the embryonic vocalizations of individual ducklings have a significant impact on their own perceptual and behavioral development, as well as on that of their broodmates. In addition to passive exposure to such social stimulation, embryos respond to each other's vocalizations (Vince, 1972), as well as to parent vocalizations prior to hatching (and incubating parents also respond to embryos' vocalizations, e.g., Hess, 1972; Johnson, 1969; Norton-Griffiths, 1969; Tuculescu & Griswold, 1983). Precocial avian embryos thus participate in a rich social milieu prenatally, despite being "isolated" from conspecifics within the egg.

The standard method of isolation rearing employed in the early studies of imprinting was adopted to prevent hatchlings from imprinting to each other, which interfered with their imprinting to other stimuli (e.g., Guiton, 1959). This neglect of intra-brood social factors by design became a standard protocol and gave rise to a skewed and overly simplistic view of both filial imprinting and the development of species identification (see Johnston & Gottlieb, 1981, 1985; Lickliter, Dyer, & McBride, 1993; Lickliter & Gottlieb, 1985). For example, Johnston and Gottlieb (1985) found that early social rearing can either facilitate or interfere with imprinting to a visual stimulus, depending upon the timing of postnatal social experience. Early social rearing (up to 48 hrs following hatching) was found to facilitate, whereas later social rearing was found to interfere with visual imprinting. Johnston and Gottlieb concluded that the effectiveness of visual imprinting had been greatly overestimated as a consequence of the non-naturalistic social isolation of birds in the majority of studies of imprinting.

Lickliter and Gottlieb (1985) similarly found that socially reared ducklings displayed a preference for a familiar mallard hen over an unfamiliar redhead duck hen at 48 and 72 hrs, a preference not shown by ducklings reared in isolation (Johnston & Gottlieb, 1981). Direct physical contact and social interaction with broodmates was found to be necessary for ducklings to show a normal preference for the familiarized hen. Ducklings reared in isolation, ducklings reared with visual but no direct physical contact with broodmates, and ducklings reared with physical contact with only a single broodmate showed no preference for the familiarized hen. Lickliter and Gottlieb (1987) also found that rearing ducklings socially prior to training with a mallard hen was insufficient for ducklings to acquire a preference for the mallard hen over a redhead duck hen. Only ducklings reared socially and tested 24 hrs (but not 1 hr) *after* training showed a significant preference for the familiar hen. The exact features of prolonged post-training social experience that gave rise to this effect are not known. However, Gottlieb (1991a) reported that one difference between isolated and group-reared ducklings is the amount of time that the birds spend sleeping: group-reared ducklings spend a great deal more time in

apparent sleep than isolated birds. Based on what is now known about the importance of sleep for the consolidation of memory (see Shank & Margoliash, 2008 for an example involving song learning in zebra finches) it is likely that this was an important mediator of the effect observed by Lickliter and Gottlieb (1987).

In subsequent studies, Gottlieb (1991a, 1993) showed both that the auditory preferences of socially reared ducklings were far more malleable than isolated ducklings and that the most significant feature of social rearing for the production of this effect was direct bodily contact with conspecifics. Ducklings that were either isolated or provided with visual and auditory access but no bodily contact with conspecifics did not prefer a species-atypical, chicken maternal call following prolonged exposure, whereas ducklings provided with the opportunity to have bodily contact with non-animate stuffed ducklings responded like normal, socially-reared ducklings, significantly preferring the species-atypical maternal call following prolonged postnatal exposure. Gottlieb (1993) went on to explore the possibility that differences in sleep and/or arousal might have been at play in producing these effects. He quantified the amount of apparent sleep ducklings were engaged in 5 min prior to a 30 min period of playback of the chicken maternal call and 25 min into this playback period. He also measured the amount of distress calling produced by ducklings as an index of arousal during two test periods. Ducklings that were raised socially showed lower levels of arousal than ducklings reared in tactile isolation. Ducklings that showed a lack of malleability (irrespective of condition) also showed higher levels of arousal than chicks from the same rearing condition that acquired a preference for the chicken maternal call. These results are similar to those obtained in studies of bobwhite quail embryos, showing that heightened arousal can interfere with the formation of auditory preferences during the prenatal period (Markham, Toth & Lickliter, 2006).

Based on these findings, Gottlieb (1991b, 1997) proposed that the canalization of behavior and the resulting decrease in plasticity or malleability over the course of early development was the result of the young organism's exposure to the range of usual or typical experiences within their species-typical developmental niche. At each stage of development, organisms are exposed to a predictable and common profile of environmental features, the stimulation and contingencies typical of typical development. Gottlieb argued that such species-typical experiences fostered the developmental of species-typical behavior, and further, buffered developing individuals from being susceptible to species-atypical forms of stimulation. Modifying species-typical rearing circumstances would result in the opposite effect, illustrating our point that *phenotypic stability and phenotypic plasticity represent differences in outcome, not differences in mechanism*.

Working at the neurophysiological level of analysis, Wallace and Stein (2007) have provided a striking example of the neural consequences of being reared in a modified, species-atypical environment. In this study, domestic cats were raised from birth to adulthood in highly controlled sensory environments that allowed the systematic manipulation of the temporal and spatial features of audio-visual

experience. Testing at 6-months of age revealed significant changes in the neural activity evoked by multisensory events, and that these changes closely reflected the structure of the cats' altered rearing circumstances. When auditory and visual stimuli were always presented simultaneously but at a fixed spatial disparity from one another over the first months of life, multisensory enhancements in neural activity were reliably seen to stimulus combinations that reflected this spatial disparity, but not to auditory-visual combinations that reflected normal (spatially collocated) audio-visual experiences. In other words, developing in a profoundly atypical sensory environment resulted in a profoundly atypical profile of neural activity and multisensory responsiveness to audio-visual events when compared to cats raised in a normal sensory environment that provided spatially coincident multisensory experiences. Such developmental plasticity provides a potent pathway for organisms to rapidly change structure and function in response to environmental changes. This plasticity potential and its implications for development and evolution have rarely been studied systematically and provide a rich area for future experimental analysis.

Further Explorations of the Malleability of Species-Typical Behavior

Operant contingency, the experience of a relationship between one's own activity and a consequence, has long been known to have a significant influence on learning and behavior across a wide range of organisms and contexts. The sequential occurrence of events in space and time signals to organisms the presence of potentially important experiential regularities (cf. Abrams & Kandel, 1988), as well as the presence of potential causal relationships (e.g., Dickinson & Shanks, 1995; Shanks, Pearson, & Dickinson, 1989). The environment regularly provides events that are time-locked with an organism's own actions, and feedback from an organism's own activity (behavioral or otherwise) has a large influence on the course of subsequent activity. Importantly for our concerns with canalization and malleability, the origin and success of emergent behavioral novelties often depend on the presence of contingencies, of being "in the right place at the right time" (Reid, 2007).

Recall that filial imprinting was long promoted to be a special type of learning, one that occurred independent of reinforcement or contingency (e.g., Lorenz, 1937). Imprinting was thus characterized as an example of *mere exposure* learning, albeit one different from traditional notions of habituation or perceptual learning. Despite the highly social nature of the developmental context of most precocial avian species, students of imprinting typically overlooked or downplayed the importance of social contingencies to the development of filial preferences and species identification. Inspired by the few previous investigations of the effects of contingency on the behavior of precocial avian hatchlings (e.g., Bateson &

Reese, 1969, Bolhuis & Johnson, 1988; Johnson, Bolhuis, & Horn, 1985; ten Cate, 1986, 1989), we recently assessed the influence of social contingency on the acquisition of auditory preferences in bobwhite quail chicks.

We exposed chicks either contingently or non-contingently to an individual variant of a bobwhite maternal assembly call and found that chicks provided with only 5 min of contingent postnatal exposure to an individual bobwhite maternal call (a single playback each time they vocalized) acquired a significant preference for that familiarized call over an unfamiliar variant of the bobwhite maternal call (Harshaw & Lickliter, 2007). In sharp contrast to this finding, our previous studies with bobwhites had shown that young chicks require 240–480 min of *passive* auditory exposure presented over several days to form significant preferences for these same maternal calls (Foushée & Lickliter, 2002; Lickliter & Hellewell, 1992). To highlight how dramatic the difference we observed between passive vs. contingent exposure was, consider that chicks receiving passive exposure to an individual maternal call required over 3,000 playbacks of the call to demonstrate a preference for that familiar call one day later. Chicks receiving exposure to an individual maternal call contingent on their own vocalization required less than 35 playbacks (about 1% of the overall exposure required by passively exposed chicks) to subsequently show a preference for that familiar call.

Given that making exposure to an auditory stimulus contingent on a chick's behavior had such a dramatic effect on the acquisition of intraspecific preferences (quickly promoting a preference for one variant of the bobwhite maternal call over another), we wondered if such contingent exposure might also have a facilitative effect on the malleability of species-specific preferences (fostering the acquisition of preferences for non-conspecific auditory stimuli). Maternally naïve bobwhite chicks normally show a strong auditory preference for their species-typical bobwhite (BW) maternal call over the maternal calls of other avian species in the days following hatching (Banker & Lickliter, 1993; Heaton, Miller, & Goodwin, 1978; Lickliter & Virkar, 1989). Could brief contingent exposure to a maternal call of another quail species significantly shift the normally robust species-specific auditory preferences of bobwhite hatchlings?

Results from a subsequent study revealed that this was indeed the case. When we provided day-old bobwhite chicks with 5 min contingent exposure to a heterospecific, Japanese quail (JQ) maternal call, they no longer showed the species-typical preference for the bobwhite (BW) maternal call over the JQ maternal call in subsequent testing (Harshaw, Tourgeman, & Lickliter, 2008). Furthermore, chicks given contingent exposure on a variable ratio (VR2) schedule, in which they heard the call on average once every two times that they vocalized, showed a reversal of their species-typical auditory preference, significantly preferring the JQ maternal call over the BW maternal call in simultaneous choice tests. In contrast, chicks given yoked, non-contingent exposure to the JQ call continued to show a significant preference for the BW maternal call in simultaneous choice tests. These results indicate that small amounts of contingent (or interactive)

exposure to a heterospecific call can be sufficient to disrupt or even reverse species-typical auditory preferences, and that the variability of the contingency appears to be a key factor in producing this dramatic modification of species-typical behavior.

In a follow up study, we found that providing chicks with 60 min of passive *prenatal* exposure to the JQ maternal call (5 min/hr over the last 12 hrs of incubation) had a significant influence on chicks' responsiveness to postnatal contingent exposure to the same call (Lickliter & Harshaw, 2008). Chicks given only prenatal exposure to the JQ call and tested 24 or 48 hrs after hatching showed no preference for the familiarized JQ call, as did naïve chicks given no prenatal exposure to the JQ call. In contrast, chicks given passive prenatal exposure to the JQ call *plus* a single (5 min) fixed ratio postnatal contingent exposure to the JQ call at 24 hrs of age showed a significant preference for the JQ maternal call over the BW maternal call. Chicks receiving only the contingent postnatal exposure, on the other hand, did not prefer the JQ over the BW maternal call. These results suggest that even relatively small amounts of prenatal auditory exposure can influence later postnatal responsiveness to the same auditory stimulus, despite the fact that prenatal exposure alone had no detectable effect on the naïve auditory preferences of hatchlings.

Our work on contingency learning in bobwhite quail emphasizes the contribution of the early social milieu typically experienced by precocial avian hatchlings to normal species-typical development. Our findings also point to the potential for such experience to induce significant shifts in species-typical outcomes. A key component of participation in a social milieu is the opportunity for contingent interaction with conspecifics, along with the varied affordances that such interaction provides for learning (Schneider & Harshaw, 2007). We have shown that even small amounts of such interactive stimulation can have a significant influence on the development of preferences for both species-typical and species-atypical auditory stimuli (Harshaw & Lickliter, 2007; Harshaw et al., 2008; Lickliter & Harshaw, 2008). It is important to note that the amounts of auditory stimulation employed in our laboratory-based studies were far less than what bobwhite quail hatchlings would experience under free-living conditions, where they would normally have prolonged contact with a maternal hen and/or father for days before and after hatching.

Under natural conditions, contingent vocal-auditory interaction with parents would also be accompanied by other reinforcing social stimuli, including the visual appearance of the parent(s), tactile contact, and warmth. Gottlieb (1971b) demonstrated that combined exposure to the visual appearance of a hen and a maternal call was superior to either stimulus employed alone for eliciting following and the formation of filial preferences by ducklings. The complete "package" of stimulation provided by a live interactive hen thus likely constitutes an optimal condition for precocial hatchlings, generating the rapid formation and canalization of filial preferences in the days following hatching (e.g., Boakes & Panter, 1985; ten Cate, 1989).

The developmental dynamics involved in the emergence of filial preferences and species identification in precocial birds illustrates the canalizing influence of normally

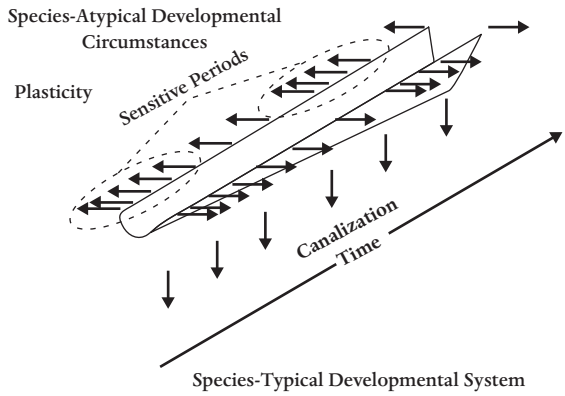

Figure 16.2. An illustration of canalization and malleability as two sides of phenotypic development. Species-typical developmental circumstances will tend to rapidly canalize phenotypes along species-typical lines. Modifications to the species-typical developmental system (the introduction of species-atypical circumstances) can produce rapidly divergent developmental outcomes. The degree of impact of such modifications tends to be larger during early development as well as during other "sensitive periods" during the lifespan (depending on the phenotype in question).

occurring experience on phenotypic outcomes. Under species-typical conditions, the presence of a live interactive hen and conspecifics of the appropriate species effectively guarantees that precocial avian hatchlings will rapidly form species-typical preferences for the visual appearance and calls of their own parent(s) and broodmates. Further, their ongoing experience with their own brood and parent(s) will strongly canalize their emerging species-identification abilities, which will also have a significant impact on their subsequent adult reproductive behavior (e.g., ten Cate & Bateson, 1988). However, under species-atypical conditions the same features of the developmental process that reliably ensured the formation of species-typical preferences results in the rapid emergence of species-atypical preferences (see Figure 16.2). In other words, the underlying experiential processes contributing to phenotypic stability are no different from those contributing to phenotypic plasticity. Canalization and malleability can be said to be two sides of the same developmental coin (see Sultan & Stearns, 2005 for additional discussion and examples).

The Organism–Environment System

The insight that the causes and control for species-typical development do not reside in any one factor or component, but rather reside in the nature and dynamics of the relations between factors *internal* and *external* to the organism, shifts thinking about development away from the pre-specified genocentric framework prevalent for most of the last century towards an appreciation of species-typical development as a situated process, dependent on resources distributed across the organism–

environment system. An organism and its environment are always fundamentally connected (Järvilehto, 2006; Lewontin, 2000) and recent research guided by this insight is providing compelling evidence that they cannot be functionally separated.

For example, work with desert locusts has recently provided illustration of the intricate links between internal and external factors contributing to the effects of experience on phenotypic plasticity. The desert locust is usually cryptic in color (green) and solitary. It typically actively avoids other locusts and flies alone at nighttime. However, under certain climatic conditions and the resulting increase in desert vegetation, their numbers can explode, triggering a rapid increase in population density that results in a dramatic transformation of their color (now bright yellow) and social behavior. Normally solitary locusts now form bands of hoppers and eventually swarms consisting of billions of locusts, causing catastrophic damage to agricultural crops. This transformation is known to involve many morphological, physiological, and behavioral changes involving numerous chemical messengers and more than 500 genes (Kang et al., 2004). Anstey, Rogers, Ott, Burrows, & Simpson (2009) have shown that the key agent in this remarkable phenotypic plasticity is the neurotransmitter serotonin, which is synthesized in the locust's thoracic nervous system in response to multiple sensory cues (touch, smell, or sight) provided by social contact with other locusts when population density increases. Within as little as two hours of proximity to other locusts, elevated serotonin levels switches behavior from mutual aversion to mutual attraction, recruiting additional chemical messengers and allowing the formation of the enormous locust swarms that can wreak havoc on human populations. Interestingly, serotonin-containing neurons in desert locusts comprise only five cell pairs in each thoracic compartment of their nervous system (Tyrer, Turner, & Altman, 1984).

The locust example highlights the interpenetration of the organism and its environment and directly challenges the long-standing notion that one can meaningfully separate genetic and environmental influences on phenotypic development. Whereas most accounts of development have traditionally focused on partitioning the organism's phenotypic characters among those that are genetically determined and those that are produced by the environment, we argue that no such partitioning is possible, even in principle. All phenotypes have a specific developmental history that explains their emergence, and this history always involves complex bidirectional traffic between genes, cells, organs, and organisms. A developmental mode of analysis is thus the only method with the potential to fully explicate the sources of the structures and functions of maturing and mature organisms.

West, King, and colleagues' decades of research on the courtship and mating patterns of brown-headed cowbirds (*Molothrus ater*) illustrate the dividends of developmental analysis for the identification of social and cultural contributions to species-typical behavioral development (e.g., Freeberg, King, & West, 1995; West, King, & Freeberg, 1994; White, King, & West, 2002). Their collective findings provide a compelling example of how knowledge of the particulars of species-typical social experience available in the cowbirds' developmental ecology helps to

explain both the generation of normal, species-typical behavior and the generation of novel behavior within members of a given cowbird population. Further, their research reminds us that any attempt to functionally separate the organism and its environment does not make biological sense.

Brown-headed cowbirds are generalist brood parasites that lay their eggs in the nests of over 200 species of birds, many of which (e.g. gnatcatchers and warblers) bear no resemblance (in size or coloration) to cowbirds. Avian brood parasitism, such as that displayed by cowbirds, seems at first glance to present a significant if not insurmountable challenge to developmental accounts of species-identification. How, after all, does a cowbird know that it is a cowbird? How does it know to interact with other cowbirds after leaving the nest of its foster parents? Such a strange system would seem to require an innate or pre-specified system of species-recognition for its continued success over successive generations. In one of their earliest studies, King and West (1977) indeed found that male cowbirds reared in isolation from other birds produced song that was atypical for cowbirds but was nonetheless highly attractive to female cowbirds – even more so than typical cowbird song. This finding strengthened an already popular idea that cowbirds had a "closed" or innately specified system of song-production and species identification. In subsequent studies, however, it became clear that despite their possession of highly attractive song, such social isolates were completely inept at courting and copulating with females (Freeberg et al., 1995). The assembly of skills involved in successful socialization and mating for male cowbirds turned out to depend upon a variety of social experiences that juvenile males receive in a typical cowbird flock setting (Freeberg, 1998; King & West, 2002).

One powerful influence on the vocal development of juvenile male cowbirds is the contingent feedback that they receive from females during their prolonged bouts of singing (King & West, 1983, 1989). Young males spend hours tirelessly singing to females, who, for the most part appear to ignore them (West, King & Arberg, 1988). Occasionally, however – when the male hits upon a particularly pleasing bit of song – the female cowbird will provide the male with a signal of positive feedback (e.g., a movement of the wing, adoption of a copulatory posture) that serves to shape his subsequent singing (West & King, 1988). Adult female cowbirds thus transmit crucial cultural information to each new generation of cowbirds by shaping the repertoires and eventual dialects of the young males who interact with them (Freeberg, 1998). A good song is, however, only one part of what a male cowbird needs to be successful at mating (King & West, 2002). As West et al. (2003) write, "song bears the imprint of a male's social and vocal history: its efficacy and use during the breeding season depends on the kind of social organization experienced during that history" (p. 620).

The species-identification skills of cowbirds have also been shown to be far more malleable than was once thought. Juvenile brown-headed cowbirds housed with canaries during their first year, for example, incorporate elements of canary song into their repertoires (King, Freeberg, & West, 1996) and vigorously pursue canaries in preference to cowbird females during their first spring (Freeberg

et al., 1995). If the filial preferences of cowbirds are so malleable, how is it that they manage to survive, given they are reared exclusively by birds of other species? Part of the answer is, of course, that in the wild young cowbirds eventually fledge and thus leave the company of their foster parents. The newly fledged cowbird looks and sounds like a cowbird and thus will attract (and be attracted to) the attention and company of other cowbirds. A nest with a cowbird egg in it is likely to be found only in an area containing similar nests and at least some of the female cowbirds that originally laid and males who fertilized these eggs. In the context of their natural ecology there is thus little danger of their high degree of malleability leading young cowbirds to "imprint" on the wrong species. Indeed, even the cowbirds housed with canaries who subsequently pursued canaries during their first year showed a reversal of this species-atypical behavior after spending an additional year housed with other cowbirds (Freeberg et al., 1995). As Freeberg and colleagues note, "such malleability would typically lead to correct recognition because the 'average expectable' environment... would contain reliable and redundant sources of information to guide learning about male and female conspecifics" (p. 364).

The cowbird research, with its focus on young birds' "developmental ecology," highlights the key point that behavioral phenotypes are generated, constrained, maintained, and reorganized through the activities and experience of an organism actively engaged with its surround. Behavioral development is self-organizing and probabilistic, a process in which pattern and order emerge and change as a result of the relations among developmentally relevant components both internal and external to the developing individual. Some of these interactions are unique or idiosyncratic to the individual (*organism-specific*) and many are common to nearly all members of a species (*species-specific*). Both types of experiences contribute to behavioral development – in the first case promoting individual variation in behavior and in the second case promoting species-typical behavior.

Concluding Remarks

The apparent seamlessness and consistency of phenotypic development within and across generations, despite the enormous complexity and variability of the environment, has led many biologists and psychologists to argue for the "innateness" of species-typical characteristics. We have argued that this nativistic view is both simplistic and incorrect. Building on the pioneering conceptual and empirical efforts of Gilbert Gottlieb, we have proposed that it is the dynamics of the individual's developmental system that is the source of both the stability and variability of phenotypic development observed within and across generations, eliminating the need for notions of preformed genetic programs to explain species-typical outcomes.

There is growing recognition that developing organisms inherit not only a genome, but an entire developmental system: a complex manifold of interacting factors, some inside and some outside the organism, including an ecological niche

in all its spatial and temporal aspects (Oyama, 1985). Early psychobiologists, including Kuo (1967) and Lehrman (1953) appreciated the importance of this systems view for the study of development, as did several zoologists of the day. For example, writing over 40 years ago, King (1968) pointed out that individuals of a species are typically raised by parents of the same species, in an environment that has been occupied by that species for many generations. King noted that this continuity of early experience from one generation to the next envelops the developing organism in a physical, biological, and social environment that is as characteristic of its species as is its genotype. Gottlieb's (1971a, 1997) research on the development of species identification in ducklings provided elegant examples of how nuanced and often non-obvious this continuity of early experience can be. His program of research in behavioral embryology carefully documented how the features and patterns of recurring prenatal sensory experience, including self-stimulation, guide and constrain young ducklings' selective attention, perception, learning, and memory during both the prenatal and postnatal periods.

Perhaps the most enduring contribution from Gottlieb's body of work was the clear demonstration that *species uniformity does not imply the absence of experiential input during development*. This demonstration effectively eliminated the dichotomy between "inherited" and "acquired" behavior promoted by many biologists and psychologists over the last century and fostered a deeper appreciation of the complex developmental dynamics involved in the realization of all phenotypic outcomes. Gottlieb was able to effectively resolve the nature-nurture dichotomy by replacing it with a synthesis of nature and nurture, a fully integrated depiction of development that takes into account the role of genes, nervous system, behavior, and environment (see Gottlieb, 1997).

Gottlieb also recognized that understanding the experiential dynamics of canalization and malleability would contribute to a fuller understanding of phenotypic development and advance both developmental and evolutionary theory. His ground breaking work on these topics (e.g., Gottlieb, 1987, 1991b, 1992) provided a new framework for making sense of how developmental and ecological dynamics contribute to stable behavioral outcomes and how developmentally based changes in behavior could contribute to the evolutionary process. In Gottlieb's view of evolution, genetic change is often a secondary or tertiary consequence of enduring transgenerational behavioral changes brought about by alterations of normal or species-typical development. From this view, alterations in the conditions of development can lead to significant change in behavior, which is often followed by changes in gene expression, hormones, physiology, and anatomy. These modifications often put individuals in new relations with their local environments, subjecting them to new selection pressures and increasing the likelihood of eventual change in the genetic composition of the population. A more complete articulation of how this evolutionary scenario might work will require more detailed description and experimentation, with the goal of explaining how each generation sets up the necessary developmental conditions

and resources for the next and how specific changes in developmental conditions lead to specific changes in behavior, anatomy, physiology, and gene expression. What we do know, drawing on decades of work by developmental psychobiologists, is that the conditions that best favor the expression of new phenotypes are species-atypical alterations in environmental conditions and contingencies early in life (e.g., Blumberg, 2008; Denenberg, 1969; Gottlieb, 1971b; Kuo, 1967; Levine, 1956; Szyf, Weaver, & Meaney, 2007; Renner & Rosenzweig, 1987; see Michel & Moore, 1995 for an overview).

Contrary to the still commonplace assumption that phenotypic stability is "biologically" based and phenotypic variability is "experience" based, a growing body of evidence indicates that there are not separate or distinct processes responsible for stability on the one hand and variability on the other. Both are the products of the bidirectional traffic among the various networks, resources, and levels of the organism–environment system. Our demonstration of the rapid redirection of the normally robust species-specific auditory preferences of bobwhite quail chicks, whereby they rapidly come to prefer the maternal call of another species in the days following hatching (Harshaw et al., 2008), illustrates the dynamic interplay between the forces of variability and the forces of stability during individual ontogeny. This interplay is particularly evident during early development, when neural, physiological, perceptual, and behavioral systems are undergoing rapid change and reorganization. An important next step in the study of phenotypic stability and plasticity will be to develop cross-level frameworks that can link and model the interactive, bidirectional processes occurring at these different levels of analysis over individual ontogeny. In our view, Gottlieb's notion of probabilistic epigenesis, with its emphasis on the fact that development is situated and historical and thus cannot have a predetermined trajectory, provides an essential conceptual framework for future investigations of the varied sources of phenotypic stability and variability within developmental systems.

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References

Abrams, T. W., & Kandel, E. R. (1988). Is contiguity detection in classical conditioning a system or a cellular property? Learning in Aplysia suggests a possible mechanism. *Trends in Neurosciences*, **11**, 128–135.

Aisner, R., & Terkel, J. (1992). Ontogeny of pine cone opening behaviour in the black rats. *Rattus rattus*. *Animal Behaviour*, **214**, 327–336.

Alberch, P. (1982). The generative and regulatory roles of development in evolution. In D. Mossakowski & G. Roth (Eds.), *Environmental adaptation and evolution* (pp. 19–36). New York: G. Fischer.

Alberts, J. R. (1994). Learning as adaptation of the infant. *Acta Paediatrica, Suppl.*, **397**, 77–85.

Alberts, J. R., & Cramer, C. P. (1988). Ecology and experience: Sources of means and meaning of developmental change. In E. M. Blass (Ed.), *Handbook of behavioral neurobiology, Vol. 9: Behavioral ecology and developmental psychobiology* (pp. 1–39). New York: Plenum Press.

Amundson, R. (2005). *The changing role of the embryo in evolutionary thought*. Cambridge: Cambridge University Press.

Anstey, M. L., Rogers, S. M., Ott, S. R., Burrows, M., & Simpson, S. J. (2009). Serotonin mediates behavioral gregarization underlying swarm formation in desert locusts. *Science*, **323**, 627–630.

Ariew, A. (1999). Innateness is canalization: In defense of a developmental account of innateness. In V. G. Hardcastle (Ed.), *Where biology meets psychology: Philosophical essays* (pp. 117–138). Cambridge, MA: MIT Press.

Arthur, W. A. (2002). The emerging conceptual framework of evolutionary developmental biology. *Nature*, **415**, 757–764.

Arthur, W. A. (2004). *Biased embryos and evolution*. Cambridge, UK: Cambridge University Press.

Avital, E., & Jablonka, E. (2000). *Animal traditions: Behavioural inheritance in evolution*. Cambridge: Cambridge University Press.

Ayala, F. J., & Valentine, J. W. (1979). *Evolving: The theory and progress of organic evolution*. California: The Benjamin Cummings Publishing Company.

Banker, H., & Lickliter, R. (1993). Effects of early and delayed visual experience on intersensory development in bobwhite quail chicks. *Developmental Psychobiology*, **26**, 155–170.

Bateson, P. P. G. (1966). The characteristics and context of imprinting. *Biological Reviews*, **41**, 177–220.

Bateson, P. P. G., & Reese, E. P. (1969). The reinforcing properties of conspicuous stimuli in the imprinting situation. *Animal Behaviour*, **17**, 692–699.

Bearhop, S., Fiedler, W., Furness, R. W., Votier, S. C., Waldron, S., Newton, J., et al. (2005). Assortative mating as a mechanism for rapid evolution of a migratory divide. *Science*, **310**, 502–504.

Bjorklund, D. F. (2006). Mother knows best: Epigenetic inheritance, maternal effects, and the evolution of human intelligence. *Developmental Review*, **26**, 213–242.

Blumberg, M. S. (2008). *Freaks of nature: What anomalies tell us about development and evolution*. New York: Oxford University Press.

Boakes, R., & Panter, D. (1985). Secondary imprinting in the domestic chick blocked by previous exposure to a live hen. *Animal Behaviour*, **33**, 353–365.

Bolhuis, J. J. (1991). Mechanisms of avian imprinting: A review. *Biological Reviews*, **66**, 303–345.

Bolhuis, J. J. (1999). Early learning and the development of filial preferences in the chick. *Behavioral. Brain Research*, **98**, 245–252.

Bolhuis, J. J., & Honey, R. C. (1998). Imprinting, learning and development: From behaviour to brain and back. *Trends in Neuroscience*, **21**, 306–311.

Bolhuis, J. J., & Johnson, M. H. (1988). Effects of response-contingency and stimulus presentation schedule on imprinting in chicks (*Gallus gallus domesticus*). *Journal of Comparative Psychology*, **102**, 61–65.

Bowlby, J. (1969). *Attachment and loss*, Vol. **1**, New York: Basic Books.

Brainard, M. S., & Doupe, A. J. (2000). Auditory feedback in learning and maintenance of vocal behaviour. *Nature Reviews Neuroscience*, **1**, 31–40.

Casey, M. B., & Karpinski, S. (1999). The development of postnatal turning bias is influenced by prenatal visual experience in domestic chicks (*Gallus gallus*). *The Psychological Record*, **49**, 67–74.

Casey, M. B., & Lickliter, R. (1998). Prenatal visual experience influences the development of turning bias in bobwhite quail chicks (*Colinus virginianus*). *Developmental Psychobiology*, **32**, 327–338.

ten Cate, C. (1986). Does behavior contingent stimulus movement enhance filial imprinting in Japanese quail? *Developmental Psychobiology*, **19**, 607–614.

ten Cate, C. (1989). Stimulus movement, hen behaviour and filial imprinting in Japanese quail (*Coturnix coturnix japonica*). *Ethology*, **82**, 287–306.

ten Cate, C. (2000). How learning mechanisms might affect evolutionary processes. *Trends in Ecology and Evolution*, **15**, 179–180.

ten Cate, C., & Bateson, P. (1988). Sexual selection: The evolution of conspicuous characteristics in birds by means of imprinting. *Evolution*, **42**, 1355–1358.

Clark, M. M., & Galef, B. G. (1977). The role of the physical rearing environment in the domestication of the Mongolian gerbil (*Meriones unguiculatus*). *Animal Behaviour*, **25**, 298–316.

Clark, M. M., & Galef, B. G. (1979). A sensitive period for the maintenance of emotionality in the Mongolian gerbil. *Journal of Comparative and Physiological Psychology*, **93**, 200–210.

Clark, M. M., & Galef, B. G. (1980). Effects of rearing environment on adrenal weights, sexual development and behavior in gerbils: An examination of Richter's domestication hypothesis. *Journal of Comparative and Physiological Psychology*, **94**, 857–863.

Clark, M. M., & Galef, B. G. (1981). Environmental influence on development, behavior, and endocrine morphology of gerbils. *Physiology and Behavior*, **27**, 761–765.

Darwin, C. (1859). *On the origin of species by means of natural selection*. London: John Murray.

Darwin, C. (1868). *The variation of animals and plants under domestication*. London: John Murray.

Davidson, E. H. (2001). *Genomic regulatory systems: Development and evolution*. New York: Academic Press.

Dawkins, R. (1976). *The selfish gene*. Oxford, UK: Oxford University Press.

de Beer, G. R. (1940). *Embryos and ancestors*. Oxford, UK: Oxford University Press.

Denenberg, V. H. (1969). The effects of early experience. In E. S. E. Hafez (Ed.), *Behavior of domestic animals* (2nd ed.).Baltimore, MD: Williams & Wilkins.

Denenberg, V. H., & Rosenberg, K. M. (1967). Nongenetic transmission of information. *Nature*, **216**, 549–550.

Deng, C., & Rogers, L. J. (2002). Prehatching visual experience and lateralization of the visual Wulst. *Behavioral. Brain Research*, **134**, 375–385.

Dickinson, A., & Shanks, D. (1995). Instrumental action and causal representation. In D. Sperber, D. Premack, & A. J. Premack (Eds.), *Causal cognition: A multidisciplinary debate* (pp. 5–25). New York: Oxford University Press.

Dobzhansky, T. (1951). *Genetics and the origin of species* (3rd ed.). New York: Columbia University Press.

Fifer, W. P., & Moon, C. M. (1995). The effects of fetal experience with sound. In J.-P. Lecanuet, W.P. Fifer, N. A. Krasnegor, & W.P. Smotherman (Eds.), *Fetal development: A psychobiological perspective* (pp. 351–366). Hillsdale, NJ: Erlbaum.

Foushée, R., & Lickliter, R. (2002). Early visual experience affects postnatal auditory responsiveness in bobwhite quail (*Colinus virginianus*). *Journal of Comparative Psychology*, **116**, 369–380.

Freeberg, T. M. (1998). The cultural transmission of courtship patterns in cowbirds. *Molothrus ater. Animal Behaviour*, **56**, 1063–1073.

Freeberg, T. M., & White, D. J. (2006). Social traditions and the maintenance and loss of geographic variation in mating patterns of brown-headed cowbirds. *International Journal of Comparative Psychology*, **19**, 206–222.

Freeberg, T. M., King, A. P., & West, M. J. (1995). Social malleability in cowbirds (*Molothrus ater artemisiae*): Species and mate recognition during the first 2 years of life. *Journal of Comparative Psychology*, **109**, 357–367.

Garstang, W. (1922). The theory of recapitulation: A critical re-statement of the biogenetic law. *Journal of the Linnean Society of Zoology*, **35**, 81–101.

Gerhart, J. C., & Kirschner, M. W. (1997). *Cells, embryos, and evolution: Toward a cellular and developmental understanding of phenotypic variation and evolutionary adaptability*. Boston, MA: Blackwell Science.

Gilbert, S. F. (2000). *Developmental Biology* (6th ed). Sunderland, MA: Sinauer Associates.

Gilbert, S. F. (2005). Mechanisms for the environmental regulation of gene expression: Ecological aspects of animal development. *Journal of Biosciences*, **30**, 65–74.

Gilbert, S. F., Opitz, J. M., & Raff, R. A. (1996). Reintegrating developmental and evolutionary biology. *Developmental Biology*, **173**, 357–372.

Goldschmidt, R. B. (1940). *The material basis of heredity*. New Haven, CT: Yale University Press.

Goodwin, B. C. (1994). *How the leopard changed its spots: The evolution of complexity*. New York: Scribners.

Gottlieb, G. (1963a). A naturalistic study of imprinting in wood ducklings (*Aix sponsa*). *Journal of Comparative and Physiological Psychology*, **56**, 86–91.

Gottlieb, G. (1963b). "Imprinting" in nature. *Science*, **139**, 497–498.

Gottlieb, G. (1965). Imprinting in relation to parental and species identification by avian neonates. *Journal of Comparative and Physiological Psychology*, **59**, 345–356.

Gottlieb, G. (1970). Conceptions of prenatal behavior. In L. R. Aronson, E. Tobach, D. S. Lehrman, & J. S. Rosenblatt (Eds.), *Development and evolution of behavior* (pp. 111–137). San Francisco: W. H. Freeman.

Gottlieb, G. (1971a). Ontogenesis of sensory function in birds and mammals. In E. Tobach, L. R. Aronson, & E. Shaw (Eds.), *The biopsychology of development* (pp. 67–128). New York: Academic Press.

Gottlieb, G. (1971b). *Development of species identification in birds: An enquiry into the prenatal determinants of perception*. Chicago, IL: University of Chicago Press.

Gottlieb, G. (1975). Development of species identification in ducklings: II. Experiential prevention of perceptual deficit caused by embryonic auditory deprivation. *Journal of Comparative and Physiological Psychology*, **89**, 675–684.

Gottlieb, G. (1987). The developmental basis of evolutionary change. *Journal of Comparative Psychology*, **101**, 262–271.

Gottlieb, G. (1991a). Social induction of malleability in ducklings. *Animal Behaviour*, **41**, 953–962.

Gottlieb, G. (1991b). Experiential canalization of behavioral development: Theory. *Developmental Psychology*, **27**, 4–13.

Gottlieb, G. (1992). *Individual development and evolution: The genesis of novel behavior*. New York: Oxford University Press.

Gottlieb, G. (1993). Social induction of malleability in ducklings: Sensory basis and psychological mechanisms. *Animal Behaviour*, **45**, 707–719.

Gottlieb, G. (1997). *Synthesizing nature–nurture: Prenatal roots of instinctive behavior*. Mahwah, NJ: Erlbaum.

Gottlieb, G. (1998). Normally occurring environmental and behavioral influences on gene activity: From central dogma to probabilistic epigenesis. *Psychological Review*, **105**, 792–802.

Gottlieb, G. (2002). Developmental–behavioral initiation of evolutionary change. *Psychological Review*, **109**, 211–218.

Gottlieb, G., & Vandenbergh, J. G. (1968). Ontogeny of vocalization in duck and chick embryos. *Journal of Experimental Zoology*, **168**, 307–325.

Gould, S. J. (1977). *Ontogeny and phylogeny*. Cambridge MA: Belknap Press.

Greenough, W. T., & Chang, F. F. (1989). Plasticity of synapse structure and pattern in the cerebral cortex. *Cerebral Cortex*, **7**, 391–399.

Guiton, P. (1959). Socialization and imprinting in brown leghorn chicks. *Animal Behaviour*, **7**, 26–34.

Hale, E. B. (1969). Domestication and the evolution of behavior. In E. S. E. Hafez (Ed.), *Behavior of domestic animals* (2nd ed., pp. 22–42). Baltimore, MD: Williams & Wilkins.

Hall, B. K. (1999). *Evolutionary developmental biology* (2nd ed.).Boston: Kluwer.

Hall, B. K., & Olson, W. M. (2003). *Keywords and concepts in evolutionary developmental biology*. Cambridge, MA: Harvard University Press.

Haraway, M. M., & Maples, E. G. (1998). Species-typical behavior. In G. Greenberg, & M.M. Haraway (Eds.), *Comparative psychology: A handbook* (pp. 191–197). New York: Garland Publishing.

Harshaw, C., & Lickliter, R. (2007). Interactive and vicarious acquisition of auditory preferences in Northern bobwhite (*Colinus virginianus*) chicks. *Journal of Comparative Psychology*, **121**, 320–331.

Harshaw, C., Tourgeman, I. P., & Lickliter, R. (2008). Stimulus contingency and the malleability of species-typical auditory preferences in Northern bobwhite (*Colinus virginianus*) hatchlings. *Developmental Psychobiology*, **50**, 460–472.

Heaton, M. B., Miller, D. B., & Goodwin, D. G. (1978). Species-specific auditory discrimination in bobwhite quail neonates. *Developmental Psychobiology*, **11**, 13–21.

Heinroth, O. (1911). Beiträge zur Biologie, namentilich Ethologie und Psychologie der Anatiden. *Proceedings of the International Ornithological Congress*, **5**, 598–702.

Hess, E. H. (1972). "Imprinting" in a natural laboratory. *Scientific American*, **227**, 24–31.

Ho, M. W., & Saunders, P. T. (1979). Beyond neo-Darwinism: An epigenetic approach to evolution. *Journal of Theoretical Biology*, **78**, 673–691.

Jablonka, E., & Lamb, M. J. (1995). *Epigenetic inheritance and evolution: The Lamarckian dimension*. Oxford University Press.

Jablonka, E., & Lamb, M. J. (2005). *Evolution in four dimensions: Genetic, epigenetic, behavioral, and symbolic variation in the history of life*. Cambridge, MA: MIT Press.

Järvilehto, T. (2006). *Behavior: Role of genes. In Encyclopedia of life sciences*. New York: John. Wiley.

Johnson, M. H., Bolhuis, J. J., & Horn, G. (1985). Interaction between acquired preferences and developing predispositions during imprinting. *Animal Behaviour*, **33**, 1000–1006.

Johnson, R. A. (1969). Hatching behavior of the bobwhite. *Wilson Bulletin*, **81**, 79–86.

Johnston, T. D., & Edwards, L. (2002). Genes, interactions, and the development of behavior. *Psychological Review*, **109**, 26–34.

Johnston, T. D., & Gottlieb, G. (1981). Visual preferences of imprinted ducklings are altered by the maternal call. *Journal of Comparative and Physiological Psychology*, **95**, 663–675.

Johnston, T. D., & Gottlieb, G. (1985). Development of visually controlled maternal preferences in Peking ducklings. *Developmental Psychobiology*, **18**, 23–36.

Johnston, T. D., & Gottlieb, G. (1990). Neophenogenesis: A developmental theory of phenotypic evolution. *Journal of Theoretical Biology*, **147**, 471–495.

King, A., Freeberg, T., & West, M. (1996). Social experience affects the process and outcome of vocal ontogeny in two populations of cowbirds. *Journal of Comparative Psychology*, **110**, 276–285.

Kang, L., Chen, X. Y., Zhou, Y., Zheng, W., Li, R. Q., Wang, J., et al. (2004). The analysis of large-scale gene expression correlated to the phase changes of the migratory locust. *Proceeding of the National Academy of Sciences*, **101**, 17611–17615.

Kaufman, I. C. (1975). Learning what comes naturally: The role of life experience in the establishment of species-typical behavior. *Ethos*, **3**, 129–142.

King, J. A. (1968). Species specificity and early experience. In G. Newton, & S. Levine (Eds.), *Early experience and behavior* (pp. 42–64). Springfield, IL: Charles C. Thomas.

King, A. P., & West, M. J. (1977). Species identification in the North American cowbird: Appropriate responses to abnormal song. *Science*, **195**, 1002–1004.

King, A. P., & West, M. J. (1983). Epigenesis of cowbird song: A joint endeavour of males and females. *Nature*, **305**, 704–706.

King, A. P., & West, M. J. (1989). Presence of female cowbirds (*Molothrus ater ater*) affects vocal improvisation in males. *Journal of Comparative Psychology*, **103**, 39–44.

King, A. P., & West, M. J. (2002). The ontogeny of competence. In D. Lewkowicz, & R. Lickliter (Eds.), *Conceptions of development* (pp. 77–103). New York: Psychology Press.

Kirschner, M. W., & Gerhart, J. C. (2005). *The plausibility of life*. New Haven, CT: Yale University Press.

Kuo, Z.-Y. (1967). *The dynamics of behavior development*. New York: Plenum.

Levine, S. (1956). A further study of infantile handling and adult avoidance learning. *Journal of Personality*, **25**, 70–80.

Lehrman, D. S. (1953). A critique of Konrad Lorenz's theory of instinctive behavior. *Quarterly Review of Biology*, **28**, 337–363.

Lewontin, R. (2000). *The triple helix*. Cambridge, MA: Harvard University Press.

Lickliter, R. (1995). Embryonic sensory experience and intersensory development in precocial birds. In J. P. Lecanuet, N. A. Krasnegor, W. Fifer, & W. P. Smotherman (Eds.), *Fetal development: A psychobiological perspective* (pp. 281–294). Hillsdale, NJ: Erlbaum.

Lickliter, R. (1996). Structured organisms and structured environments: Developmental systems and the construction of learning capacities. In J. Valsiner, & H.G. Voss (Eds.), *The structure of learning processes* (pp. 86–108). Norwood, NJ: Ablex.

Lickliter, R. (2000). An ecological approach to behavioral development: Insights from comparative psychology. *Ecological Psychology*, **12**, 319–334.

Lickliter, R. (2005). Prenatal sensory ecology and experience: Implications for perceptual and behavioral development in precocial birds. In P. Slater, J. Rosenblatt, C. Snowden, T. Roper, H. J. Brockmann, & M. Naguib (Eds.), *Advances in the Study of Behavior*, Vol. **35** (pp. 235–274). New York: Academic Press.

Lickliter, R. (2008). Theories of attachment: The long and winding road to an integrative developmental science. *Integrative Psychological and Behavioral Science*, **42**, 397–405.

Lickliter, R., & Gottlieb, G. (1985). Social interaction with siblings is necessary for visual imprinting of species-specific maternal preferences in ducklings (*Anas platyrhynchos*). *Journal of Comparative Psychology*, **99**, 371–379.

Lickliter, R., & Gottlieb, G. (1987). Retroactive excitation: Posttraining social experience with siblings consolidates maternal imprinting in ducklings (*Anas platyrhynchos*). *Journal of Comparative Psychology*, **101**, 40–46.

Lickliter, R., & Harshaw, C. (2008). *Prenatal experience, contingency, and the malleability of species-specific auditory preferences*. Paper presented at the annual meeting of the *International Society for Developmental Pyschobiology, Washington, DC*.

Lickliter, R., & Hellewell, T. (1992). Contextual determinants of auditory learning in bobwhite quail embryos and hatchlings. *Developmental Psychobiology*, **25**, 17–31.

Lickliter, R., & Honeycutt, H. (2009). Rethinking epigenesis and evolution in light of developmental science. In M. Blumberg, J. Freeman, & S. Robinson (Eds.), *Oxford handbook of developmental behavioral neuroscience* (pp. 30–47). New York: Oxford University Press.

Lickliter, R., & Ness, J. W. (1990). Domestication and comparative psychology: Status and strategy. *Journal of Comparative Psychology*, **104**, 211–218.

Lickliter, R., & Virkar, P. (1989). Intersensory functioning in bobwhite quail chicks: Early sensory dominance. *Developmental Psychobiology*, **22**, 651–667.

Lickliter, R., Dyer, A. B., & McBride, T. (1993). Perceptual consequences of early social experience in precocial birds. *Behavioural Processes*, **30**, 185–200.

Lorenz, K. (1937). The companion in the bird's world. *Auk*, **54**, 245–273.

Maynard-Smith, J., Burian, R., Kaufman, S., Alberch, P., Campbell, J., Goodwin, B., et al. (1985). Developmental constraints and evolution. *Quarterly Review of Biology*, **60**, 265–287.

Mayr, E. (1982). *The growth of biological thought*. Cambridge, MA: Harvard University Press.

Mayr, E., & Provine, W. B. (1980). *The evolutionary synthesis*. Cambridge, MA: Harvard University Press.

Markham, R., Toth, G., & Lickliter, R. (2006). Prenatally elevated physiological arousal interferes with perceptual learning in bobwhite quail (*Colinus virginianus*) embryos. *Behavioral Neuroscience*, **120**, 1315–1325.

Matsuda, R. (1987). *Animal evolution in changing environments, with special reference to abnormal metamorphosis*. New York: John Wiley.

Michel, G. F., & Moore, C. L. (1995). *Developmental psychobiology: An interdisciplinary science*. Cambridge, MA: MIT Press.

Miller, D. B. (1997). The effects of nonobvious forms of experience on the development of instinctive behavior. In C. Dent-Reed, & P. Zukow-Goldring (Eds.), *Evolving explanations of development* (pp. 457–507). Washington, DC: American Psychological Association.

Newman, S. A., & Müller, G. B. (2000). Epigenetic mechanisms of character origination. *Journal of Experimental Zoology*, **288**, 304–317.

Nijhout, H. F. (2003). Development and evolution of adaptive polyphenisms. *Evolution & Development*, **5**, 9–18.

Norton-Griffiths, M. (1969). The organization, control and development of parental feeding in the oystercatcher. *Behaviour*, **34**, 55–114.

Oyama, S. (1985). *The ontogeny of information: Developmental systems and evolution*. Cambridge: Cambridge University Press.

Oyama, S. (1993). Constraints and development. *Netherlands Journal of Zoology*, **43**, 6–16.

Payne, R. B., Payne, L. L., Woods, J. L., & Sorenson, M. D. (2000). Imprinting and the origin of parasite-host associations in brood-parasitic indigobirds (*Vidua chalybeata*). *Animal Behaviour*, **59**, 69–81.

Pedersen, P. E., & Blass, E. M. (1982). Prenatal and postnatal determinants of the first suckling episode in albino rats. *Developmental Psychobiology*, **15**, 349–355.

Pigliucci, M. (2007). Do we need an extended evolutionary synthesis? *Evolution*, **61**, 2743–2749.

Price, E. O. (1999). Behavioral development in animals undergoing domestication. *Applied Animal Behaviour Science*, **65**, 245–271.

Price, E. O., & King, J. A. (1968). Domestication and adaptation. In E. S. E. Hafez (Ed.), *Adaptation of domestic animals* (pp. 34–45). Philadelphia, PA: Lea and Febiger.

Raff, R. A. (2000). Evo-devo: The evolution of a new discipline. *Nature Reviews: Genetics*, **1**, 74–79.

Reid, R. G. B. (1985). *Evolutionary theory: The unfinished synthesis*. Beckenham: Crom Helm.

Reid, R. G. B. (2007). *Biological emergences: Evolution by natural experiment*. Cambridge, MA: MIT Press.

Renner, M. J., & Rosenzweig, M. R. (1987). *Enriched and impoverished environments. Effects on brain and behavior*. New York: Springer-Verlag.

Robert, J. S. (2004). *Embryology, epigenesis, and evolution: Taking development seriously*. Cambridge, UK: Cambridge University Press.

Rogers, L. J. (1995). *The development of brain and behaviour in the chicken*. Wallingford, UK: CAB International.

Ronca, A. E., & Alberts, J. R. (1994). Sensory stimuli associated with gestation and parturition evoke cardiac and behavioral responses in fetal rats. *Psychobiology*, **22**, 270–282.

Rosenzweig, M. R., & Bennett, E. L. (1972). Cerebral changes in rats exposed individually to an enriched environment. *Journal of Comparative and Physiological Psychology*, **80**, 304–313.

Rossiter, M. C. (1996). Incidence and consequence of inherited environmental effects. *Annual Review of Ecology and Systematics*, **27**, 451–476.

Russell, E. S. (1930). *Interpretation of development and heredity*. New York: Clarendon Press.

Sapp, J. (2003). *Genesis: The evolution of biology*. Oxford, UK: Oxford University Press.

Schmalhausen, I. I. (1949). *Factors of evolution: The theory of stabilizing selection*. Chicago: University of Chicago Press.

Schneirla, T. C. (1956). Interrelationships of the "innate" and "acquired" in instinctive behavior. In P. P. Grasse (Ed.), *L'Instinct dans le comportment des animaux et de l'homme* (pp. 387–452). Paris: Masson.

Shank, S. S., & Margoliash, D. (2008). Sleep and sensorimotor integration during early vocal learning in a songbird. *Nature*, **458**, 73–77.

Shanks, D. R., Pearson, S. M., & Dickinson, A. (1989). Temporal contiguity and the judgment of causality by human subjects. *The Quarterly Journal of Experimental Psychology Section B*, **41**, 139–159.

Schneider, S. M., & Harshaw, C. (2007). Whence malleability? Gottlieb's ducklings, operant contingency, and the social manifold. *European Journal of Developmental Science*, **1**, 233–237.

Siegal, M. L., & Bergman, A. (2002). Waddington's canalization revisited: Developmental stability and evolution. *Proceedings of the National Academy of Sciences*, **99**, 10528–10532.

Smotherman, W. P., & Robinson, S. R. (1990). The prenatal origins of behavioral organization. *Psychological Science*, **1**, 97–106.

Spalding, D. A. (1873). Instinct. With original observations on young animals. *Macmillan's Magazine*. **27**, 282–293.

Spear, N. E. (1984). Ecologically determined dispositions control the ontogeny of learning and memory. In R. Kail, & N. E. Spear (Eds.), *Comparative perspectives on the development of memory* (pp. 325–358). Hillsdale, NJ: Erlbaum.

Spear, N. E., & McKinzie, D. L. (1994). Intersensory integration in the infant rat. In D. J. Lewkowicz, & R. Lickliter (Eds.), *The development of intersensory perception: Comparative perspectives* (pp. 133–161). Hillsdale, NJ: Erlbaum.

Sultan, S. E., & Stearns, S. C. (2005). Environmentally contingent variation: Phenotypic plasticity and norms of reaction. In B. K. Hall, & B. Hallgrimsson (Eds.), *Variation: A hierarchical examination of a central concept in biology* (pp. 303–332). London: Elsevier Academic Press.

Szyf, M., Weaver, I., & Meaney, M. (2007). Maternal care, the epigenome and phenotypic differences in behavior. *Reproductive Toxicology*, **24**, 9–19.

Terkel, J. (1996). Cultural transmission of feeding behavior in the black rat (*Rattus rattus*). In C. M. Heyes, & B.G. Galef, Jr. (Eds.), *Social learning in animals: The roots of culture* (pp. 17–48). New York: Academic Press.

Thompson, E. (2007). *Mind in life: Biology, phenomenology, and the sciences of mind*. Cambridge, MA: Harvard University Press.

Tuculescu, R. A., & Griswold, J. G. (1983). Prehatching interactions in domestic chickens. *Animal Behaviour*, **31**, 1–10.

Tyrer, N. M., Turner, J. D., & Altman, J. S. (1984). Identifiable neurons in the locust central nervous system that react with antibodies to serotonin. *Journal of Comparative Neurology*, **227**, 313–330.

Vince, M. A. (1972). Communication between quail embryos and the synchronization of hatching. In K. H. Voous (Ed.), *Proceedings of the XVth International Ornithological Congress* (pp. 357–362). Leiden, The Netherlands: E. J. Brill.

Waddington, C. H. (1942). Canalization of development and the inheritance of acquired characters. *Nature*, **150**, 563–565.

Waddington, C. H. (1975). *The evolution of an evolutionist*. Edinburgh: Edinburgh University Press.

Wallace, M. T., & Stein, B. E. (2007). Early experience determines how the senses will interact. *Journal of Neurophysiology*, **97**, 921–926.

Weaver, I. C., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., et al. (2004). Epigenetic programming by maternal behavior. *Nature Neuroscience*, **7**, 847–854.

West, M. J., & King, A. P. (1987). Settling nature and nurture into an ontogenetic niche. *Developmental Psychobiology*, **20**, 549–562.

West, M. J., & King, A. P. (1988). Female visual displays affect the development of male song in the cowbirds. *Nature*, **334**, 244–246.

West, M. J., King, A. P., & Arberg, A. A. (1988). The inheritance of niches: The role of ecological legacies in ontogeny. In E. M. Blass (Ed.), *Developmental psychobiology and behavioral ecology* (pp. 41–62). New York: Plenum Press.

West, M. J., King, A. P., & Freeberg, T. M. (1994). The nature and nurture of neophenotypes. In L. Real (Ed.), *Behavioral mechanisms in evolutionary ecology* (pp. 238–257). Chicago: University of Chicago Press.

West, M. J., King, A. P., & White, D. J. (2003). The case for developmental ecology. *Animal Behaviour*, **66**, 617–622.

West-Eberhard, M. J. (1989). Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics*, **20**, 249–278.

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. New York: Oxford University Press.

White, D. J., King, A. P., & West, M. J. (2002). Plasticity in adult development: Experience with young males enhances mating competence in adult male cowbirds (*Molothrus ater*). *Behaviour*, **139**, 713–728.

Williams, G. C. (1966). *Adaptation and natural selection*. Princeton, NJ: Princeton University Press.

Wimsatt, W. C. (1986). Developmental constraints, generative entrenchment, and the innate–acquired distinction. In B. W. Bechtel (Ed.), *Integrating scientific disciplines* (pp. 185–208). Dordrecht: Martinus-Nijhoff.

Part IV
Applications to Development

17

Gene-Parenting Interplay in the Development of Infant Emotionality

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Why do some infants become happy, even-tempered, and agreeable children, whereas others become irritable, anxious, or emotionally intense and labile? These qualities of emotionality are often apparent in early infancy and reflect processes that are integral components of temperament (e.g., Buss & Plomin, 1984; Goldsmith & Campos, 1982; Rothbart & Derryberry, 1981). Temperament is generally thought to be a set of inherited personality traits that are observable and stable from early infancy onward (Buss & Plomin, 1984; Rothbart, Ahadi, & Evans, 2000). Despite the emphasis on the genetic underpinnings of temperament and its stability across the lifespan, theory and empirical research suggest that core components of temperament, particularly emotionality, can be substantially modified by early environmental experiences (e.g., Kagan, 1989).

The origins of infant emotion reactivity and regulation, fundamental components of temperament, have been studied from various perspectives over the past several decades. Emotion reactivity is defined as an individual's response to a stimulus change, or an alteration in the environment, which is reflected in changes in the somatic, endocrine, and autonomic nervous systems. This change is observed in the excitability or arousability of response systems in both temporal features (e.g., how fast responses begin after stimuli, how rapidly they escalate, how long they last, how slowly they go away) and intensity features (e.g., how strongly responses are expressed, how sensitive they are to stimulation). Emotion regulation has been defined as "extrinsic and intrinsic processes responsible for monitoring, evaluating, and modifying emotional reactions, especially their intensive and temporal features, to accomplish one's goals" (Thompson, 1994, p. 27–28).

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Developmental theories focus on experiences within families, especially parenting, as the primary influence on child development. During the first years of life, parent--infant relationships may have an impact on the development of emotionality by altering biological processes that are involved with fundamental component processes of reactivity and regulation, including genetic activity (e.g., Caspi et al., 2003), physiological or hormonal responses (e.g., Gunnar & Donzella, 2002), and neural networking (e.g., Schore, 2000). Various theoretical perspectives propose that sensitive and responsive parenting may facilitate the organization of infants' physiological systems needed to support optimal emotion reactivity and effective emotion regulation (Panksepp, 1986; Schore, 2000; Siegel, 2001).

Despite the primacy of parenting in developmental theories, causality cannot be attributed to one factor as the origin of a given developmental outcome. Instead, as Gottlieb proposed in his theoretical expositions of a developmental systems model, it is the coaction among environmental, behavioral, neural, and genetic levels over time that pushes development forward (Gottlieb, 1991, 1998; Gottlieb & Halpern, 2002). The bidirectional movement of traffic within and among several levels of analyses results in probabilistic epigenesis, that is, outcomes that are probabilistic, rather than pre-determined (Gottlieb, 1970). Consequently, organisms with a similar genetic makeup may sustain very different outcomes based on coactions with diverse life experience (Caspi et al., 2002; Heim et al., 2000).

Chapter Structure

In this chapter, we use Gottlieb's developmental systems model to frame discussion of coactions between genetic mechanisms and parenting as contributors to development of infants' temperament, specifically via endophenotypic development of physiological processes that are related to emotion reactivity and regulation. We propose that the coaction of parenting and genetic processes affects the expression of infants' temperament through their impact on rapidly developing physiological systems of emotion reactivity and emotion regulation. The impact of parenting during developmentally sensitive periods, such as early infancy, may have an important adaptive function, affording a plasticity that prepares individuals to adapt most successfully to the environment into which they are born (e.g., Boyce & Ellis, 2005).

Consistent with Gottlieb's delineation of the limited utility of broad measures of heritability for developmental analysis and the proposal of Moffitt, Caspi, and Rutter (2006, p. 9) that the search for gene-environment mechanisms should be theoretically driven beginning with "plausible triads" (i.e., gene, environmental factor, behavioral phenotype), in this chapter we focus on literature that has examined specific candidate genes, on parenting as an environmental factor, and on

stability and change in infants' emotionality, indexed by physiological and behavioral functioning, as phenotype.

First we briefly define the mechanisms of gene-environment correlation (rGE) and gene-environment interaction (G × E), where E is defined here as parenting, and discuss how these mechanisms may affect the development of infants' emotionality. A consideration of gene-environment correlation is necessary in analyses of gene-environment interactions. Next we describe genetic literature related to dopamine and serotonin, and interactions between relevant candidate genes and parenting in relation to elements of temperament. We then provide an explanation of physiological processes associated with emotional regulation, as an element of temperament, and describe literature linking these processes with infant behavior and development. We also describe how these processes may mediate the coactional contributions of parenting and genetic expression to infant emotional development. We conclude with a discussion of implications for future research.

Gene-Environment Correlation

Gene-Environment Correlation and Stability In Infants' Emotionality

Genetic contributions to a child's emotionality may be related to measures of parenting because of rGE. Although three types of rGE are defined: passive, active, and evocative (e.g., Scarr & McCartney, 1983), evocative rGE, described below, is most relevant to the current discussion of coactions between children's genetic makeup and parenting. Passive rGE occurs when genes shared between the child and parent contribute to both infant emotionality and parenting. Active rGE occurs when genetic characteristics of the child contribute to his or her active choice of environmental experiences. Because the genetics of parenting are beyond the focus of this chapter (see Ganiban, Leve, Moore, & Neiderhiser, 2008 for a recent discussion of this topic) and because infants are not able to make active choices about the parenting environments into which they are born, passive and active rGE will not be considered further in this chapter.

Evocative rGE occurs when genetically influenced characteristics of the child, such as emotionality, elicit specific responses from the parent. For example, a child who shows a high level of negative reactivity to events and is difficult to soothe may have a parent who becomes overwhelmed or frustrated and, in response, is more negative or intrusive and therefore less sensitive. Passive and evocative rGE may be difficult to disentangle. For example, a child whose parent has a genetically influenced tendency towards negative emotionality that affects his or her parenting, may inherit those genetic factors, increasing the likelihood of displaying behaviors that elicit negative, insensitive parenting. In this case, parenting does not necessarily have a causal influence on the expression of child emotionality. Rather,

the two may be associated because of genetic influences shared between parent and child, or because of evocative rGE.

Research provides evidence for evocative rGE. O'Connor, Neiderhiser, Reiss, Hetherington, & Plomin (1998) found that children born to mothers with higher rates of antisocial behaviors, who are more likely than other children to inherit negative emotionality, were more likely to receive negative parenting from their adoptive caregivers, and that this association was mediated by children's disruptive behaviors. Ge, Conger, Cadoret, & Neiderhiser (1996), also using an adoption design, found comparable results, whereas Riggins-Caspers, Cadoret, Knutson, & Langebehn (2003) found that such evocative child effects were primarily found in adoptive families with high cumulative social risk (e.g., alcohol abuse, depression, divorce). Although adoption studies have been fruitful in identifying children's genetic variability as a potential source of variation in parenting behaviors, the ability to examine directly candidate genes and their contributions to the family system is a relatively new approach to examining coactions among genes and environments (Rutter, Moffitt, & Caspi, 2006).

Molecular genetics research has made many advances in recent developmental and family studies. Research by Propper, Willoughby, Halpern, Carbone, & Cox (2007), Propper et al. (2008) and Mills-Koonce et al. (2007) suggest that children with specific polymorphic variations in two dopamine receptor genes (DRD2 and DRD4) that are associated with behaviors indicative of problematic emotion reactivity and regulation (e.g., novelty seeking, impulsivity; see Cloninger, 1987; Ebstein et al., 1998; Noble et al., 1998; Suhara et al., 2001) receive lower levels of sensitive caregiving from their mothers. Mills-Koonce et al. (2007) found that this association was not due to maternal variations in the DRD2 polymorphism, suggesting that this may be an evocative rather than passive gene-environment correlation.

Regardless of whether any given correlation between child genotype and parental behavior is passive or evocative in nature, an appreciation of gene-environment correlations within a research sample may be critical for understanding the implications (and potential limitations) of specific interactions between genes and environment. Furthermore, these associations highlight the importance of differentiating the statistical term, *interaction*, from the epigenetic term, *coaction*. In this light, it is important to remember that child genotype and parental phenotypic behavior are both part of one family (Cox & Paley, 2003) and one developmental epigenetic (Gottlieb, 1998) system, and that the co-dependencies between these separate genetic and behavioral subsystems must be considered with respect to how they jointly organize the function and activities of each member of the family system (Cairns, 1997).

Nevertheless, to the extent that infant emotionality is influenced by genetic factors, in most cases we would expect mechanisms of rGE to *increase* stability of emotionality over time. Examining mechanisms of gene-environment interaction ($G \times E$) may provide a clearer understanding of *change* in emotionality and how

parenting could modify aspects of infant emotionality that are initially genetically influenced.

Gene-Environment Interaction and Change in Infant Emotionality

Gene-environment interaction occurs when individuals with different genotypes have differential responses to the same environmental factor. For example, the impact of harsh, negative parenting may be greater (or lesser) on a child with a genetic risk for negative emotionality. Here it is important to note that processes of rGE and G × E can occur simultaneously. Passive rGE and evocative rGE, as described above, and G × E may all be operating in the following example: A parent and child have a shared genetic tendency toward negative emotionality (passive rGE), the parents' negative emotionality is elicited frequently by the child's negative emotionality (evocative rGE), and the child's genotype may make him differentially sensitive to the harmful effects of harsh, negative parenting (G × E). Again, however, we would expect these processes working in tandem to result in substantial stability in infants' emotionality. Thus, in a context where rGE is expected, that is, biological parents rearing their children, developmental theories should take into account that parenting may not have a consistently strong effect on *change* in a child's emotionality.

One of the lessons of much of the behavioral genetics research of the past decade is that for children who do not carry specific risk genotypes, some presumably negative environmental experiences, such as harsh, negative parenting, may have little, or variable, effects on aspects of their functioning. For example, recent research found that infants who did *not* carry a specific form of the dopamine receptor gene DRD2 (*taq*1 A1) that has been associated with negative outcomes showed effective physiological regulation of emotion (measured as change in vagal tone in response to maternal separation) regardless of parenting sensitivity. Infants who *did* carry the *taq*1 A1allele showed ineffective physiological regulation at 3 and 6 months, again regardless of parenting sensitivity, but, by 12 months, showed effective physiological regulation if their mothers were rated as being higher in sensitivity (Propper et al., 2008; see Figure 17.1). This example of G × E illustrates a differential sensitivity to parenting as a function of genotype.

The G × E interaction described above unfolds over time. At 3 and 6 months, the presence of the *taq*1 A1 DRD2 allele was associated with atypical lack of vagal withdrawal in a challenging situation, suggesting less effective physiological regulation. This pattern represents a fixed strategies pattern (Belsky, 2005) with a main effect for genotype. By 12 months, the statistical interaction between genotype and parenting was evident, such that only infants at dual risk, genetically and environmentally, showed the atypical lack of vagal withdrawal. This unfolding of an interaction between genotype and parenting over time illustrates the concept of coactions between genes and environment.

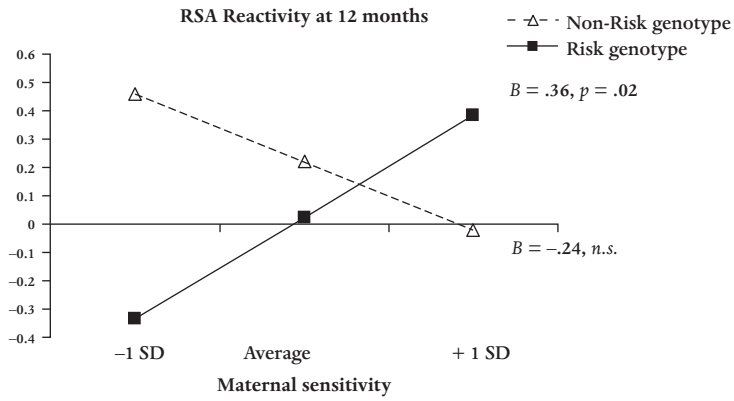

Figure 17.1. Gene-parenting interaction effect: Amount of infants' RSA reactivity in a challenge task as a function of maternal sensitivity.

Differential Susceptibility Hypothesis

In a variation of G × E theory, the differential susceptibility hypothesis (e.g., Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2007), individuals with a specific genotype are proposed to be more susceptible than other children to environmental influences, whether those influences act as risk or protective factors. This formulation of G × E proposes that specific genotypes may make some children show enhanced negative effects when interacting with environmental risk factors, but also show enhanced positive effects when interacting with environmental protective factors.

Assume, hypothetically, in the research previously discussed, that infants with the *taq*1 A1 DRD2 allele that appears to confer risk for ineffective physiological regulation, showed a differential *susceptibility* to parenting. In this case, infants in the risk genotype group with mothers rated low in sensitivity would show significantly lower values of the expected physiological response than all other infants, regardless of genotype, whereas infants in the risk genotype group with highly sensitive mothers would show significantly higher values of the expected physiological response than all other infants, including those without the "risk" allele. This pattern of results would support the differential susceptibility hypothesis, providing evidence that infants with a particular genotype showed increased negative effects when exposed to an environmental risk factor (insensitive parenting) and increased positive effects when exposed to an environmental protective factor (sensitive parenting). In this hypothetical example, as in the actual research, the physiological regulation of children without the risk genotype is not affected by parenting, although that is not a condition of the differential susceptibility model.

The differential susceptibility model differs from the traditional G × E sensitivity model in that it does not assume that a particular genotype confers a susceptibility to environmental influences in only one direction (i.e., either risk or protective), but that a particular genotype may confer a heightened susceptibility to *both* positive and negative rearing influences (Belsky et al., 2007). Furthermore, the model suggests that specific genes may have been selected over time because of the differential advantage they confer in favorable environments. Of note, this model emphasizes that a more appropriate way of conceptualizing risk or protective factors is in terms of coactions between genes and environment over time rather than conceptualizing either genes or environments as risk or protective factors in and of themselves.

We propose that genetic contributions to emotionality can be modified by early experience in parent-child relationships and that this process may occur primarily through G × E mechanisms, with some genotypes being more sensitive or susceptible than others to parenting. As before, we do not rule out the impact of rGE on emotionality, but suggest that, to the extent that emotionality is a function of genotype, rGE may influence emotionality in the direction of stability. Next we review evidence for the association between specific genotypes and elements of infants' emotionality, followed by evidence for interactions between genes and parenting on developing emotionality. Fundamental to this discussion is the supposition that emotion reactivity and emotion regulation are critical processes involved in human adaptation that influence individuals' interactions with the social environment.

Genetics of Infants' Emotionality

Using a molecular genetics approach, which attempts to make associations between complex behavioral traits and specific genes that regulate neurotransmitter systems, the first step in understanding the genetic underpinnings of infant emotionality has been through an examination of the genes commonly associated with adult personality characteristics. Several recent studies have investigated these associations in samples of infants and young children, however, many questions remain regarding the pathways between these genes and the physiological and behavioral characteristics to which they are linked. The following discussion outlines research in this area, with a specific focus on two of the most commonly studied genes in relation to infant and adult behavior: dopamine and serotonin.

Dopamine

Dopaminergic system genes have been the most consistently studied in relation to behavior, personality, and temperament. Dopamine has been suggested as an

underlying neurotransmitter influencing the behavioral system of approach (Cloninger, 1987; Gray, 1982; Zukerman, 1994) and the activation and intensity of physiological responses in reward situations (Panksepp, 1986). In adults (Benjamin et al., 1996; Ebstein, et al., 1996) and animals (Bailey, Breidenthal, Jorgensen, McCracken, & Fairbanks, 2007; Grandy & Kruzich, 2004; Livak, Rogers, & Lichter, 1995) specific polymorphisms of dopamine genes have been related to higher levels of novelty seeking and exploratory behavior.

One of the first groups to look at a dopamine gene in relation to emotionality in an infant sample examined the D4 dopamine receptor gene (Auerbach, Faroy, Ebstein, Kahana, & Levine, 2001; Auerbach et al., 1999; Ebstein et al., 1998). This gene contains a repeated sequence polymorphism (i.e., variations in DNA) within its coding sequences that changes the length of the receptor protein, which has been shown to have functional significance (Asgahari et al., 1994). There is a long polymorphism (L-DRD4; 6-8 repeats *or* presence of 7-repeat allele) and a short polymorphism (S-DRD4; 2-5 repeats *or* absence of 7-repeat allele); the shorter the allele, the more efficient the receptor is in binding dopamine (Plomin & Rutter, 1998). Infants with the long polymorphism of DRD4 (L-DRD4) were rated as lower on negative emotionality and distress to limitations at 2 months of age (Ebstein et al., 1998) and exhibited lower levels of anger-related negative emotionality at 12 months of age (Auerbach et al., 2001) than those possessing the short polymorphism of DRD4 (S-DRD4). Furthermore, at age 12-months, infants with L-DRD4 also showed higher levels of activity during a free play situation than those with S-DRD4. In sum, this line of research found that infants possessing the long version of the DRD4 gene were less negative, not as easily distressed, and more active than those with the short version of this gene, suggesting (as does the adult literature) that this gene may be related to exploratory or stimulus-seeking behavior even before the age of one. Similarly, other studies in children have found associations between L-DRD4 and approach, or novelty-seeking, behaviors such as externalizing problems (LaHoste et al., 1996; Sunohara et al., 2000), oppositional defiant behaviors (Kirley et al., 2004), and aggression (Schmidt, Fox, Rubin, Hu, & Hamer, 2002).

Serotonin

Another neurotransmitter that has been found to be linked to human behavior and emotion is serotonin. The human serotonin transporter (5-HTT) gene has a polymorphism in the promoter region called the serotonin transporter gene-linked polymorphic region (5-HTTLPR), which has been associated with the regulation of mood and emotional states in adults (see review in Westernberg, Murphy, & Den Boer, 1996) through inhibition of behavioral and emotional responses (Sourbrie, 1986). Human adult studies have found that low levels of serotonin are associated with depression, alcohol abuse, impulsivity, risk taking, aggression,

and difficulties in regulatory and emotional functioning (Barr et al., 2004; Suomi, 2000). The serotonin transporter gene has also been associated with withdrawn behaviors and anxiety-related symptoms (Lesch et al., 1996). Studies illustrate that there is less gene transcription, and therefore less protein production, in individuals with the short allele (s) than the long allele (l) of the serotonin transporter gene (5-HTTLPR) (Greenberg et al., 1999; Heinz et al., 2000; Lesch et al., 1996). Therefore, low 5-HTTLPR expression may lead to lower serotonergic function.

Initial studies looked at bivariate associations between serotonin and infant and child behavioral functioning. Findings of one study revealed that the short allele of serotonin was associated with shyness in third and fourth grade children (Battaglia et al., 2005). Similarly, preschool-aged children carrying the short alleles of the 5-HTTLPR gene were also rated (by mothers) as significantly shyer than those carrying at least one of the long alleles (Hayden et al., 2007). These findings, consistent with the adult literature, confirm the role of this genotype in mechanisms of emotion reactivity and regulation related to internalizing types of behaviors.

Although studies of dopamine and serotonin in infants and children have revealed some interesting links that parallel work in the adult and animal literature, there have been many mixed results and failed replications. For example, one study found the opposite association between L-DRD4 and aggressive behavior in 6-year-old children than the ones reported above (i.e., elevated externalizing problem scores in the absence of L-DRD4; Birkas et al., 2005). Similarly, several studies have found no relationship between the short allele of serotonin and shyness or other internalizing behaviors in children (Arbelle et al., 2003; Schmidt et al., 2002; Young, Smolen, Stallings, Corley, & Hewitt, 2003). These studies share a common limitation, namely the adoption of a main effects modeling approach in which polymorphisms were used as predictors of behavioral outcomes without consideration of relevant experience or other genetic effects.

Gene x Parenting Interactions

These initial studies are important in that they identify candidate genes for various emotional characteristics in infants and young children, however, these studies looked at genes in relation to outcomes without taking environmental factors into account. Although identifying associations between a specific gene and outcome is an important starting point, it is becoming increasingly clear that it is impossible to understand genetic effects without taking into account environmental factors and life experiences. This limitation may be one reason for mixed results and failed replications in the literature.

For example, one line of research found that as early as 1-month of age, infants possessing the L-DRD4 genotype exhibited significantly less difficulty in modifying their reactions to a change in stimuli (i.e., adaptation, perhaps a rudimentary form of emotion regulation), such as objects, food, or clothes, than those with the

S-DRD4 form (DeLuca et al., 2001). However, a follow-up study at 5-months-old, and again at 3-years-old, evaluated individual differences in adaptability behavior and results at both time points failed to find an association between the DRD4 polymorphism with the measured trait (DeLuca et al., 2003). Although there was no main effect of DRD4 at the two later time points, the possibility of an interaction with parenting cannot be ruled out. Over time, infants' increased interactions with the external environment and their caregivers may provide the necessary experience to effect change in genetic expression.

The first studies that examined gene by environment interactions were conducted with non-human animals and human adults. Several studies found that rhesus monkeys who possessed the short allele of serotonin and were raised by peers, rather than their mothers, exhibited more behavioral and physiological problems (i.e., aggression, alcohol consumption, stress reactivity) than those who did not possess the short allele (Barr et al., 2004; Bennett et al., 2002). Similarly, Caspi et al. (2003) found that adults carrying the short allele of serotonin were more likely to be depressed when they experienced stressful life events than adults without the short allele or those with the short allele who did not experience stressful life events.

Recent research of infant and child emotionality has examined interactions between genes and parenting and found consistent results across studies. The interaction of the L-DRD4 polymorphism and insensitive maternal caregiving during infancy predicted increased externalizing behaviors, including oppositional and aggressive behaviors, in preschool-age children (Bakermans-Kranenburg & van IJzendoorn, 2006). Consistent with this research, the interaction of S-DRD4 and higher maternal warm-responsive parenting in another recent study predicted decreased externalizing behavior in African American children by 30 months of age (Propper et al., 2007). A third study that examined these relations in younger children found that children 18–21 months of age possessing the L-DRD4 polymorphism who received lower quality parenting were reported, by caregivers, as exhibiting more sensation seeking behavior than children with the L-DRD4 allele who received higher quality parenting (and those children with the S-DRD4 and high or low quality parenting) (Sheese, Voelker, Rothbart, & Posner, 2007). Interestingly, those children with the L-DRD4 polymorphism who received higher quality parenting were reported to have the least sensation seeking behavior of all the groups. Furthermore, children who did not possess the L-DRD4 polymorphism were unaffected by parenting, potentially providing support for the differential susceptibility hypothesis discussed above.

Similar studies of the interaction between the serotonin gene and the early caregiving environment have been done in samples of children, although, to date, none have examined serotonin-parenting interactions in infancy. One study found that observed behavioral inhibition and mother- reported shyness in children were associated with the short allele of 5-HTTLPR only when families reported low levels of social support (Fox et al., 2005), which is associated with greater difficulties

in parenting. Stein, Schork, and Gelernter (2007) found that children who experienced emotional or physical maltreatment, and were homozygous for the short allele of 5-HTTLPR, displayed higher levels of anxiety-sensitivity than those without the short allele. Consistent with that research, Kaufman et al. (2004) found that maltreated children with two short alleles (s/s) of 5-HTTLPR and no positive support, from parent, relative, other adult, or friend, scored almost twice as high on depression ratings as those possessing the long allele, or those with the s/s genotype and positive support. Finally, in a non-risk sample, an interaction between serotonin and attachment security predicted electrodermal reactivity during a psychosocial stress task in 7-year-old children (Gilissen, Bakermans-Kranenburg, van IJzendoorn, & Linting, 2008). Although no direct association was found between 5-HTTLPR and electrodermal reactivity in that study, children with secure attachment relationships who also possessed two long alleles of 5-HTTLPR (l/l) experienced less stress than did children in every other genotype/attachment group (i.e., short allele/secure, short allele/insecure, long allele/insecure).

In summary, these findings provide consistent evidence for the importance of parenting as a moderator of genetic effects on behavioral and physiological indices of infant emotionality. An important next direction in the study of gene-parenting interactions is to understand the mediating mechanisms by which parenting may moderate genetic effects. We propose that, in infancy, parenting may moderate genetic effects through its impact on infants' developing physiological systems of emotion reactivity and emotion regulation. In the next section, we discuss research supporting this theory. First we discuss research that describes the physiological basis of infant emotionality. Then we provide evidence for parenting effects on infant emotionality and for genetic effects on infant emotionality. Finally, we discuss research on gene-parenting interactions in the study of infants' physiological mechanisms of emotionality.

Infant Emotionality and Physiological Responses

In infancy, much of the research on physiological processes associated with infants' temperament and emotionality has focused on vagal tone functioning. The term vagal tone refers to control of the heart via the vagus nerve and is typically measured as the amplitude of respiratory sinus arrythima (RSA), which represents parasympathetic influence on heart rate variability (Porges, 1996; Porges & Byrne, 1992). The vagal system has been suggested as one possible biological substrate for regulation of arousal state and reactivity underlying individual differences in temperament (Fox & Stifter, 1989; Stifter, 1995; Stifter & Fox, 1990; Stifter, Fox, & Porges, 1989). In the extant literature, RSA is commonly equated with vagal tone, however, it is important to remember that RSA is only one

component of vagal tone and that there are many other influences on heart rate variability (Grossman & Taylor, 2007). Of these various components, however, RSA has been the most consistently examined in relation to dimensions of temperament and emotionality in infants.

Baseline RSA

Baseline or resting RSA is considered to be a stable neurophysiological mechanism underlying autonomic and behavioral reactivity that provides a measure of the potential capacity of the system to react to emotion eliciting stimuli, with higher baseline RSA indicating flexibility and greater capacity to respond. Baseline RSA is also thought to reflect an infant's regulatory capacity, independent of caregivers' assistance, to return to behavioral and physiological homeostasis after a stressor (Porges, 1996). Research has explored the relationship between baseline RSA and infant emotionality during positive, novel, and mildly stressful situations and found positive correlations between the two measures.

Developmentally, neonates who cried in response to pacifier withdrawal had higher resting RSA than those who did not cry and, at 5 months, were more likely to cry in response to arm restraint (Stifter & Fox, 1990). Five-month-old infants who cried in response to arm restraint had higher resting RSA than those who did not cry, and infants who cried as neonates and at 5 months in response to stressors had higher resting RSA than infants who cried at neither age, and they were rated by their mothers as being more easily distressed by limitations (Stifter & Fox, 1990). Similarly, at 14, 20, and 26 weeks of age, infants with higher baseline RSA tended to be more behaviorally reactive (DiPietro, Larson, & Porges, 1987; Porges, Doussard-Roosevelt, Portales, & Suess, 1994) and exhibited more frequent approach behaviors. This work suggests that, in early infancy, higher resting RSA is associated with behavioral indices of negative emotionality, greater emotion reactivity, and temperamental difficulty.

However, longitudinal investigations have identified shifts in associations between RSA and functional behaviors. Although Stifter and Fox (1990) observed that at 5 months of age, negative behavioral reactivity to arm-restraint occurred more quickly and intensely in infants with higher RSA than those with lower RSA, at 14 months of age, during an interaction with an unfamiliar adult, infants with higher RSA showed more positive approach behaviors towards the stranger and towards a novel toy than infants with lower RSA. One explanation for the unexpected findings that higher RSA is associated with negative emotionality and reactivity at 5 months and with positive and sociable behavior at 14 months is that parenting modified the expression of infants' temperamental emotionality over time. Young infants with higher RSA may react more strongly to emotion-eliciting stimuli in the early months of life and influence the way in which their caregivers respond to them, an example of evocative rGE. Highly reactive infants that respond

appropriately to aversive stimuli (with distress and crying) may be better able to signal their caregivers and receive the parenting support that they need, which may lead to more socially appropriate and effective behavioral expressions of emotionality later in development.

An alternate interpretation is that the different contexts at the two different ages account for the findings, with infants with high RSA showing the more appropriate and effective responses for the specific context at each age, regardless of intervening parenting. Measures of parenting in longitudinal research examining the associations between infants' physiological functioning and observed emotionality are needed to clarify the meaning of these findings.

There is consistent evidence of a normative developmental increase in mean levels of baseline RSA as physiological systems become more organized over time. Research has shown that mean baseline RSA increases from infancy through middle childhood (Bornstein & Suess, 2000; Izard et al., 1991; Porges et al., 1994; Richards & Cameron, 1989; Stifter et al., 1989). However, there is inconsistent evidence for stability of baseline RSA over time. Several studies have found no stability over the first year of life (Porter, Bryan, & Hsu, 1995; Stifter et al., 1989), yet other studies have reported stability from 3 months to 3 years of age (Izard et al., 1991; Porges et al., 1994; Porter et al., 1995), and moderate stability from 4 months to 4 years of age (Bar-Haim, Marshall, & Fox, 2000). Once more, these mixed findings may be due to the lack of attention given to the influence that experience in parent-infant relationships exerts on the developing physiological systems from birth onward that may help to maintain or modify existing RSA response patterns.

RSA Reactivity

Vagal tone functioning represents a dynamic physiological process. Because of this, measures of RSA are sensitive to environmental demands or challenge. Porges' (2007) polyvagal theory of social engagement asserts that the autonomic nervous system enhances restoration and growth by regulating the "vagal brake," which slows down the heart (i.e., activated vagal tone) during situations that do not present a challenge or in some contexts that elicit calm, focused attention. A decrease in RSA, or RSA withdrawal, however, typically occurs when an individual is involved in an activity that requires active coping (Porges, 1991, 1996), at which time the vagal "brake" is withdrawn (i.e., vagal tone is inhibited) to support an increase in heart rate. When environmental demands have ceased, the brake is reengaged (i.e., vagal tone is activated) to promote decreases in metabolic output and a return to a calm state. Thus, effective RSA reactivity has been related to the ability to maintain homeostasis in the face of changing demands by allowing a shift from attention on internal demands to external ones that include the use of coping strategies to regulate affective or behavioral arousal.

RSA reactivity in terms of RSA withdrawal during a challenging situation has been related to positive outcomes in infants and children, including higher soothability (Huffman et al., 1998), more attentional control (Huffman et al., 1998; Suess, Porges, & Plude, 1994), and better emotion regulation (Calkins, 1997). One study of 9-month-olds found that infants with lower levels of RSA withdrawal during a social/attentional task exhibited more behavioral problems at 3 years of age than peers who showed higher levels of RSA withdrawal (Porges, Doussard-Roosevelt, Portales, & Greenspan, 1996). Thus, Posner and Rothbart (2000) asserted that dynamic RSA reactivity may be an index of social and attentional control that underlies the behavioral strategies necessary for regulation of emotionality. Although supporting data (Bazhenova, Plonskaia, & Porges, 2001; Moore & Calkins, 2004) are not conclusive, empirical research has led to the assumption that greater RSA withdrawal during a challenge condition reflects more effective dynamic regulation, whereas, as previously discussed, baseline RSA reflects the potential capacity of the individual to respond effectively.

Parenting, Infants' RSA Regulation, and Emotionality

In early infancy, caregivers facilitate physiological homeostasis as they assist infants in attaining a balance between endogenous needs and exogenous stimuli (Hofer, 1987). Due to the rapid amount of neurological growth during this stage, parenting may have long-term effects on development of physiological systems related to emotionality (Black & Greenough, 1986; Cichetti & Lynch, 1995). The way in which caregivers respond to the needs of their infants may have an impact on infants' ensuing abilities to modulate physiological reactivity and develop more adaptive methods of regulation over time (Derryberry & Rothbart, 1984).

RSA functioning, therefore, is a potential mediator of the relation between parenting and changes in the expression of infant emotionality across development. A number of studies have explored developing patterns of RSA functioning in relation to qualities of parent-infant interaction. Infants of dyads that spent more time in joint communicative states with responsive adjustment to each other's behaviors, had higher baseline RSA than those in dyads that did not display this pattern of mutual dyadic regulation (Porter, 2003). Moore and Calkins (2004) found that as early as 3 months of age, infants of dyads exhibiting lower behavioral synchrony showed higher RSA withdrawal during a normal play episode and less RSA withdrawal during a situation meant to elicit distress, both of which were atypical responses. Following an experimentally disrupted social interaction at 6 months of age (the still-face procedure), only infants of sensitive mothers and their mothers showed a significant decrease in RSA from baseline, suggesting that their mutual RSA withdrawal may have been a function of mutual behavioral

responsiveness and that both were equally active and engaged in repairing their social interaction (Moore et al., 2009).

Attachment theorists (e.g., Cassidy, 1994) propose that the attachment relationship consolidates, over time, the behavioral and physiological responses used by infants to successfully interact and cope with stress within the parent-child relationship. Consistent with this theory, infants classified as insecure-avoidant display greater cardiac arousal and RSA withdrawal during separation and reunion episodes of the Strange Situation procedure than do infants classified as securely attached (Hill-Soderlund et al., 2008). According to attachment theory, a mismatch between a high level of RSA withdrawal and a low level of observed behavioral distress may occur if infants have developed the need to rely on self-regulation rather than caregiver support for regulation of distress.

Support for the theory that parenting influences the consolidation of infants' physiological regulation extends into toddlerhood. In a sample of two-year-olds, maternal negative control was associated with lower infant RSA withdrawal in an emotion-eliciting task, reflecting less effective physiological regulation (Calkins, Smith, Gill, & Johnson, 1998). The authors proposed that children with more controlling mothers may be less able to regulate their own physiological arousal because they are accustomed to receiving high amounts of external regulation or are not exposed to a range of effective regulatory strategies for use in different situations. Alternatively, mothers may exert more negative control with children who have difficulty regulating their emotions, another example of evocative rGE. Cross-sectional studies of biological families are unable to disentangle these various interpretations.

Longitudinal research, however, does provide evidence for the influence of early parent-infant relationships on later physiological functioning. Burgess, Marshall, Rubin, and Fox (2003) found that, although attachment classification at 14 months of age was not concurrently associated with heart period or baseline RSA, it did predict these autonomic measures at 4 years of age. More specifically, children classified as having insecure-avoidant attachment relationships, assessed at age 14 months, had significantly higher heart period (i.e., lower heart rate) and higher baseline RSA at 4 years than did children classified as having secure or insecure-ambivalent relationships. One possible explanation for this change may be that hyperactivation of the vagal system in early infancy, due to the lack of caregiver support for infant regulation, may lead to a "burn out" phenomenon (see Hill-Soderlund et al., 2008). As described above, infants classified as insecure-avoidant may regulate their own distress, rather than openly communicate to their caregivers, which may be more physiologically challenging (e.g., Hill-Soderlund et al., 2008; Spangler & Grossman, 1993). Although in the short term this may be an adaptive response, this increased physiological load may lead to poor functioning in the long term. As the infant matures, the vagal system may become autonomically under-reactive, which may in turn lead to under-controlled behaviors.

Physiological regulatory fatigue has been demonstrated in adults over short periods of time in conditions that require self-control (Muraven & Baumeister, 2000; Muraven & Slessareva, 2003), suggesting that chronic activation of self-regulatory mechanisms could lead to a diminished reactivity and also detract from resources that could be utilized to promote optimal social and cognitive development. Prospective longitudinal work is valuable in that it supports the possibility that autonomic functioning at age 4 may be the result of, rather than the cause of, the quality of parent-child relationships.

Depression

Mothers with high levels of depressive symptoms are less likely to provide contingent responsive behavior towards their infants during interactions (Field, Healy, Goldstein, & Guthertz, 1990; Forbes, Cohn, Allen, & Lewinsohn, 2004), in part due to less matching of affective states and more matching of negative states (Cohn, Campbell, Matias, & Hopkins, 1990; Field et al., 1990). Mothers with higher levels of depressive symptoms have been found to display lower levels of synchrony during face-to-face interaction with their infants than other mothers (Moore & Calkins, 2004). Compared to other infants, infants of mothers with higher levels of depressive symptoms may receive less positive feedback, less exposure to a range of emotions, or may need to do more work to engage their mothers, which in turn may create long term changes in the vagal system. This type of interaction may inhibit the development of behavioral and physiological responses necessary for effective emotion regulation. Consistent with this theory, infants of depressed mothers did not show the normative increase in baseline RSA between 3 and 6 months of age (Field, Pickens, Fox, & Nawrocki, 1995).

Maternal depression is associated with several influences, including genetic and prenatal factors, that may affect offspring. Future research should examine infant emotion development in a way that differentiates impaired neurological functioning due to such genetic or prenatal circumstances from long term changes that may occur due to parenting.

Parent Conflict

In older children, vagal regulation is related to behavioral adjustment to conflict between parents. Higher measures of RSA withdrawal appeared to buffer children from the negative effects of inter-parent conflict (Katz & Gottman, 1997) and a relation between high levels of inter-parent conflict and child externalizing behaviors was found for children with low baseline RSA, but not for children with high baseline RSA (Katz & Gottman, 1995). These results were replicated and

extended to show that higher baseline RSA moderated relations between exposure to inter-adult conflict and child internalizing behavior problems and health problems (El-Sheikh, Harger, & Whitson, 2001).

Only two studies of which we are aware have examined infants' RSA in relation to parent conflict. The first found that 6-month-old infants in families reporting higher marital conflict showed lower baseline RSA, suggesting a decreased capacity for effective regulation (Porter, Wouden-Miller, Silva, & Porter, 2003). In that study, infants with lower baseline RSA also showed lower levels of behavioral regulation as assessed by the Behavior Rating Scales (Bayley, 1993). In a second study, 6-month-old infants in higher conflict families showed diminished RSA withdrawal in a challenge condition relative to other infants and unexpected RSA withdrawal when interacting with mothers (Moore, 2010), although no differences in baseline RSA. This pattern of findings suggests atypical RSA regulation and a reliance on self-regulation for infants in families with moderate levels of parent conflict. In contrast, Gottman and Katz (1989) found that preschool-aged children in families reporting greater parent conflict showed higher RSA than other children. Gottman and Katz (1989) proposed that this unexpected finding represented a temporary adaptation that, over the course of development, could overburden children's capacities for self-regulation.

Exposure to parent conflict may increase demands on infants to regulate arousal and/or higher conflict between parents in a family may diminish parents' abilities to respond sensitively to their infant's need for support in regulating behavior and emotions, requiring the child to become more dependent on self-regulation (e.g., Davies & Cummings, 2006; Donovan, Leavitt, & Walsh, 1998). More frequent demands to regulate arousal and the necessity of relying on self-regulation could either overwhelm infants' rudimentary abilities to regulate RSA, consistent with the regulatory fatigue theory discussed earlier (Muraven & Baumeister, 2000; Muraven & Slessareva, 2003). Thus, as Gottman and Katz (1989) suggested, a pattern of RSA regulation that may be an effective adaptation in early childhood, with time could become a maladaptive regulatory response.

On the other hand, the physiological toughness model (Dienstbier, 1989; Dienstbier & Zillig, 2002) proposes that experiencing intermittent and mild to moderate stress has a beneficial effect on some biological systems. Dienstbier (1989), for example, found that the neuroendocrine system increases availability of epinephrine under intermittent and moderate stress conditions, which protects against depletion of epinephrine when the individual encounters more intense or more chronic stressors in the future. Therefore, increased frequency of the need to employ RSA regulation and a need to rely more on self-regulation than external support from parents, which may occur in environments with higher parent conflict, could facilitate the effectiveness of a child's RSA functioning, particularly if he or she is living in an environment where conflict is mild but common. This could explain why some children develop effective RSA regulation in families with high conflict, which then buffers them from negative behavioral outcomes

typically associated with parent conflict later in development (El-Sheikh et al., 2001; Katz & Gottman, 1995, 1997).

It is also possible that infants' difficulty regulating their own emotion may lead to parental irritability, creating more stress within the marital relationship (evocative rGE), or that parents who have difficulty regulating their own emotions and thus engage in more conflict have infants who have inherited a physiological vulnerability to problems with emotion regulation (passive rGE). Consistent with this, a study using the Children of Twins design found that genetic influences mediated the association between marital conflict and conduct problems (Harden et al., 2007).

In summary, the importance of the parent-infant relationship as an agent of change in infant emotionality over time has been supported by numerous studies utilizing a variety of research designs, including longitudinal, at-risk samples, and twin studies. However, it is less clear from that body of research whether parenting modifies physiological mechanisms or behavioral expression of emotionality related to these physiological mechanisms. Research is needed to clarify those relations. In addition to understanding whether parenting serves as an environmental influence on the development of infants' emotion reactivity and regulation, it is important to also consider the genetic contributions to these physiological mechanisms, and how genetic processes and parenting interact.

G × E (Parenting) Predictors of RSA

A recent study provides support for the importance of this approach by taking into account the effect of both genotype *and* infant-caregiver relationships on physiological change over time. Findings revealed that the direct effect of the dopamine receptor gene D2 (DRD2) on infant physiological responses to stress changed over the first year of life in relation to infants' experiences with caregivers (Propper et al., 2008). At 3 and 6 months of age, infants with the *taq*1 A1 polymorphism of the DRD2 gene, which has been found in adolescents and adults to be associated with impulse control problems and sensation seeking behaviors, did not exhibit expected physiological regulation during an age-appropriate stressful situation (i.e., separation from mother) as measured by vagal reactivity (i.e., decrease in respiratory sinus arrhythmia, or RSA). However, results revealed that maternal caregiving behavior moderated the change in RSA response to stress by 12 months of age. Those infants possessing the *taq*1 A1 polymorphism of DRD2, who were also exposed to sensitive maternal caregiving over the first year of life, exhibited a more optimal and expected RSA response to stress at 12 months of age, comparable to the RSA reactivity of those infants possessing the non-risk version of the gene. At this time, this study is the only one of which we are aware that has looked at the interplay of genes and parenting as predictors of RSA reactivity in infants.

However, these findings provide a promising new direction for examining individual differences in trajectories of physiological functioning. Furthermore, studies such as this one contribute to our understanding of the origins of infant temperament through the identification of specific genes and parenting behaviors that influence infant physiological processes associated with emotion reactivity and regulation.

Conclusion

In summary, recent studies have made great strides towards understanding the origins of infant temperament and emotionality. Although there have been numerous studies of the influence of parenting and genetics on infant behavior and physiology, many questions still remain. The developmental systems approach that we have described here seeks to examine infant behavioral and physiological phenotypes by taking into account *all* of these predictors through the exploration of the interplay between specific candidate genes and the environment (i.e., infant-parent relationship) over time. This multi-level methodology has yielded some exciting results and has provided a new and encouraging direction for research on infant temperament. Extant research has supported the theory that parenting behavior may moderate genetic effects on temperament, whether directly via behavior or through the impact on the endophenotypic development of physiological systems, as we have proposed, leading to individual differences in emotion reactivity and regulation.

The univariate approach (studying only genes or environment or using single measures as outcomes) that has often been used in the past to predict complex behavioral and physiological outcomes is oversimplified. In order to truly understand the contributing mechanisms and developmental pathways of infant behavior and physiology over time, we must begin to examine in one model relations between infant behaviors (reactivity and regulation), multiple candidate genes, multiple measures of autonomic and neuroendocrine functioning (e.g., vagal tone, cortisol, alpha-amalyase), and neurological variables (e.g., assessed via ERP, EEG), along with sophisticated measurement of the environment. Future research using this developmental systems approach could become increasingly productive by incorporating even more precise measurement at various levels (Moore et al., 2009). Global ratings or self-reports of parenting are likely to provide less meaningful measures of the environment when analyzed in relation to specific, dynamic physiological processes of infant functioning, as global ratings and self-reports of parenting tend to reflect trait-like characteristics of parents rather than reflecting a parent's unique responses to an individual child's behavior in a specific context. On a different temporal scale, the same properties that Gottlieb describes as emerging across development from coactions among environmental,

behavioral, neural, and genetic levels (Gottlieb, 1991, 1998; Gottlieb & Halpern, 2002) may also occur in the everyday, moment-to-moment interactions between parents and children.

Furthermore, it has become increasingly clear that the study of gene-environment correlations and interactions is an integral part of understanding developmental outcomes. The conceptual differentiation of the statistical term, *interaction*, from the epigenetic term, *coaction*, is particularly important in this regard. To this effect, appreciating that the notion of *risk* and *resiliency* must be understood as properties of gene-environment coaction, rather than of individual genotypes and environments, is paramount for understanding and accurately representing the complexities of infant temperament and emotion development. As described previously in this chapter, it is important to remember that child genotype and parental behavior are both part of one family (Cox & Paley, 2003) and one developmental epigenetic (Gottlieb, 1998) system. Only when our theoretical and methodological approaches achieve sufficient sophistication to incorporate multiple relations across time will we better model the dynamic complexity of development.

References

Arbelle, S., Benjamin, J., Golin, M., Kremer, I., Belmaker, R. H., & Ebstein, R. P. (2003). Relation of shyness in grade school children to the genotype for the long form of the serotonin transporter promoter region polymorphism. *American Journal of Psychiatry*, **160**, 671–676.

Asgahari, V., Schoots, O., van Kats, S., Ohara, K., Jovanovic, V., Guan, H. C., et al. (1994). Dopamine D4 receptor repeat: Analysis of different native and mutant forms. *Molecular Pharmacology*, **46**, 364–373.

Auerbach, J. G., Faroy, M., Ebstein, R., Kahana, M., & Levine, J. (2001). The association of the dopamine D4 receptor gene (DRD4) and the serotonin transporter promotor gene (5-HTTLPR) with temperament in 12-month-old infants. *The Journal of Child Psychology and Psychiatry*, **6**, 777–783.

Auerbach, J., Geller, V., Lezer, S., Shinwell, E., Belmaker, R. H. Levine, J., et al. (1999). Dopamine D4 receptor (D4DR) and serotonin transporter promoter (5-HTTLPR) polymorphisms in the determination of temperament in 2-month-old infants. *Molecular Psychiatry*, **4**, 369–373.

Bailey, J. N., Breidenthal, S. E., Jorgensen, M. J., McCracken, J. T., & Fairbanks, L. A. (2007). The association of DRD4 and novelty seeking is found in a nonhuman primate model. *Psychiatric Genetics*, **17**, 23–27.

Bakermans-Kranenburg, M. H., & van IJzendoorn, M. J. (2006). Gene-environment interaction of the dopamine D4 receptor (DRD4) and observed maternal insensitivity predicting externalizing behavior in preschoolers. *Developmental Psychobiology*, **48**, 406–409.

Bar-Haim, Y., Marshall, P. J., & Fox, N. (2000). Developmental changes in heart period and high-frequency heart period variability from 4 months to 4 years of age. *Developmental Psychobiology*, **37**, 44–56.

Barr, C. S., Newman, T. K., Lindell, S., Shannon, C., Champoux, M. Lesch, K. P., et al. (2004). Interaction between serotonin transporter gene variation and rearing condition in alcohol preference and consumption in female primates. *Archives of General Psychiatry*, **61**, 1146–1152.

Battaglia, M., Ogliari, A., Zanoni, A., Citterio, A., Pozzoli, U., Giorda, R., et al. (2005). Influence of the serotonin transporter promoter gene and shyness on children's cerebral responses to facial expressions. *Archives of General Psychiatry*, **62**, 85–94.

Bayley, N. (1993). *Bayley scales of infant development* (2nd ed.). San Antonio, TX: Psychological Corporation.

Bazhenova, O. V., Plonskaia, O., & Porges, S. W. (2001). Vagal reactivity and affective adjustment in infants during interaction challenges. *Child Development*, **72**, 1314–1326.

Belsky, J. (2005). Differential susceptibility to rearing influence: An evolutionary hypotheses and some evidence. In B. Ellis, & D. Bjorklund (Eds.), *Origins of the social mind: Evolutionary psychology and child development* (pp. 139–163). New York: Guilford.

Belsky, J., Bakermans-Kranenburg, M. J., & van IJzendoorn, M. H. (2007). For better and for worse: Differential susceptibility to environmental influences. *Association for Psychological Science*, **16**(6), 300–304.

Benjamin, J., Li, L., Patterson, C., Greenberg, B. D., Murphy, D. L., & Hamer, D. H. (1996). Population and familial association between the D4 dopamine receptor gene and measures of novelty seeking. *Nature Genetics*, **12**, 81–84.

Bennett, A. J., Lesch, K. P., Heils, A., Long, J., Lorenz, J., Shoaf, S. E., et al. (2002). Early experience and serotonin transporter gene variation interact to influence primate CNS function. *Molecular Psychiatry*, **7**, 118–122.

Birkas, E., Lakatos, K., Nemoda, Z., Ney, K., Toth, I., Novak, A., et al. (2005). Effects of the D4 dopamine receptor gene variation on behavior problems at 6 years of age. *Neuropsychopharmacologia Hungarica*, **7**, 125–131.

Black, J. E., & Greenough, W. T. (1986). Induction of pattern in neural structure by experience: Implications for cognitive development. In M. E. Lamb, A. L. Brown, & B. Rogoff (Eds.), *Advances in developmental psychology* (pp. 1–50). Hillsdale, NJ: Erlbaum.

Bornstein, M. H., & Suess, P. E. (2000). Physiological self-regulation and information processing in infancy: Cardiac vagal tone and habituation. *Child Development*, **2**, 273–287.

Boyce, W. T., & Ellis, B. J. (2005). Biological sensitivity to context: I. An evolutionary–developmental theory of the origins and functions of stress reactivity. *Development and Psychopathology*, **17**, 271–301.

Burgess, K. B., Marshall, P. J., Rubin, K. H., & Fox, N. A. (2003). Infant attachment and temperament as predictors of subsequent externalizing problems and cardiac physiology. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, **44**, 819–831.

Buss, A. H., & Plomin, R. (1984). *Temperament: Early developing personality traits*. Hillsdale, NJ: Erlbaum.

Cairns, R. B. (1997). The making of developmental psychology. In W. Damon (Ed.-in-chief) and R. M. Lerner (Vol. Ed.), *Handbook of child psychology* (Vol. **1**, 5th ed., pp. 25–106). New York: Wiley.

Calkins, S. D. (1997). Cardiac vagal tone indices of temperamental reactivity and behavioral regulation in young children. *Developmental Psychobiology*, **31**, 125–135.

Calkins, S. D., Smith, C. L., Gill, K., & Johnson, M. C. (1998). Maternal interactive style across contexts: Relations to emotional, behavioral and physiological regulation during toddlerhood. *Social Development*, **7**, 350–369.

Cassidy, J. (1994). Emotion regulation: Influences of attachment relationships. In N. A. Fox (Ed.), *The development of emotion regulation: Biological and behavioral considerations. Monographs of the Society for Research in Child Development, 59* (2–3, Serial No. 240), 228–283.

Caspi, A., McClay, J., Moffitt, T. E., Mill, J., Martin, J., Craig, I. W., et al. (2002). Role of genotype in the cycle of violence in maltreated children. *Science*, **297**, 851–854.

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., et al. (2003). Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science*, **301**, 386–389.

Cichetti, D., & Lynch, M. (1995). Failures in the expectable environment and their impact on individual development: The case of child maltreatment. In D. Cicchetti and D. J. Cohen (Eds.), *Developmental psychopathology, Volume 2: Risk, disorder, and adaptation* (pp. 32–71). New York: Wiley.

Cloninger, C. (1987). A systematic method for clinical description and classification of personality variants. *Archives of General Psychiatry*, **44**, 573–588.

Cohn, J., Campbell, S., Matias, R., & Hopkins, J. (1990). Face-to-face interactions of postpartum depressed and nondepressed mother-infant pairs at 2 months. *Developmental Psychology*, **26**, 15–23.

Cox, M. J., & Paley, B. (2003). Understanding families as systems. *Current Directions in Psychological Science*, **12**, 193–196.

Davies, P. T., & Cummings, E. M. (2006). Interparental discord, family process, and developmental psychopathology. In D. Cicchetti & D. Cohen (Eds.), *Developmental psychopathology: Vol. 3. Risk, disorder, and adaptation* (2nd ed., pp. 86–128). New York: Wiley.

DeLuca, A., Rizzardi, M., Buccino, A., Alessandroni, R., Salvioli, G. P., Filograsso, N., et al. (2003). Association of dopamine D4 receptor (DRD4) exon III repeat polymorphism with temperament in 3-year-old infants. *Neurogenetics*, **4**, 207–212.

DeLuca, A., Rizzardi, M., Torrente, I., Alessandroni, R., Salvioli, G. P., Filograsso, N., et al. (2001). Dopamine D4 receptor (DRD4) polymorphism and adaptability trait during infancy: A longitudinal study in 1- to 5-month-old neonates. *Neurogenetics*, **3**, 79–82.

Derryberry, D., & Rothbart, M. K. (1984). Emotion, attention, and temperament. In C. E. Izard, J. Kagan, & R. B. Zajonc (Eds.), *Emotions, cognition, and behavior* (pp. 132–166). Cambridge: Cambridge University Press.

Dienstbier, R. (1989). Arousal and physiological toughness: Implications for mental and physical health. *Psychological Review*, **96**, 84–100.

Dienstbier, R. A., & Zillig, L. M. P. (2002). Toughness. In C. R. Snyder & S. J. Lopez (Eds.), *Handbook of positive psychology* (pp. 515–527). New York: Oxford University Press.

DiPietro, J. A., Larson, S. K., & Porges, S. W. (1987). Behavioral and heart-rate pattern differences between breast-fed and bottle-fed neonates. *Developmental Psychology*, **23**, 467–474.

Donovan, W. L., Leavitt, L. A., & Walsh, R. O. (1998). Conflict and depression predict maternal sensitivity to infant cries. *Infant Behavior and Development*, **23**, 505–517.

Ebstein, R. P., Levine, J., Geller, V., Auerbach, J., Gritsenko, I., & Belmaker, R. H. (1998). Dopamine D4 receptor and serotonin transporter promoter in the determination of neonatal temperament. *Molecular Psychiatry*, **3**, 238–246.

Ebstein, R. P., Novick, O., Umansky, R., Priel, B., Osher, Y., Blaine, D., et al. (1996). Dopamine D4 receptor (D4DR) exon III polymorphism associated with the human personality trait of novelty seeking. *Nature Genetics*, **12**, 78–80.

El-Sheikh, M., Harger, J., & Whitson, S. (2001). Exposure to interparental conflict and children's adjustment and physical health: The moderating role of vagal tone. *Child Development*, **72**, 1617–1636.

Field, T., Healy, B., Goldstein, S., & Guthertz, M. (1990). Behavior-state matching and synchrony in mother-infant interactions of nondepressed versus depressed dyads. *Developmental Psychology*, **26**, 7–14.

Field, T., Pickens, J., Fox, N. A., & Nawrocki, T. (1995). Vagal tone in infants of depressed mothers. *Development & Psychopathology*, **7**, 227–231.

Forbes, E. E., Cohn, J. F., Allen, N. B., & Lewinsohn, P. M. (2004). Infant affect during parent-infant interaction at 3 and 6 months: Differences between mothers and fathers and influence of parent history of depression. *Infancy*, **5**, 61–84.

Fox, N. A., Nichols, K. E., Henderson, H. A., Rubin, K., Schmidt, L., Hamer, D., et al. (2005). Evidence for a gene-environment interaction in predicting behavioral inhibition in middle childhood. *Psychological Science*, **16**, 921–926.

Fox, N. A., & Stifter, C. A. (1989). Biological and behavioral differences in infant reactivity and regulations. In G. Kohnstamm, J. Bates, & M. Rothbart (Eds.), *Handbook of temperament in childhood*. Chichester: Wiley.

Ganiban, J. M., Leve, L. D., Moore, G. A., & Neiderhiser, J. M. (2008). Strategies for understanding the mechanisms of mothering and fathering. In R. Bridges (Ed.), *Neurobiology of the parental brain* (pp. 391–404). New York: Elsevier.

Gilissen, R., Bakermans-Kranenburg, M. J., van IJzendoorn, M. H., & Linting, M. (2008). Electrodermal reactivity during the Trier Social Stress Test for children: Interaction between the serotonin transporter polymorphism and children's attachment representation. *Developmental Psychobiology*, **50**(6), 615–625.

Ge, X., Conger, R. D., Cadoret, R. J., & Neiderhiser, W. (1996). The developmental interface between nature and nurture: A mutual influence model of child antisocial behavior and parent behaviors. *Developmental Psychology*, **32**, 574–589.

Goldsmith, H. H., & Campos, J. J. (1982). Toward a theory of infant temperament. In R. N. Emde & R. J. Harmon (Eds.), *The development of attachment and affiliative systems*. New York: Plenum Press.

Gottlieb, G. (1970). Conceptions of prenatal behavior. In L. R. Aronson, E. Tobach, D. S. Lehrman, & J. S. Rosenblatt (Eds.), *Development and evolution of behavior: Essays in memory of T. C. Schneirla* (pp. 111–137). San Francisco: W. H. Freeman.

Gottlieb, G. (1991). The experiential canalization of behavioral development: Theory. *Developmental Psychology*, **27**, 4–13.

Gottlieb, G. (1998). Normally occurring environmental and behavioral influences on gene activity: From central dogma to probabilistic epigenesis. *Psychological Review*, **105**, 792–802.

Gottlieb, G., & Halpern, C. T. (2002). A relational view of causality in normal and abnormal development. *Development and Psychopathology*, **14**, 421–435.

Gottman, J., & Katz, L. (1989). Effects of marital discord on young children's peer interaction and health. *Developmental Psychology*, **25**, 373–381.

Grandy, D. K., & Kruzich, P. J. (2004). Molecular effects of dopamine receptors on attention. In M. I. Posner (Ed.), *Cognitive Neuroscience of Attention*, (pp. 260–268). New York: Guilford Press.

Gray, J. (1982). *The neuropsychology of anxiety*. New York: Oxford University Press.

Greenberg, B. D., Tolliver, T. J., Huang, S., Li, Q., Bengel, D., & Murphy, D. L. (1999). Genetic variation in the serotonin transporter promoter region affects serotonin uptake in human blood platelets. *American Journal of Medical Genetics*, **88**, 83–87.

Grossman, P., & Taylor, E. W. (2007). Toward understanding respiratory sinus arrhythmia: Relations to cardiac vagal tone, evolution and biobehavioral functions. *Biological Psychology*, **74**(2), 263–285.

Gunnar, M. R., & Donzella, B. (2002). Social regulation of the cortisol levels in early human development. *Psychoneuroendocrinology*, **27**, 199–220.

Harden, K. P., Turkheimer, E., Emery, R. E., D'Onofrio, B. M., Slutske, W. S., Heath, A. C., et al. (2007). Marital conflict and conduct problems in children of twins. *Child Development*, **78**, 1–18.

Hayden, E. P., Dougherty, L. R., Maloney, B., Durbin, C. E., Olino, T. M., Nurnberger, J. I. Jr., et al. (2007). Temperamental fearfulness in childhood and the serotonin transporter promoter region polymorphism: A multimethod association study. *Psychiatric Genetics*, **17**(3), 135–142.

Heim, C., Newport, D. J., Heit, S., Graham, Y. P., Wilcox, M., Bonsall, R., et al. (2000). Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. *Journal of the American Medical Association*, **284**, 592–597.

Heinz, A., Jones, D. W., Mazzanti, C., Goldman, D., Ragan, P., Hommer, D., et al. (2000). A relationship between serotonin transporter genotype and in vivo protein expression and alcohol neurotoxicity. *Biological Psychiatry*, **47**, 643–649.

Hill-Soderlund, A. L., Mills-Koonce, W. R., Propper, C., Calkins, S., Granger, D., Moore, G., et al. (2008). Parasympathetic and sympathetic responses to the strange situation in infants and mothers from avoidant and securely attached dyads. *Developmental Psychobiology*, **50**, 361–367.

Hofer, M. A. (1987). Early social relationships: A psychobiologist's view. *Child Development*, **58**, 633–647.

Huffman, L. C., Bryan, Y., Del Carmen, R., Pederson, F., Doussard-Roosevelt, J., & Porges, S. W. (1998). Infant temperament and cardiac vagal tone: Assessments at twelve weeks of age. *Child Development*, **69**, 624–635.

Izard, C. E., Porges, S. W., Simons, R. F., Haynes, O. M., Hyde, C., Parisi, M., et al. (1991). Infant cardiac activity: Developmental changes and relations with attachment. *Developmental Psychology*, **27**, 432–439.

Kagan, J. (1989). *Unstable ideas: Temperament, cognition, and self*. Cambridge: Harvard University Press.

Katz, L. F., & Gottman, J. M. (1995). Vagal tone protects children from marital conflict. *Development and Psychopathology*, **7**, 83–92.

Katz, L. F., & Gottman, J. M. (1997). Buffering children from marital conflict and dissolution. *Journal of Clinical Child Psychology*, **26**, 157–171.

Kaufman, J., Yang, B., Douglas-Palumberi, H., Houshyar, S., Lipschitz, D., Krystal, J., et al. (2004). Social supports and serotonin transporter gene moderate depression in maltreated children. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 17316–17321.

Kirley, A., Lowe, N., Mullins, C., McCarron, M., Daly, G., Waldman, I., et al. (2004). Phenotype studies of the DRD4 gene polymorphisms in ADHD: Association with oppositional defiant disorder and positive family history. *American Journal of Medical Genetics: Neuropsychiatric Genetics*, **131**, 38–42.

LaHoste, G. J., Swanson, J. M., Wigal, S. B., Glabe, C., Wigal, T., King, N., et al. (1996). Dopamine D4 receptor gene polymorphism is associated with attention deficit hyperactivity disorder. *Molecular Psychiatry*, **1**, 83–84.

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*, **274**, 1527–1531.

Livak, K. J., Rogers, J., & Lichter, J. B. (1995). Variability of dopamine D4 receptor (DRD4) gene sequence within and among nonhuman primates. *Proceedings of the National Academy of Science of the USA*, **92**, 427–431.

Mills-Koonce, W. R., Propper, C. B., Gariepy, J. -L., Blair, C., Garrett-Peters, P., & Cox, M. J. (2007). Bidirectional genetic and environmental influences on mother and child behavior: The family system as the unit of analyses. *Development and Psychopathology*, **19**, 1073–1087.

Moffitt, T. E., Caspi, A., & Rutter, M. (2006). Measure gene-environment interactions in psychopathology: Concepts, research strategies, and implications for research, intervention, and public understanding of genetics. *Perspectives on Psychological Science*, **1**, 5–27.

Moore, G. A. (2010). Parent conflict predicts infants' vagal regulation in social interaction. *Development and Psychopathology*, **22**(1), 23–33.

Moore, G. A., & Calkins, S. D. (2004). Infants' vagal regulation in the still-face paradigm is related to dyadic coordination of mother-infant interaction. *Developmental Psychology*, **40**, 1068–1080.

Moore, G. A., Hill-Soderlund, A., Propper, C., Calkins, S., Mills-Koonce, W. R., & Cox, M. (2009). Mother-infant vagal regulation in the face-to-face and still-face paradigm is moderated by maternal sensitivity. *Child Development*, **80**, 209–223.

Muraven, M., & Baumeister, R. F. (2000). Self-regulation and depletion of limited resources: Does self-control resemble a muscle? *Psychological Bulletin*, **126**, 247–259.

Muraven, M., & Slessareva, E. (2003). Mechanisms of self-control failure: Motivation and limited resources. *Personality and Social Psychology Bulletin*, **29**, 894–906.

Noble, E. P., Ozkaragoz, T. Z., Ritchie, T. L., Zhang, X., Belin, T. R., & Sparkes, R. S. (1998). D2 and D4 dopamine receptor polymorphisms and personality. *American Journal of Medical Genetics: Neuropsychiatric Genetics*, **81**, 257–267.

O'Connor, T. G., Neiderhiser, J. M., Reiss, D., Hetherington, E. M., & Plomin, R. (1998). Genetic contributions to continuity, change, and co-occurrence of antisocial and depressive symptoms in adolescence. *Journal of Child Psychology and Psychiatry*, **39**, 323–336.

Panksepp, J. (1986). The neurochemistry of behavior. *Annual Review of Psychology*, **37**, 77–107.

Plomin, R., & Rutter, M. (1998). Child development, molecular genetics, and what to do with genes once they are found. *Child Development*, **69**, 1223–1242.

Porges, S. W. (1991). Vagal tone: An autonomic mediatory of affect. In J. A. Garber, & K. A. Dodge (Eds.), *The development of affect regulation and dsyregulation* (pp. 11–128). New York: Cambridge University Press.

Porges, S. W. (1996). Physiological regulation in high-risk infants: A model for assessment and potential intervention. *Development and Psychopathology*, **8**, 43–58.

Porges, S. W. (2007). The polyvagal perspective. *Biological Psychology*, **74**, 116–143.

Porges, S. W., & Byrne, E. A. (1992). Research methods for measurement of heart rate and respiration. *Biological Psychology*, **34**, 93–130.

Porges, S. W., Doussard-Roosevelt, J. A., Portales, A. L., & Greenspan, S. I. (1996). Infant regulation of the vagal "brake" predicts child behavior problems: A psychobiological model of social behavior. *Developmental Psychobiology*, **29**, 697–712.

Porges, S. W., Doussard-Roosevelt, J. A., Portales, A. L., & Suess, P. E. (1994). Cardiac vagal tone: Stability and relation to difficultness in infants and 3-year-olds. *Developmental Psychobiology*, **27**, 289–300.

Porter, C. L. (2003). Coregulation in mother-infant dyads: Links to infants' cardiac vagal tone. *Psychological Reports*, **92**, 307–319.

Porter, C. L., Bryan, Y. E., & Hsu, H. (1995). Physiological markers in early infancy: Stability of one- to six-month vagal tone. *Infant Behavior and Development*, **18**, 365–369.

Porter, C. L., Wouden-Miller, M., Silva, S. S., & Porter, A. E. (2003). Marital harmony and conflict: Links to infants' emotional regulation and cardiac vagal tone. *Infancy*, **4**, 297–307.

Posner, M. I., & Rothbart, M. K. (2000). Developing mechanisms of self-regulation. *Development and Psychopathology*, **12**, 427–441.

Propper, C., Moore, G. A., Mills-Koonce, W. R., Halpern, C. T., Hill-Soderlund, A. L. Calkins, S. D., et al. (2008). Gene-environment contributions to the development of

infant vagal reactivity: The interaction of dopamine and maternal sensitivity. *Child Development*, **79**, 1378–1395.

Propper, C., Willoughby, M., Halpern, C. T., Carbone, M. A., & Cox, M. (2007). Parenting quality, DRD4, and the prediction of externalizing and internalizing behaviors in early childhood. *Developmental Psychobiology*, **49**, 619–632.

Richards, J. E., & Cameron, D. (1989). Infant heart rate variability and behavioral developmental status. *Infant Behavior and Development*, **12**, 45–58.

Riggins-Caspers, K. M., Cadoret, R. J., Knutson, J. F., & Langebehn, D. (2003). Biology-environment interaction and evocative biology-environment correlation: Contributions of harsh discipline and parental psychopathology to problem adolescent behaviors. *Behavior Genetics*, **33**, 205–220.

Rothbart, M. K., Ahadi, S. A., & Evans, D. E. (2000). Temperament and personality: Origins and outcomes. *Journal of Personality and Social Psychology*, **78**(1), 122–35.

Rothbart, M. K., & Derryberry, D. (1981). Development of individual differences in temperament. In M. E. Lamb & A. L. Brown (Eds.), *Advances in developmental psychology*. (pp. 37–86). Hillsdale, NJ: Erlbaum.

Rutter, M., Moffitt, T. E., & Caspi, A. (2006). Gene-environment interplay and psychopathology: Multiple varieties but real effects. *Journal of Child Psychology and Psychiatry*, **47**, 226–261.

Scarr, S., & McCartney, K. (1983). How people make their own environments: A theory of genotype (leading to) environmental effects. *Child Development*, **54**, 424–35.

Schmidt, L. A., Fox, N. A., Rubin, K. H., Hu, S., & Hamer, D. H. (2002). Molecular genetics of shyness and aggression in preschoolers. *Personality and Individual Differences*, **33**, 227–238.

Schore, A. N. (2000). Attachment and the regulation of the right brain. *Attachment & Human Development*, **2**, 23–47.

Sheese, B. E., Voelker, P. M., Rothbart, M. K., & Posner, M. I. (2007). Parenting quality interacts with genetic variation in dopamine receptor D4 to influence temperament in early childhood. *Development and Psychopathology*, **19**, 1039–1046.

Siegel, D. J., (2001). Toward an interpersonal neurobiology of the developing mind: Attachment relationships, "mindsight," and neural integration. *Infant Mental Health Journal*, **22**, 67–94.

Sourbrie, P. (1986). Reconciling the role of central serotonin neurons in human and animal behavior. *Behavioral and Brain Sciences*, **9**, 319–364.

Spangler, G., & Grossman, K. E. (1993). Biobehavioral organization in securely and insecurely attached infants. *Child Development*, **64**, 1439–1450.

Stein, M. B., Schork, N. J., & Gelernter, J. (2007). Gene-by-Environment (serotonin transporter and childhood maltreatment) interaction for anxiety sensitivity, an intermediate phenotype for anxiety disorders. *Neuropsychopharmacology*, **33**, 312–319.

Stifter, C. A. (1995). Approach/withdrawal processes in infancy: The relationship between parasympathetic tone and infant temperament. In K. Hood, E. Tobach, & G. Greenberg (Eds.), *Approach/withdrawal theory and behavioral development*. Hillsdale, NJ: Erlbaum.

Stifter, C. A., & Fox, N. A. (1990). Infant reactivity: Physiological correlates of newborn and 5-month temperament. *Developmental Psychology*, **26**, 582–588.

Stifter, C. A., Fox, N. A., & Porges, S. W. (1989). Facial expressivity and vagal tone in 5- and 10-month-old infants. *Infant Behavior and Development*, **12**, 127–137.

Suess, P. E., Porges, S. W., & Plude, D. J. (1994). Cardiac vagal tone and sustained attention in school-age children. *Psychophysiology*, **31**, 17–22.

Suhara, T., Yasuno, F., Sudo, Y., Yamamoto, M., Inoue, M., Okubo, Y., et al. (2001). Dopamine D_2 receptors in the insular cortex and the personality trait of novelty seeking. *Neuroimage*, **13**, 891–895.

Sunohara, G. A., Roberts, W., Malone, M., Schachar, R. J., Tannock, R., Basile, V. S., et al. (2000). Linkage of the dopamine D4 receptor gene and attention-deficit/hyperactivity disorder. *Journal of the American Academy of Child and Adolescent Psychiatry*, **39**, 1537–1542.

Suomi, S. J. (2000). A biobehavioral perspective on developmental psychopathology. In A. J. Sameroff, M. Lewis, & S. M. Miller (Eds.), *Handbook of developmental psychopathology* (pp. 237–256). New York: Kluwer Academic/Plenum.

Thompson, R. A. (1994). Emotion regulation: A theme in search of a definition. In N. A. Fox (Ed.), *Emotion regulation: Behavioral and biological considerations*, *Monographs of the Society for Research in Child Development* (Nos. 2–3, Serial No. 240), 25–52.

Westernberg, H. G., Murphy, D. L., & Den Boer, J. A. (Eds.). (1996). *Advances in the neurobiology of anxiety disorders*. New York: Wiley.

Young, S. E., Smolen, A., Stallings, M. C., Corley, R. P., & Hewitt, J. K. (2003). Sibling-based association analyses of the serotonin transporter polymorphism and internalizing behavior problems in children. *The Journal of Child Psychology and Psychiatry*, **44**(7), 961–967.

Zukerman, M. (1994). Impulsive unsocialized sensation seeking: The biological foundations of a basic dimension of personality. In J. Bates & T. Wachs (Eds.), *Temperament: Individual differences at the interface of biology and behavior* (pp. 219–255). Washington, DC: American Psychological Association.

18

Genetic Research in Psychiatry and Psychology

A critical overview

Jay Joseph

Introduction

The current consensus position in psychiatry and psychology is that, in addition to environmental factors, genes play an important role in causing psychiatric disorders and variation in "continuously distributed" psychological traits (such as personality and IQ). The fields of psychiatric genetics and behavior genetics have produced much of the research supporting this position, which consists of two broad areas of investigation. The first explores how a trait is distributed among various types of biologically related (and sometimes unrelated) people. The most common approaches have been the studies of families, of twins, and of people who have been adopted. These methods are sometimes grouped together under the heading *kinship research*. The second area is molecular genetic research, which attempts to pin down the actual genes that researchers believe underlie various traits and disorders.

Contrary to most academic and popular accounts of this research, in this chapter we will see that studies of families, twins, and adoptees are greatly flawed on several critical dimensions. Moreover, countless sensationalized media reports notwithstanding, researchers have failed to discover the genes they believe underlie DSM-defined psychiatric disorders, and normal variation in psychological traits. Due to space considerations, we will look only at the most frequently cited research methods.

If a trait is caused or influenced by hereditary factors, we would expect to find a greater concentration of it among members of the same family, roughly proportional to their degree of genetic relatedness. For example, first-degree biological relatives (such as the siblings, children, or parents of an individual) should manifest the trait more often, or resemble each other to a greater degree, than second-degree

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biological relatives (such as an individual's aunts, uncles, grandparents, grandchildren, nieces, nephews, or half-siblings). In addition, monozygotic (MZ, identical) twin pairs should resemble each other to a greater degree than dizygotic (DZ, fraternal) twin pairs.

A major aim of this chapter, however, is to show that although relatives frequently manifest traits in patterns predicted by genetic theories, these patterns frequently match the predictions made by theories of *non*-genetic causation as well. Thus, it is frequently difficult or impossible to disentangle these potential influences.

In the context of the upcoming analysis, the term *environment* refers to all non-genetic factors that could contribute to or cause the appearance of traits. Environmental factors and influences include parenting styles, peer groups, abuse, neglect, oppression, toxic chemicals, viruses, accidents, culture, attachment disturbance, racism, and so on. The term *confound* refers to unforeseen or uncontrolled-for factors that threaten the validity of conclusions researchers draw from their studies. In genetic research, potential confounds are usually environmental.

Heritability estimates (coefficients) are not evaluated in this chapter because they are misleading and widely misunderstood (Joseph, 2004, chapter 5). Genetic researchers produce such estimates (ranging from 0 to 100%) in reference to psychiatric disorders and psychological traits, and imply that as the percentage increases, the importance of genetic influences on the trait correspondingly increases. In particular, the "heritability of IQ" topic has been fiercely debated for decades. However, critics have argued, correctly in my view, that heritability estimates cannot tell us "how much" genes influence a given trait. Critical behavior geneticist Jerry Hirsch (1997, 2004) argued that a heritability estimate is not a "nature/nurture ratio" of the relative contributions of genes and environment. Other critics (e.g., Block, 1995; Chaufan, 2008; Feldman & Lewontin, 1975; Greenberg, 2005; Joseph, 2004; Lewontin, 1987; Moore, 2001; Schönemann, 1997; Stoltenberg, 1997; Wahlsten, 1990, 1994) have also detailed problems with the heritability concept. According to critical behavior geneticist Douglas Wahlsten (1990, p. 119), "The only practical application of a heritability coefficient is to predict the results of a program of selective breeding." Moreover, heritability estimates are derived from the flawed research methods discussed in this chapter.

Another important yet rarely discussed issue in genetic research is the validity and reliability of concepts such as "schizophrenia," "IQ," "personality," "bipolar disorder," "criminality," "ADHD," etc. Establishing the validity of these constructs, and the ability to reliably identify and define them, is an important part of any research project. Yet the validity and reliability of psychiatric disorders and psychological traits is open to question (Boyle, 2002; Hill, 1983; Kirk & Kutchins, 1992; Kutchins & Kirk, 1997; Mensh & Mensh, 1991; Richardson, 2000). Psychologist Richard Bentall has rejected the concept of discrete mental disorders: "There is no clear boundary between mental health and mental illness. Psychological complaints exist on a continuum with normal behaviors and experiences" (Bentall, 2003, p. 143). And it is widely understood that there is no consensus definition of "intelligence" (Neisser et al., 1996).

In the following sections we will look at four major areas of genetic research: Family studies, twin studies, adoption studies, and molecular genetic studies. The upcoming analysis differs from the accounts of most textbooks and popular works, whose authors usually endorse twin and adoption studies as valid instruments for the detection of genetic factors. Furthermore, these accounts sometimes erroneously claim that researchers have already discovered genes for IQ, personality traits, and the major mental disorders. One purpose of this chapter is to encourage you to develop a healthy dose of skepticism about such claims.

Family studies constitute the first step in the process of determining whether hereditary factors underlie psychiatric disorders and psychological traits. Let us now turn to a discussion of how these studies are performed, and of how we should interpret their results.

Family Studies

Background

Traits and conditions have been known to run in families since biblical times (Weissman et al., 1986). The question of what *causes* this to occur has been the subject of debate ever since. These questions have come to form what is sometimes called the ''nature-nurture'' controversy. Why do some traits run in families? Is it the result of the common familial (nurture), social, and physical environments shared by families, by the common genes shared by family members (nature), or perhaps a combination of both? The current consensus in psychiatry and psychology holds that both genes and environment play an important role, although the authors of many authoritative works emphasize genetic factors over environmental factors.

The first ostensibly scientific attempt to determine whether traits run in families was the *family pedigree study*, in which researchers mapped a person's family through several generations and noted which members were affected by the trait or disorder in question. The first few decades of the 20th century saw many publications containing pedigree charts of families manifesting traits such as ''insanity,'' ''feeblemindedness,'' ''genius,'' ''criminality,'' and pellagra. Figure 18.1 shows a five-generation 1911 family pedigree chart of the mating of a ''feeble-minded'' woman and an ''alcoholic'' man. The author, Charles B. Davenport, was a leading eugenicist in the early part of the 20th century. Davenport and other proponents of eugenics used such charts to support their argument that psychological traits and socially disapproved behaviors show ''a strong hereditary bias'' (Davenport, 1911, p. 83).

Moving on from family pedigree studies, the first type of systematic study of relatives was the *family study method*. In family (and adoption) studies, researchers identify persons manifesting the trait in question (called ''cases''; psychiatric geneticists call such persons ''probands''), and attempt to determine whether their biological relatives manifest the trait more often than the general population expectation. These relatives comprise the ''index'' group (a term also used in adoption research). In the

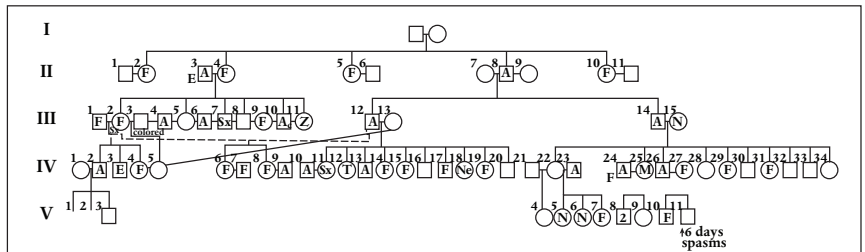


Fig. 49.—Pedigree of a Massachusetts family comprising much feeble-mindedness or imbecility, F; associated with alcoholism, *A*; criminality, *C*; sex immorality, *Sx*; epilepsy, *E*; *M*, migrainous; *Ne*, neurotic; *T*, tubercular. Note the association of alcoholism with imbecility.

I, 1, a basket-maker, alcoholic, married a feeble-minded woman. Of their 5 children (II, 2–10) 4 were feeble-minded and the other, II, 8, shiftless and an alcoholic. II, 4, married an epileptic alcoholic and they had 7 children. The oldest Amanda, III, 2, feeble-minded and sexually immoral, married, first, a feeble-minded man, III, 1, by whom she had 3 children, 1 alcoholic and immoral, 1 epileptic and a cripple, 1 feeble-minded; secondly, by a colored man, III, 3, she had 1 illegitimate colored child, IV, 5; thirdly, by an alcoholic, she had 2 other feeble-minded illegitimate offspring, both of whom married, the first a feeble-minded man, the second an alcoholic consumptive. The second daughter, III, 5, married twice, both alcoholics, but had no offspring; III, 7, was sexually immoral as was also the imbecile son, III, 8. III, 10, was alcoholic and criminal and two daughters, III, 11, were normal. II, 8, a shiftless alcoholic known as "Woodchuck Pete" married twice. By his first wife, a normal woman, he had two sons, both alcoholic. The oldest, III, 12, we have met above as the illegitimate husband of III, 2. He married later her daughter, IV, 4, they had 11 children. The oldest daughter, sexually immoral, IV, 11, married an alcoholic but had no children. IV, 12, was a consumptive; IV, 13, an alcoholic, and of the others, 4 are imbeciles and 1 neurotic, 4 being state wards. III, 14 married a normal woman and they had 10 children. IV, 22, married first a normal but shiftless man whom she left for non-support; her second husband was an alcoholic wanderer, by whom she had 2 normal and 1 feeble-minded child. IV, 25, migraine and immoral, married an alcoholic imbecile by whom she had 3 normal but scrofulous children. IV, 26, an alcoholic, married a feeble-minded woman and had 2 children, 1 feeble-minded, 1 died at 6 days of spasms. Of remaining 7 children, 2 are feeble-minded. F. W., 1.

Source: Davenport, 1911, p. 84

Figure 18.1. Five generation pedigree of the offspring of a ''feeble minded'' woman and an ''alcoholic'' man, published in 1911.

past few decades, researchers more often compare index relatives to a control group consisting of the biological relatives of people that do not manifest the trait.

If a condition is found to aggregate in families, it is said to be familial. In the past, researchers considered the results of family studies (called ''eugenical family studies'' in the early 20th century) as proof positive that psychiatric disorders were caused by hereditary factors. In many cases, such interpretations were put forward in support of eugenics and compulsory eugenic sterilization programs (Black, 2003; Proctor, 1988). This is seen in the early German psychiatric genetic schizophrenia family studies of Ernst Rüdin (1916), and Franz J. Kallmann (1938). According to Kallmann (1938, p. xiv), ''The principal aim of our investigations was to offer conclusive proof of the inheritance of schizophrenia and to help, in this way, to establish a dependable basis for the clinical and eugenic activities of psychiatry.'' (See Joseph, 2004, chapter 4 for further documentation of Kallmann's and Rüdin's enthusiastic support for eugenic practices.)

However, contrary to the views of these early researchers, ''familial'' is not the same as ''genetic.'' Unfortunately, many people view these terms as being synonymous, when in fact they are not. Moreover, some researchers, and more frequently reports in the popular media, continue to mistakenly cite family data in support of genetics (see Joseph, 2006).

The following description, analysis, and critique of family, twin, and adoption research draws on five works published since 1999 by authors who are among the world's leading behavioral genetic and psychiatric genetic theorists and researchers.

The five publications are *Genetics of Mental Disorders*, by psychiatric geneticists Steven V. Faraone, Ming T. Tsuang, and Debby W. Tsuang (Faraone, Tsuang, & Tsuang, 1999), *Genes, Environment, and Psychopathology*, by psychiatric geneticists Kenneth S. Kendler and Carol A. Prescott (Kendler & Prescott, 2006), *Genes and Behavior*, by behavioral geneticist Michael Rutter (Rutter, 2006), *Behavioral Genetics*, the standard behavioral genetics textbook by Robert Plomin, John C. DeFries, Gerald E. McClearn, and Peter McGuffin (Fifth Edition; Plomin, DeFries, McClearn, & McGuffin, 2008), and ''Genetic and Environmental Influences on Human Psychological Differences,'' by behavioural geneticists Thomas J. Bouchard, Jr. and Matt McGue (Bouchard & McGue, 2003). Subsequent references to the ''five publications'' refer to the works of these authoritative authors/researchers.

The authors of the five publications agree that a family study, while constituting an important stage of genetic research, is unable to disentangle the potential role of genetic and environmental factors. In other words, because family members share a common environment as well as common genes, a trait ''running in the family'' can be completely explained by genetic *or* environmental factors. Family studies, therefore, do not supply evidence in support of genetics. The authors of the five publications weighed in on the issue as follows:

> Resemblance among relatives can be ascribed to shared environment (nurture) or shared genes (nature). (Kendler, 2000, p. 1149. Kendler & Prescott did not address family studies in their 2006 publication)

> Showing that a disorder runs in families does not conclusively establish that genes cause the disorder. Although family studies are indispensable for establishing the familial transmission of disorders, they cannot by themselves establish the causes of disorders. (Faraone et al., 1999, p. 21)

> Family studies differ from twin and adoptee designs in the key respect that they do not permit a clear separation of genetic and non-genetic influences. (Rutter, 2006, p. 58)

> Many behaviors ''run in families,'' but family resemblance can be due to either nature or nurture. (Plomin et al., 2008, p. 70)

> Family studies by themselves cannot disentangle genetic and environmental influences. (Plomin et al., 2008, p. 151)

> Correlations between biological relatives (i.e., IQ correlations between siblings or parents and offspring) reared together are etiologically ambiguous. Behavior geneticists are quick to point out that ''familial does not equate to genetic.'' (Bouchard & McGue, 2003, p. 5)

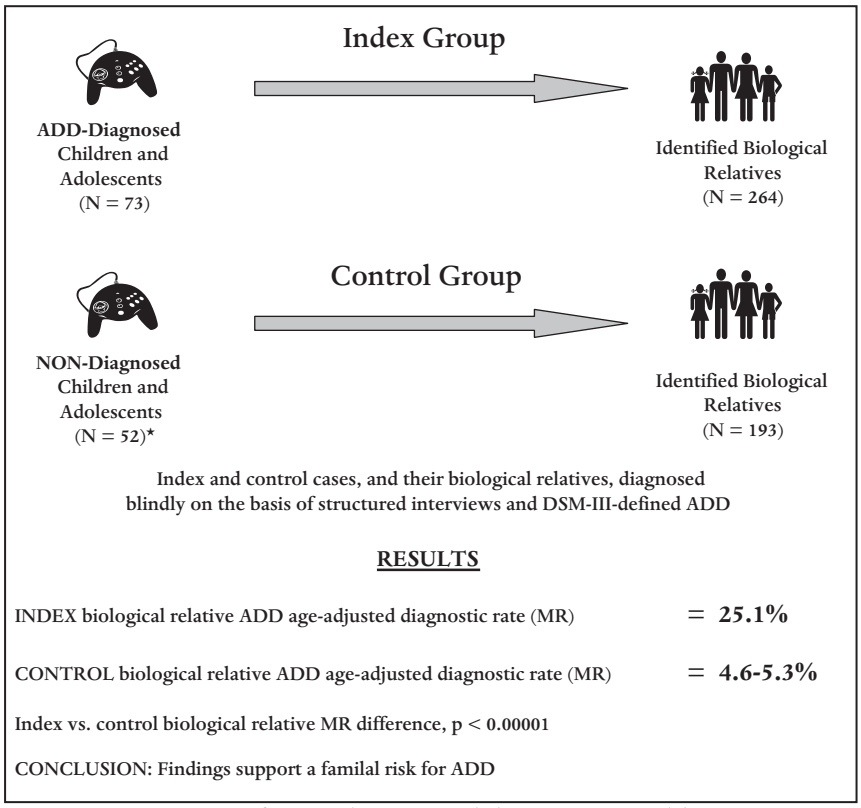
How Family Studies are Performed

Modern family studies in psychiatry employ DSM-defined diagnoses, control groups, structured diagnostic interviews, and blind diagnoses. These techniques

were largely absent in studies published before the 1970s. Researchers now compare the index group to a control group in order to rule out the possibility that faulty diagnostic methods were used, as opposed to the previous practice of comparing diagnostic rates against the general population expectation (Faraone et al., 1999). Ideally, the control cases would be similar to the index case in terms of age, sex, and other matching criteria, and would differ only in diagnostic status. Structured interviews are employed in order to standardize diagnostic procedures based on accepted criteria. For continuously distributed traits such as IQ and personality, standardized testing instruments can serve this purpose. Blind diagnostic procedures mean that the diagnosticians, raters, or scorers have no knowledge of whether family members are related to the index or control cases. Most of the earlier researchers were strong proponents of genetic theories, and it is likely that the non-blinded diagnoses they made were biased by their pre-existing views on genetics and eugenics.

Although the early studies contained the biases I have just described, their results continue to be presented to students in textbooks and in widely reproduced graphics such as Irving Gottesman's Figure 10 on the lifetime risk of schizophrenia, from his book *Schizophrenia Genesis* (Gottesman, 1991, p. 96). The familial risk percentages in this figure appear to be heavily weighted by the poorly executed, non-blinded, large sample-size studies of a previous era. Modern studies using blind diagnoses, structured interviews, and control groups find much lower schizophrenia rates among first-degree biological relatives (Joseph, 2006; Joseph & Leo, 2006). However, in general both environmental and genetic theories of mental disorders predict the finding of higher rates of the disorder among the biological relatives of index cases versus controls, or versus the general population lifetime prevalence (approximately 0.5%–1.5% in the case of schizophrenia; APA, 2000).

A 1990 family study of ADD (attention deficit disorder; now called "ADHD") by psychiatric genetic investigators Joseph Biederman and colleagues (Biederman, Faraone, Keenan, Knee, & Tsuang, 1990) provides an example of a modern psychiatric family study. As seen in Figure 18.2, the index cases consisted of 73 consecutively-ascertained outpatient children and adolescents diagnosed with ADD on the basis of DSM-III criteria and structured psychiatric interviews. A control group consisting of 52 children and adolescents not diagnosed with ADD was also established. The index and control cases produced 457 first-degree biological relatives (index $N = 264$, control $N = 193$). These relatives were evaluated and diagnosed by blinded raters, again using structured diagnostic interviews. The results showed an age-corrected lifetime risk for ADD (calculated as a "morbid risk" or "MR" by the investigators) of 25.1% for the index biological relatives, versus a rate of 4.6%–5.3% among the biological relatives of controls. This is a statistically significant difference ($p < 0.00001$). The researchers concluded that "ADD is a highly familial disorder" (p. 532), while recognizing that "familial aggregation does not necessarily imply genetic risk" (p. 531).



Notes : ADD = Attention Deficit Disorder. MR = Morbid Rate (age-corrected diagnostic estimate).
* Control children and adolescents consisted of 26 normal controls + 26 psychiatric controls.
Source : Based on methods, results, and conclusions from Biederman et al., 1990.

Figure 18.2. Psychiatric family study using modern methods.

Bouchard and McGue (1981) reported that the pooled IQ correlations of the studies they surveyed were .45 for non-twin siblings reared together, .24 for siblings reared apart, and .385 for single parent and offspring. Personality test score correlations are somewhat lower (Plomin et al., 2008). In general, as expected by people with such diverging viewpoints as behavior geneticists, psychoanalysts, hereditarian theorists, critics of hereditarian theories, and family therapists, people sharing a common family environment resemble each other more for psychological traits that do randomly selected members of the population.

Thus, genetic researchers have turned to twin and adoption studies in an attempt to clearly separate the potential role of genes and environment. We will see, however, that this separation is far more difficult to accomplish than is currently believed.

Twin Studies

Introduction

Some ways that twins have been used for research purposes include:

- The twin method (twins reared-together)
- Studies of twins reared-apart
- The co-twin control method
- Genetic studies of the offspring of discordant monozygotic twin pairs
- Studies of discordant monozygotic twin pairs (investigating environmental differences)

After a brief description of these methods, the remaining portion of this section is devoted to an analysis of the twin method, and of twins reared-apart studies.

The Twin Method

The 1920s saw the development of the ''classical twin method,'' more commonly known as the ''twin method.'' The twin method compares the trait resemblance of reared-together MZ twin pairs (also known as monozygotic, or identical), who share 100% genetic similarity, versus the resemblance of reared-together same-sex DZ twin pairs (also known as dizygotic, or fraternal), who average a 50% genetic similarity. Twins' trait resemblance is usually measured with concordance rates or correlations. Twin pairs are *concordant* when both are diagnosed with the same disorder, and *discordant* when only one member of the pair is diagnosed. Based on the theoretical assumption that the childhood and adult environments of both types of twins are comparable, known as the *equal environment assumption* (or ''EEA''), twin researchers attribute to genetic factors the usual finding of a significantly greater resemblance among MZ versus same-sex DZ twin pairs. The EEA is, by far, the most controversial twin method assumption. The main theoretical assumptions of the twin method are outlined in Figure 18.3.

Twins Reared-Apart

In 1937, American researcher Horatio Newman and his colleagues (Newman, Freeman, & Holzinger, 1937) published the first systematic study of ''reared-apart'' twins. More recently, the Minnesota reared-apart twin studies of Thomas Bouchard and colleagues (Bouchard, Lykken, McGue, Segal, & Tellegen, 1990)

Monozygotic (MZ) Twin Pairs	Same-Sex Dizygotic (DZ) Twin Pairs
	
Share **100%** of the same genes. Reared together in the same home	Share on average **50%** of the same genes. Reared together in the same home

A significantly greater concordance rate or correlation among MZ versus same-sex DZ twin pairs is attributable to genetic factors and is generalizable to the non-twin population, *as long as all of the following five theoretical assumptions are true*:

Most Controversial: The Equal Environment Assumption (EEA)

- MZ twin pairs and same-sex DZ twin pairs experience the same emotional and psychological bond with each other, as well as experiencing roughly the same social, treatment, and physical environments.

Other Assumptions

- There are only two types of twins, MZ and DZ
- Investigators are able to distinguish between MZ and DZ twin pairs
- The prevalence or distribution of the trait in question is the same among twins and non-twins (generalizability)
- The prevalence or distribution of the trait in question is the same among individual MZ twins as a population, versus individual DZ twins as a population

Source : Adapted with revisions from Joseph, 2004, p. 22

Figure 18.3. The twin method and its assumptions.

have influenced the field of psychology and have been put forward as strongly supporting the case for genetics.

Reared-apart twin studies have assessed twin resemblance for psychological traits such as IQ and personality, but have not assessed twin concordance for psychiatric disorders. This is due to the difficulty of obtaining a large enough sample of reared-apart twins to perform such studies. The reports that have been published consist of case histories of individual twin pairs judged concordant or discordant for particular psychiatric disorders.

Other Types of Twin Studies

Another way of studying twins is the *co-twin control method*. This method looks at environmental factors that might lead to different outcomes for twins. For example, researchers might wish to study the smoking habits of a pair of MZ twins, one of whom has been diagnosed with lung cancer. As a team of twin researchers observed, ''Co-twins serve as exceptionally well-matched controls, removing or substantially reducing the effects of genetics, age, and race, as well as many unmeasured factors, such as pre-adult home environment, schools, religious upbringing, and so forth'' (Herrell et al., 1999, p. 869). Other twin research approaches include *genetic studies of the offspring of discordant MZ pairs* (Fischer, 1971; Gottesman & Bertelsen, 1989; Kringlen & Cramer, 1989; for a critique of these studies, see Joseph, 2004, chapter 6; Torrey, 1990), and the *study of discordant MZ pairs* to assess for possible environmental differences (e.g., Mosher, Pollin, & Stabenau, 1971), or physiological differences (e.g., Torrey, Bowler, Taylor, & Gottesman, 1994) between members of the pair.

The Twin Method (Twins Reared Together)

The twin method provides the most frequently cited evidence in support of important genetic influences on psychological traits and psychiatric disorders. Indeed, genetic researchers look upon the twin method as one of the two ''workhorses of human behavioural genetics'' (Plomin et al., 2008, p. 38; the authors cited adoption studies as the other ''workhorse''). Yet, as I will attempt to show, the twin method is no more able to disentangle potential genetic and environmental influences than is a family study. We will see, however, that although both research methods are clearly confounded by environmental influences, genetic researchers approach and interpret twin studies very differently than they approach and interpret family studies.

The twin method has been used widely to assess the role of genetic factors for IQ, personality, medical diseases, and psychiatric disorders. Researchers use correlations to measure the association (relationship) of continuously distributed traits, such as twins' scores on personality or IQ tests. A positive correlation is expressed as a coefficient ranging from 0.0 to 1.0. Concordance rates are used with ''qualitative'' traits such as schizophrenia, bipolar disorder (previously known as manic-depressive disorder), and autism. In these cases the researchers must determine whether the disorder is present or is not present, as opposed to continuously distributed traits, where test scores fall on a continuum.

Researchers ascertain a group of MZ and same-sex DZ pairs for their studies. The names of these twins are obtained from sources which include resident hospital populations, national or local twin registers, lists of twins consecutively

admitted to a facility, and clinical referrals. The researchers then determine zygosity, which refers to the method used to determine whether pairs are MZ or DZ. The ability to accurately make such a distinction is an essential aspect of twin research. Like family studies, most twin studies performed before the 1970s failed to make diagnoses blindly, and failed to use standard diagnostic procedures (or even adequately define the trait in question in many cases). Moreover, twin studies are subject to biases relating to the ascertainment procedures used (Rosenthal, 1962b).

If all twin method assumptions are valid (see Figure 18.3), significantly greater MZ versus same-sex DZ pair trait resemblance can be attributed to genetic factors. For personality traits measured with psychometric tests, MZs correlate at roughly .48, while DZs correlate at .23 (Bouchard, 1997a). IQ correlations are higher, with Bouchard and McGue reporting a pooled MZ correlation of .85, and a DZ pooled correlation of .58 (Bouchard & McGue, 1981; see Lerner, 1986, for a critical review of this publication). Although textbooks frequently report pooled MZ concordance for schizophrenia as 50%, it is closer to 25% in the more methodologically sound studies (Joseph, 2006; Walker, Downey, & Caspi, 1991). In any case, MZ concordance for schizophrenia is roughly 3–4 times greater than the concordance rate for same-sex DZ pairs. Many other psychiatric disorders show a similar pattern.

The results of twin studies in psychology and psychiatry are widely reported in textbooks, in popular books about genetics, and in the media. In the vast majority of cases the authors of these publications accept, with little or no criticism, twin researchers' claims that the twin method provides unambiguous evidence in favor of genetics. We will soon see, however, that the plausibility of the equal environment assumption (EEA) is in serious doubt. If this assumption is false – and I will argue that it is false – studies utilizing the twin method may have recorded nothing more than the greater environmental similarity and psychological bond of MZ twin pairs as compared with same-sex DZ pairs.

Critics have argued since the 1920s that using the twin method to assess the role of genetics is dubious, since MZ twin pairs, in addition to being more genetically similar than DZ pairs, experience much more similar *environments* than DZ pairs. For example, twin researcher Harold Carter wrote in 1940 (p. 247) that "the assumption that the nurture influences are approximately equal for fraternal and identical twins . . . seems untenable to anyone who has had much contact with twins in their own social environment." Carter went on to observe,

> Identical twins obviously like each other better; they obviously have the same friends more often; they obviously spend more time together; and they are obviously treated by their friends, parents, teachers, and acquaintances as if they were more alike than fraternal twins are. (Carter, 1940, p. 247)

Family systems pioneer Don Jackson (1960) went further, and argued forcefully that schizophrenia twin research (and by implication twin research in psychology

and other areas of psychiatry) may have recorded little more than the greater environmental similarity and "ego fusion" experienced by MZ versus DZ twin pairs. Jackson's most telling point was that – among pairs with the same genetic relationship to each other – those pairs experiencing a more similar environment and a closer emotional bond were consistently more concordant for schizophrenia. This suggests that MZ-DZ concordance rate differences could be explained on environmental grounds (in addition to methodological bias). Although some twin researchers subsequently adjusted their methods on the basis of Jackson's criticism (e.g., Gottesman & Shields, 1972), most continued to uphold the validity of the twin method. Today, Jackson's critique is a forgotten document in the sense that the twin method is more popular than ever, even though none of his arguments against the twin method have ever been refuted (Joseph, 2001a, 2004).

Apart from the decades-old controversy over what the twin method actually measures (the EEA debate), critics of the twin method have pointed to a series of methodological problems. These include:

- The acceptance of unsupported theoretical assumptions
- The lack of an adequate and consistent definition of the trait or disorder under study
- The questionable reliability and validity of the trait or disorder under study
- The use of non-blinded diagnoses
- The use of diagnoses that were made on the basis of inadequate information
- The use of unreliable methods of zygosity determination (whether a pair is MZ or DZ)
- That hospital psychiatrists might have given MZ twins similar diagnoses because they were influenced by their knowledge of the twins' common genetic heritage
- The unnecessary use of age-correction formulas
- The use of non-representative sample populations
- Small sample sizes
- The lack of an adequate description of the methods
- Investigator bias in favor of genetic conclusions

Still, despite these problems, there is little doubt that MZ pairs resemble each other more than same-sex DZ pairs for most behavioral traits and psychiatric disorders. However, we have seen that the decisive question is: *What factors explain this difference*? The answer to this question depends on the validity of the twin method's equal environment assumption. In previous publications I have argued in detail that the EEA is not valid, and that, like family studies, the twin method is unable to disentangle the potential roles of genetic and environmental influences (see Joseph, 1998, 2000, 2002, 2004, 2006).

There is overwhelming evidence that MZ twin pairs experience much more similar environments than DZ pairs (Joseph, 2004, 2006). Perhaps more important,

MZ pairs experience a much stronger psychological bond than DZs, and more often experience what Jackson (1960) characterized as ''identity confusion.'' In a 1967 Norwegian twin study, for example, schizophrenia twin researcher Einar Kringlen found that 90% of his MZ twins (N = 75 pairs) had experienced ''identity confusion'' in childhood, whereas only 10% of his same-sex DZ pairs (N = 42 pairs) had this experience (Kringlen, 1967, p. 115). Clearly, the greater environmental similarity experienced by MZ versus DZ twin pairs could completely explain the former's greater behavioral resemblance.

Contemporary twin researchers usually concede the point that MZ twin pairs experience more similar environments (see below), yet continue to uphold the validity of the twin method and the equal environment assumption (EEA) on the basis of two key arguments.

The *first argument* is that, although twin researchers recognize that MZ and DZ twin pair environments are in fact different, it is the responsibility of *critics* of the twin method to identify the ''trait-relevant'' environmental factors for which these two types of twins experience dissimilar environments. A group of prominent investigators provide an example of twin researchers placing the burden of proof onto critics: ''it would seem that the burden of proof rests with critics of the twin method to demonstrate that 'trait-relevant' environmental factors are more similar for identical than same-sex fraternal twins'' (Lyons, Kendler, Provet, & Tsuang, 1991, p. 126). Other examples of genetic researchers attempting to reverse the burden of proof from themselves to critics include Bouchard (1993b), and Faraone & Biederman (2000). By ''trait relevant,'' twin researchers mean aspects of the environment that have been shown to contribute to the trait in question. (For example, exposure to trauma contributes to post-traumatic stress disorder.)

However, as psychologist Scott Lilienfeld and his colleagues pointed out, in the context of separating science from pseudoscience,

> a basic tenet of science is that the burden of proof always falls squarely on the claimant, not the critic ... Consequently, it is up to the proponents of these techniques to demonstrate that they work, not up to the critics of these techniques to demonstrate the converse. (Lilienfeld, Lynn, & Lohr, 2003, p. 3)

At other times, twin researchers point to a body of evidence purporting to have tested and upheld the validity of the EEA (e.g., Kendler, 1983; for a critical review of the ''EEA test'' literature, see Joseph, 2006; Pam, Kemker, Ross, & Golden, 1996; Richardson, 1998). Moreover, although faced with a similar problem, twin researchers do not make the ''trait relevant'' argument when discussing potential environmental confounds in *family studies*. In this case they are willing to concede that, because family members share a common environment (''trait-relevant'' or not), one cannot draw valid conclusions in favor of genetic influences on the basis of the family resemblance of a trait.

The *second argument* twin researchers put forward in defense of the twin method is that MZ pairs tend to ''create'' more similar environments for themselves by

virtue of their greater genetically-caused behavioral resemblance. Therefore, according to this argument, the twin method's validity is based on determining why – not whether – MZs experience more similar environments than DZs. In a key 1983 article on twin studies in psychiatry, Kendler based much of his argument in support of the EEA on this position: "Although the similarity in environment might make MZ twins more similar," thereby invalidating the twin method, the genetically-caused "similarity in behavior of MZ twins might *create* for themselves more similar environments" (Kendler, 1983, p. 1416, italics in original). Following Kendler, ADHD twin researchers David Hay and colleagues wrote that, although MZ twins "may well be treated more similarly" than DZs, "this is far more a consequence of their genetic similarity in behaviour (and of ensuing responses by parents and others) than a cause of such similarity" (Hay, McStephen, & Levy, 2001, p. 12).

However, those who make this argument (including the authors of the five publications, see below) fail to understand that *the reason MZ pairs experience more similar environments than DZ pairs, be it environmental or genetic, is irrelevant in assessing the validity of the EEA*. For example, suppose that schizophrenia is caused solely by exposure to the chemical mercury. Because MZ pairs spend much more time together than DZ pairs, it is much more likely that both members of an MZ twin pair will be exposed to mercury, and subsequently be diagnosed with schizophrenia, than it is that both members of a DZ pair will be exposed and diagnosed. Let us further imagine that MZ twins are more genetically predisposed than DZs to enjoy spending time at the beach. Although MZ pairs may well show much higher concordance for skin cancer than DZs, this does not mean that skin cancer is a genetically-based disease.

On a psychological level, the theorized genetically-programmed behavioral resemblance of MZ pairs, and the "ensuing responses by parents and others," could create more similar abusive, abandoning, or traumatizing parental behavior that could lead to higher concordance for childhood or adult disorders such as, for example, anxiety, depression, or psychosis (Bentall, 2003; Read, Fink, Rudegeair, Felitti, & Whitfield, 2008; Read, Mosher, & Bentall, 2004). In this case it is not heredity, but rather abuse, abandonment, or trauma that plays a major role in causing psychiatric disorders.

Thus, even if MZ pairs do indeed "create" more similar environments for themselves than do DZ pairs on the basis of their greater genetic similarity, it would be erroneous to conclude that higher MZ versus DZ concordance for schizophrenia, skin cancer, depression, or anxiety constitutes evidence that these conditions have a genetic basis. In the first example – regardless of *why* MZ pairs are together more often – higher concordance for schizophrenia among MZ pairs is caused solely their greater likelihood of being similarly exposed to mercury than DZ pairs.

Moreover, the "twins create their own environment" position illogically implies that parents are able to change their behavior on the basis of their children's (twins') behavior, but that children do not change *their* behavior on the basis of their parents' behavior (Joseph, 1998).

Finally, proponents of the "twins create their own environment" position use circular reasoning, that is, they *assume* what they need to *demonstrate*. Moreover, their claim that twins' behavioral resemblance is caused by genetics *is based implicitly on the results of previous twin studies*. In other words, modern twin researchers circularly rely on the twin method to validate the twin method, and in the process they circularly *assume* that twins' behavioral resemblance is caused by genetics, in order to *conclude* that twins' behavioral resemblance is caused by genetics.

Thus, the only relevant question in assessing the validity of the twin method and the EEA is *whether* – not why – MZ twin pairs experience more similar environments than DZ pairs (Joseph, 2004).

The Five Publications on the Validity of the Equal Environment Assumption

I will elaborate on the above-stated arguments against the validity of the equal environment assumption (EEA) in the context of a critical analysis of the five publications mentioned earlier (Bouchard & McGue, 2003; Faraone et al., 1999; Kendler & Prescott, 2006; Plomin et al., 2008; Rutter, 2006). All of these authors have attempted to validate the EEA and twin method, and most have themselves conducted twin research. If the EEA were valid, we would expect these experts to present a convincing argument that this indeed is the case.

Agreement on the Importance of the EEA, and Agreement That MZs Experience More Similar Environments

The authors of the five publications are in agreement that the EEA is a critical theoretical assumption of the twin method. For example, Rutter (2006, p. 41) wrote that in order to infer that genetic influences explain MZ-DZ differences,

> it is necessary to rely on what has been called the "equal environments assumption" (EEA). In other words, one has to assume that the contrast between MZ and DZ pairs can be wholly attributable to genes because the environmental variation within MZ pairs should be much the same as within DZ pairs.

The authors also concede that the evidence shows that MZ twin pairs experience more similar environments than DZ pairs:

> MZ twins are more likely than DZ twins to share friends and parental treatment in adolescence. (Bouchard & McGue, 2003, p. 9)

> Several studies have found that the social environments of MZ twins are more similar than those of DZ twins. For example, habits, activities, personal preferences, parental treatment, and self-image tend to be more similar between MZ twins. Moreover, MZ twins are more likely to be dressed alike and are more likely to be confused for one another in childhood. (Faraone et al., 1999, p. 38)

> Consistent with other studies, we found evidence that some aspects of the environment of members of MZ pairs are, on average, more similar than those of members of DZ pairs. (Kendler & Prescott, 2006, p. 124)

> At first sight, [the EEA] seems a most implausible assumption. It is obvious, for example, that MZ twins are more likely to be dressed alike than are DZ twins. Also (for genetic reasons) MZ twins (within any pair) are more likely than DZ twins to be similar in their behavior, attitudes, and interests. It may safely be assumed that this is almost bound to lead to their choosing more similar experiences and also eliciting more similar patterns of interaction with other people. (Rutter, 2006, p. 41). [We will examine Rutter's insertion of the phrase ''for genetic reasons'' a bit later]

Plomin and colleagues (2008) did not state explicitly that MZ environments are more similar, but did allude to possible environmental differences between the two types of twins which they, like the others, believe are the result of genetics and therefore do not invalidate the twin method. In a stronger statement on the differing environments of MZ and DZ twin pairs, behavioral genetic twin researcher David Rowe wrote in 1994, ''the question is not whether MZ twins receive more similar treatments (they do, and to claim otherwise would be foolish), but whether these treatments influence a particular trait'' (Rowe, 1994, p. 45). And as early as 1979, twin researchers Sandra Scarr and Louise Carter-Saltzman (1979, p. 528) concluded, ''the evidence of greater environmental similarity for MZ than DZ twins is overwhelming.'' Indeed, it is.

Trait Relevant Definition of the EEA

While it is clear that MZ pairs experience more similar environments than same-sex DZs, all authors continued to uphold the validity of the twin method and the EEA. Four of the five publications did so on the basis of the ''trait relevant'' definition of the EEA:

> Twin studies of psychiatric disorders would . . . be in some trouble if MZ pairs had more similar environments than DZ pairs *and* if we could show that these environments altered risk for a particular psychiatric disorder. (Kendler & Prescott, 2006, p. 116, italics in original)

> Twin studies may overestimate heritability if [MZ vs. DZ] differences in environmental similarity are etiologically relevant to the disorder under study. (Faraone et al., 1999, p. 38)

> The EEA will not be violated if that [MZs eliciting more similar environments] is all that is occurring. That is because if the environments are being entirely driven by genes, it is reasonable to attribute the effects to genes provided, and only provided, that the environments that differ between MZ and DZ pairs do not have an effect on the trait being studied. (Rutter, 2006, pp. 41–42)

> Behavioral geneticists call this assumption the ''equal environmental similarity assumption,'' a term that is somewhat misleading in that the issue is not whether MZ twins experience more environmental similarity than DZ twins, but rather whether they are more likely to share trait-relevant features of their environments. (Bouchard & McGue, 2003, p. 9)

It is worth noting that until the mid-1960s, virtually all twin researchers defined the equal environment assumption as the *unqualified* assumption that MZ and same-sex DZ twin pairs experience roughly equal environments. This is the ''traditional definition'' of the equal environment assumption. Until that time, most researchers either denied that MZ and DZ environments differed, or simply ignored the issue. As late as 1966, the authors of a World Health Organization (WHO) assessment of twin research concluded that ''most shared post-natal experiences of MZ twins are probably not qualitatively different from those shared by DZ partners or even by sibs'' (World Health Organization, 1966, p. 115). As Kendler (1983, pp. 1413–1414) described the definition of the EEA during the first four decades of the twin method's existence, ''According to the traditional view, because monozygotic and same-sex dizygotic twins share environmental factors to approximately the same extent, differences in concordance between the two twin types must be due to the influence of genetic factors.''

However, by the mid-1960s twin researchers were faced with the growing evidence of MZ-DZ environmental differences, with Jackson's irrefutable (1960) critique, and with a good dose of common sense. Many began to realize that the 40-year-old critical theoretical assumption of the twin method, as it had been defined until then, *was false*. As one of many examples, in 1963 veteran Swedish psychiatric genetic twin researcher Erik Essen-Möller wrote as follows:

> Quite obviously, then, the logical evidence furnished by the classical twin method is not unambiguous, as originally believed. A greater concordance in monozygotics must not invariably depend on their genetic identity, since also their environment may have been more similar (Essen-Möller, 1963, p. 69; for many more examples, see Joseph, 2004, pp. 171–175).

One could argue that twin researchers and others should have relegated the twin method to a place alongside the discarded pseudosciences of bygone eras. Or at minimum, they could have concluded that both family studies *and* the twin method were hopelessly confounded by environmental factors, and that results from these studies proved nothing about genetics. One highly regarded psychiatric genetic researcher did indeed appear to move toward this position near the end of his career. In 1979, David Rosenthal (1979, p. 25) concluded that both family studies and the twin method are ''confounded,'' and that ''one can draw conclusions about them only at considerable risk.''

But the twin method lived on. What happened was that twin researchers began to subtly redefine the EEA away from the traditional definition to the new ''trait-relevant'' configuration. One of the first examples of this shift is found in a 1966 publication by twin researchers Irving Gottesman and James Shields, who wrote that the twin method would indeed have problems if the ''environments of MZ twins are systematically more alike than those of DZ twins in features which can be *shown* to be of etiological significance in schizophrenia'' (Gottesman & Shields, 1966, pp. 4–5, italics in original). Unfortunately, hardly anyone noticed or challenged this critical change in definition, which constituted an *ad hoc* hypothesis used to plug a gaping hole in twin method theory.

The trait relevant condition, however, means that the twin method and family studies *have precisely the same problem*, since both are subject to unavoidable environmental confounds. Yet twin researchers and popularizers of their work approach family studies and twin studies as if they were completely different animals. The logical fallacy is that the arguments they put forward in support of inferring a role for genetic factors from ''trait-relevant'' twin studies could just as easily be made in support of inferring a role for genetic factors from the creation of ''trait-relevant family studies.'' From the standpoint of environmental confounds, family studies and the twin method are not different animals. They are the same animal.

EEA Test Literature

Most of the authors argued that, although MZ twin pairs do indeed experience more similar environments than DZ pairs, the ''EEA Test'' research suggests that this does not constitute a major environmental bias in twin studies. This was Kendler and Prescott's (2006) main defense of the EEA, which led them to conclude that the twin method derived heritability estimates of psychiatric disorders they presented in their book ''are substantially correct'' (p. 125). Further references to the EEA test literature include:

> There have been various attempts to look for possible violations of the EEA with respect to twin studies of schizophrenia and other major mental disorders, with the conclusion that the EEA is not violated. (Rutter, 2006, p. 44)

> The equal environments assumption has been tested in several ways and appears reasonable for most traits. (Plomin et al., 2008, p. 79)

> Tests of the equal environmental similarity assumption have repeatedly shown that it is valid in most instances. (Bouchard & McGue, 2003, p. 9)

Interestingly, several EEA test studies (e.g., Borkenau, Riemann, Angleitner, & Spinath, 2002; Kaprio, Koskenvuo, & Rose, 1990; Kendler & Gardner, 1998;

LaBuda, Svikis, & Pickens, 1997; Lytton, 1977; Morris-Yates, Andrews, Howie, & Henderson, 1990; Scarr, 1968; Scarr & Carter-Saltzman, 1979) found that MZ pairs experience more similar environments than DZ pairs. The authors of these studies usually argue, however, that the greater environmental similarity of MZ pairs does not contribute to their greater behavioral resemblance, or if it does, that MZ's greater behavioral resemblance is caused by their greater genetic similarity.

This follows Kendler's (1983) position that "the behavioral similarity of monozygotic versus dizygotic twins cannot be ascribed to differences in treatment of the twins by the social environment" (p. 1416). This position is unsustainable because, among other reasons, it *must generalize to mean that no one's behavior (whether twins or non-twins) is influenced by his or her social environment* (Joseph, 2006). Individual twins, like individual non-twins (singletons), are human beings who receive treatment in their social, cultural, and familial environments. Yet Kendler argued that the social environments experienced by twins *as individuals* do not influence their behavior. It must therefore follow, for Kendler's EEA theory to hold, that *no-one's* behavior is influenced by his or her social, cultural, or familial environment.

Thus, the widely recognized greater environmental similarity of MZ versus DZ twin pairs invalidates the twin method on its face. The twin method, therefore, is contaminated by environmental factors regardless of what EEA-test researchers have claimed. What they must demonstrate – without qualification – is that MZ and DZ pairs experience roughly equal environments.

Twins Creating Their Own Environments

As we have seen, twin researchers have defended the twin method on the grounds that MZ twin pairs create (elicit) more similar environments for themselves on the basis of their more similar behavioral characteristics, which twin researchers attribute to their greater genetic similarity. In the past, twin researchers such as Kendler (1983), Scarr (1968), Shields, (1954), and Zerbin-Rüdin (1972) defended the twin method and the equal environment assumption on this basis. In fact, much of Kendler's earlier defense of the EEA was based on his position that "the similar phenotypes in monozygotic twins are caused by their genetic similarity" (Kendler, 1983, p. 1414; reaffirmed as recently as Kendler, 2000), and that MZ twins create more similar environments for themselves on the basis of their greater genetic similarity.

However, we have already seen that the "twins create their own environment" argument does not hold up: (a) because even if it were true, it does little to support the EEA; (b) because it illogically implies that parents – but not twins – are able to change their behavior on the basis of others' behavior; and (c) because twin researchers circularly assume that twins' behavioral resemblance is genetic in order to conclude the very same thing. Thus, Kendler and other twin researchers simultaneously *assume and conclude* that "the similar phenotypes in monozygotic twins are caused by their genetic similarity."

Thus, twin researchers' interpretations of MZ-DZ correlational or concordance rate differences as supporting a role for genetics is tautological. They argue, in essence, that the twin method is valid because . . . the twin method was previously shown to be valid. Furthermore, they seem to argue that the EEA is valid (a) if MZ and DZ pairs experience equal environments, *or* (b) if MZ and DZ pairs experience far different environments. This "heads I win, tails you lose" argument has little scientific validity.

It is worth noting that Kendler and Prescott (2006) appear to have abandoned the "twins create their own environments" argument, upon which Kendler had earlier (1983) placed so much importance. Others, such as Rutter, claimed that "(for genetic reasons) MZ twins (within any pair) are more likely than DZ twins to be similar in their behavior, attitudes, and interest" (2006, p. 41). But why add the phrase "for genetic reasons"? Rutter merely proclaimed this to be the case (again, implicitly basing his argument on the results of previous twin studies), and then concluded, "the EEA will not be violated if that is all that is occurring" (pp. 41–42). In fact, as we saw earlier in the hypothetical example of schizophrenia being caused by mercury, and in the real examples of skin cancer being caused by sunbathing, and depression, anxiety and psychosis being caused by trauma and abuse, the EEA *will* be violated even if this is occurring.

According to Plomin et al. (2008, p. 79), "some experiences may be driven genetically. Such differences between identical and fraternal twins in experience are not a violation of the equal environments assumption because the differences are not caused environmentally." And Bouchard and colleagues had earlier argued,

> Adult MZ twins . . . tend to remain in closer contact than DZ twins or other siblings, but we believe that this additional contact does not "cause" them to become more alike. We suggest instead, as the most plausible hypothesis, that MZs especially enjoy each other's company because they are so [genetically] similar in personality, interests, and attitudes. (Lykken, McGue, Bouchard, & Tellegen, 1990, p. 560)

For the reasons I have already outlined, circular arguments of this type do little to uphold the validity of the equal environment assumption.

Equalizing MZ and DZ Environments Through Rhetoric

Several authors implied that there are environmental aspects of the MZ twinship that might make such pairs *differ* from one another. Kendler (1983) referred to this as a possible "reverse bias" in schizophrenia twin research. While biases of this type may well exist, the message the authors convey is that similarity biases and differentiating biases might cancel each other out. An earlier attempt to create such a rhetorical balance is found in a publication by Gottesman and Shields (1966, p. 55),

who implied that ''the same proportion of potential schizophrenics are held back from overt illness by identifying with a normal twin as those who became ill by identifying with a normal one.''

We find similar unsubstantiated claims in three of the five publications. Apart from discussions of prenatal and obstetric factors, the authors provide no citations in support of their claims:

> The greater physical and environmental similarity of MZ twins may actually lead to a decrease in behavioral similarity. (Faraone et al., 1999, p. 38)

> A second possible threat to the EEA is provided by circumstances in which the experiences of MZ twins within the same pair tend to be *less* alike than those of DZ twins. When this is the case the violation of the EEA will lead to a misleading *under*estimate of genetic effects if the environmental influences have effects on the trait or disorder being studied. The main circumstance in which this could be the case concerns obstetric factors. (Rutter, 2006, p. 42; italics in original)

> Prenatally, identical twins may experience greater environmental *differences* than fraternal twins ... To the extent that identical twins experience less similar environments, the twin method will underestimate heritability. (Plomin et al., 2008, p. 79; italics in original)

Again, while it is possible that ''reverse biases'' in twin research exist, the authors provide no reason to reject the idea that obvious biases in the direction of creating more similar twin pairs are massively larger.

Global or Trait-Specific Acceptance or Rejection of the EEA?

The authors of three of the five publications maintained that the acceptance or rejection of the equal environment assumption is not a global evaluation, but that the EEA must be tested on a trait-by-trait basis:

> There is no such thing as a ''generic'' violation of the EEA. Potential violations of the EEA must be evaluated disorder by disorder. (Kendler & Prescott, 2006, p. 117)

> Whether or not the EEA is, or is not, violated will vary by traits. There cannot be any general conclusions on the EEA. (Rutter, 2006, p. 43)

> Good scientific practice ... requires that the [EEA] be repeatedly tested for each trait under investigation and particular findings that depend on the assumption be replicated in designs that do not make the assumption. (Bouchard & McGue, 2003, p. 9)

Once again, the authors imply a qualitative distinction between family studies and twin studies, when no such distinction is warranted. I am unaware of any

behavior genetic or psychiatric genetic researcher arguing in support of testing for environmental confounds in *family studies*, or that family studies should be tested ''disorder by disorder'' for such confounds. On the contrary, we have seen that a simple understanding that families experience common environmental influences is sufficient for these researchers to indeed reach the ''general conclusion'' that family studies are hopelessly confounded by environmental factors. Their argument in support of the twin method appears to be based on the logical fallacy of ''special pleading,'' which refers to the application of standards, principles, and rules to others while claiming to be exempt, without providing adequate justification for the exemption.

Do Other Types of Studies Validate the Twin Method?

Faraone and colleagues emphasized several times that twin method results, by themselves, prove little about genetics: ''Any conclusion about the role of genes and environment must rely not on a single study or class of study but on the converging evidence provided by a variety of research paradigms'' (Faraone et al., 1999, p. 43; the entire sentence was italicized in the original). Elsewhere they wrote that a ''key point'' in psychiatric genetic research is, ''No one study proves or disproves anything. Scientists require a pattern of converging evidence from multiple studies before they can reasonably conclude that genes play a role in causing the disease'' (Faraone et al., 1999, p. 12).

According to Bouchard and McGue (2003, p. 10), ''inferences about the nature and existence of genetic and environmental influences on individual differences in behavior do not rest solely with twin studies.'' Rutter, while recognizing the ''limitations'' of twin research (2006, p. 59), argued that the ''overall pattern'' of behavior genetic findings ''demands the acceptance of the importance of genetic influences'' (p. 60). These researchers echoed one of Gottesman's earlier positions, where he wrote that although schizophrenia family, twin, and adoption studies each ''contribute to the genetic argument . . . No one method alone yields conclusive proof or disproof'' (Gottesman, 1991, p. 93).

Lilienfeld et al. (2003) addressed this ''holism'' argument, pointing out that pseudoscience proponents ''typically maintain that scientific claims can be evaluated only within the context of broader claims and therefore cannot be judged in isolation'' (p. 9). An example they gave was the response of proponents of the Rorschach Inkblot Test to their critics. Supporters of the Rorschach sometimes caution that its results should not be interpreted in isolation, but instead should be considered along with other information obtained in a psychological evaluation. For Lilienfeld et al., this means that ''proponents of the Rorschach and other techniques can readily avoid subjecting their claims to the risk of falsification.'' This allows them to protect their techniques through the ''heads I win, tails you lose''

(p. 9) position, whereby proponents can point to positive research in support of the technique, while dismissing negative findings on the grounds that the technique should never be judged in isolation.

Clearly, while recognizing the ''limitations'' of the twin method, the twin researchers cited above have helped immunize the method from criticism by attempting to validate it through the results provided by the ''converging evidence'' allegedly supplied by other types of studies. Their error lies in this: If the EEA and other assumptions were truly sound, the twin method – standing alone – would indeed provide conclusive evidence in favor of genetics.

Would the Falseness of the EEA Invalidate the Twin Method, or Merely Lead to an ''Overestimation of the Genetic Effects?''

In a 1993 article on psychiatric twin studies, Kendler wrote, ''The EEA is crucial because, if the EEA is incorrect, excess resemblance of MZ twins compared with DZ twins ascribed to genetic factors could be partly *or entirely* due to environmental effects'' (Kendler, 1993, p. 906, italics added). Kendler's accurate assessment summarizes the point that critics of the twin method have made for over three generations. The main difference between Kendler and the critics is that Kendler affirms the validity of the EEA, whereas critics have argued the opposite position.

By 2006, however, Kendler would allow only that ''Failure of the assumptions of the twin model can lead to incorrect genetic and environmental estimates'' (Kendler & Prescott, 2006, p. 114). The authors of three of the four other publications made similar arguments:

> MZ twins are more likely to be dressed alike and are more likely to be confused for one another in childhood. Thus, twin studies may overestimate heritability if these differences in environmental similarity are etiologically relevant to the disorder under study. (Faraone et al., 1999, p. 38)

> With respect to these traits, some of the difference in similarity between MZ and DZ pairs will be due to environmental influences. This means that, to a degree, [the] EEA is violated . . . it will mean that the standard way of measuring heritability will tend to overestimate the genetic effect. (Rutter, 2006, p. 43)

> If the assumption [EEA] were violated because identical twins experience more similar environmental than fraternal twins, this violation would inflate estimates of genetic influence. (Plomin et al., 2008, p. 79)

It appears that twin researchers have further immunized the twin method from falsification in the sense that, as opposed to invalidating genetic interpretations of twin method results in general, they have transformed a finding that the EEA is invalid to indicate only a degree of error that leads to an overestimation – rather than a negation – of genetic effects.

The Failure to Address the Specific Arguments of EEA Critics

Another problem found in the five publications, and indeed in the behavioral genetic and psychiatric genetic literature in general, is the frequent failure to address the specific arguments of critics of the twin method. Although genetically-oriented authors sometimes mention unnamed ''critics,'' they rarely quote them or take their specific objections seriously. In their discussions of twin method theory and practice, Kendler and Prescott, Faraone et al., and Plomin et al. did not even mention that critics exist, even though there have been many such critics (for example, Bleuler, 1978; Boyle, 1990, 2002; Breggin, 1991; Charney, 2008; Hoffman, 1985, 1991; Jackson, 1960; Joseph, 1998, 2000, 2002, 2004, 2006; Kamin, 1974; Kamin, in Eysenck vs. Kamin, 1981; Laing, 1981; Lewontin, Rose, & Kamin, 1984; Marshall, 1990; Neel & Schull, 1954; Pam, 1995; Pam et al., 1996; Phillips, 1993; Richardson, 1998;Richardson & Norgate, 2005; Rosenthal, 1960, 1961, 1962a, 1962b). Only Rutter (2006) referenced a critic who pointed to post-natal environmental influences that might lead MZ twins to resemble each other more than DZs.

A Failure to Recognize the Unique Psychological Bond of MZ Twin Pairs

Twin researchers assess the childhood environments of twins by asking questions such as ''Did you share the same room,'' ''Did you dress alike,'' and ''Did you and your twin have the same friends'' (Kendler & Prescott, 2006, p. 117). What they tend to overlook is that the MZ twinship is a unique human relationship, which involves an extreme level of closeness, mutual association, and difficulty in maintaining a separate identity from one's co-twin. ''Identity formation,'' wrote Ricardo Ainslie (1985, p. 50) in his book on twinship, ''is often considered the cornerstone of any discussion of the psychology of twinship. The idea that twins encounter difficulties in the process of identity formation is as pervasive in scientific writings on twinship as it is in popular culture.''

Although the problem of identity formation might not greatly impact twins' correlations for traits such as IQ, we could expect it to have a major impact on twin personality correlations, and on twin concordance rates for psychotic disorders such as schizophrenia (Jackson, 1960; Kringlen, 1967).

Conclusion

The twin method has supplied the most frequently cited evidence in support of important genetic influences on psychiatric disorders and psychological trait variation. The results of these studies have been put forward, largely uncritically, in countless textbooks and popular works, in review articles, and in the media.

However, the evidence suggests that, like family studies, the twin method is unable to disentangle the possible roles of genes and environment. There is, in fact,

little reason to accept that the twin method has measured anything other than the more similar treatment, greater environmental similarity, and closer psychological association experienced by MZ versus same-sex DZ pairs. Qualifications regarding ''trait-relevance'' or twins ''creating their own environments,'' or other arguments put forward by the authors of the five publications, do little to alter this conclusion.

Studies of Reared-Apart Twins

The past few decades have seen a great deal of attention paid to studies of reared-apart twins. The intuitive appeal of these studies is understandable, since studying twins separated at birth and reared apart in different families would appear to overcome the problems of environmental confounds in the twin method. Yet, we will see that these studies are also subject to environmental confounds and other biases.

Twins reared-apart (known as ''TRA'') studies compare the psychological trait resemblance of reared-apart MZ pairs (known as ''MZAs'') to the resemblance of reared-together MZs (known as ''MZTs''), the latter serving as a control group. Some studies have included a group of reared-apart DZ pairs (''DZAs''). TRA researchers usually conclude that, because MZA correlations are far greater than zero and are comparable to MZT correlations, their results support important genetic influences on psychological trait differences. Others have cited the results of TRA studies in support of the validity of the twin method (e.g., Alford, Funk, & Hibbing, 2005), and in support of the claim that family environment has a negligible influence on human psychological development (e.g., Harris, 1998; Rowe, 1994).

Although many anecdotal reports of individual MZA pairs have been published since the 1920s, through 1979 there had been only three systematic studies of MZAs: Newman et al. (1937), who studied 19 pairs (and used MZTs and DZTs as controls); Shields (1962), who studied 44 pairs (and used MZTs and DZTs as controls); and Juel-Nielsen (1965/1980), who studied 12 MZA pairs (and other types of twins). Newman et al. reported MZA IQ correlations of MZA .67, and .91 MZT, and personality score correlations MZA .58, and MZT .56. Shields reported IQ correlations of .77 for MZAs, and .76 for MZTs. For personality, he reported MZA and MZT ''Neuroticism'' correlations of .53 and .38 respectively, and .61 and .42 for ''Extraversion.'' Juel-Nielsen reported an MZA IQ correlation of .62, but did not calculate total sample personality test correlations. The MZA IQ correlations reported by Cyril Burt (1966) are largely discredited due to allegations of fraud or unreliability, and the data he reported are no longer accepted in the scientific literature (Bouchard & McGue, 1981; Hearnshaw, 1979; Kamin, 1974).

The Minnesota Study of Twins Reared Apart (MISTRA) was initiated in 1979 by Thomas J. Bouchard, Jr. and his colleagues at the University of Minnesota. The study concluded in 2000. Since 1988 (Tellegen et al., 1988), the MISTRA group has produced numerous publications reporting test score correlations for MZAs,

MZTs, DZAs, and other types of twins. Two other TRA studies began reporting data in the 1980s – a study from Finland (Langinvainio, Kaprio, Koskenvuo, & Lönnqvist, 1984), and the Swedish Adoption/Twin Study on Aging (SATSA; Pedersen, Plomin, McClearn, & Friberg, 1988).

According to the MISTRA researchers, in their widely cited 1990 *Science* publication (Bouchard et al., 1990), TRA studies "provide the simplest and most powerful method for disentangling the influence of environmental and genetic factors on human characteristics" (p. 223). The researchers reported MZA and MZT IQ correlations of .69 and .88 respectively, and personality correlations of .48 and .49 (see Figure 18.4). They concluded that, because "monozygotic twins reared apart are about as similar as are monozygotic twins reared together," their findings supported "the strong heritability of most psychological traits" (p. 223). The MISTRA results have been popularized and promoted in many popular works (e.g., Harris, 1998; Pinker, 2002; Ridley, 2003; L. Wright, 1997; W. Wright, 1998).

MZA Group Correlation vs. MZT Group Correlation

	MINNESOTA MZAs		MZTs	
	R	N	R	N
IQ Tests				
WAIS–Full	.69	48	.88	40
Raven, Mill Hill	.78	42	.76	37
Personality Tests				
MPQ	.50	44	.49	217
CPI	.48	38	.49	99

Notes : MZA = monozygotic twins reared-apart. MZT = monozygotic twins reared-together. N = number of twin pairs. R = interclass correlation. WAIS = Wechsler Adult Intelligence Scale. MPQ = Multidimensional Personality Questionnaire. CPI = California Personality Inventory. MZAs and MZTs were the only twin types reported by Bouchard et al. in this publication.

Source : Based on descriptions and results reported in Bouchard et al., 1990.

Figure 18.4. The Minnesota Study of Twins Reared Apart (MISTRA) model and selected IQ and personality test results: The 1990 *Science* publication.

Critique of TRA Studies

Critics have pointed to several key methodological problems with TRA studies (see Farber, 1981; Joseph, 2001b, 2004; Kamin, 1974; Kamin & Goldberger, 2002; Lewontin et al., 1984; Taylor, 1980). These include: (a) it is doubtful that most studied MZAs deserve the status of having been ''reared-apart,'' since most pairs had significant contact with each other for many years; (b) in several studies, there were biases favoring the recruitment of MZA pairs who resembled each other more for behavioral traits than MZA pairs as a population; (c) the Minnesota researchers failed to publish life history information for the twins under study, and then denied independent reviewers access to raw data and other unpublished information (Joseph, 2004); (d) there is controversy about whether ''intelligence'' and ''personality'' are valid and quantifiable constructs; and (e) the impact that the researchers' bias in favor of genetic explanations may have had on their results and conclusions.

Similarity bias Kamin (1974) showed that the Newman et al. and Shields TRA (twins reared-apart) studies recruited twins on the basis of similarity and of their pre-existing knowledge of each other, which meant that these MZA samples were biased toward similarity. Susan Farber, author of the exhaustive *Identical Twins Reared Apart: A Reanalysis* (Farber, 1981), found that, due to ascertainment bias, ''approximately 90 percent of the known cases of separated MZ twins have been studied precisely because they were so alike'' (p. 36). Therefore, according to Farber, the original researchers' conclusions that genetic factors explain MZA resemblance were based on ''circular reasoning'' (p. 36).

Common environmental factors shared by MZAs Although rarely mentioned in popular accounts of TRA research, there are many *environmental* (non-genetic) factors shared by MZA pairs that would lead them to resemble each more than pairs of randomly selected members of the world's population. We would expect a sample of the latter, of course, to correlate near zero for psychological traits on either environmental or genetic grounds. Non-genetic (in part non-familial) influences experienced by MZA pairs are seen in Box 18.1.

Thus, the fact that MZA pairs share the common factors seen in Box 18.1 would lead to us to expect them to correlate *well above zero* for most psychological traits. As MISTRA researchers McGue and Bouchard have recognized, age and sex effects alone can have a ''substantial'' impact on MZA IQ and personality correlations (McGue & Bouchard, 1984, p. 325).

The myth of the separated twins Following up on the final bulleted point in Box 18.1, it is a *myth* that TRA researchers studied twins whom we can legitimately regard as having been ''reared-apart'' (Joseph, 2004; Taylor, 1980). In her analysis of the TRA

Box 18.1. Environmental (in part non-familial) influences shared by reared-apart monozygotic twin pairs (MZAs).

- They are exactly the same age (birth cohort)
- They are the same sex
- They are almost always the same ethnicity
- Their appearance is strikingly similar (which will elicit more similar treatment)
- They usually are raised in the same socioeconomic class
- They usually are raised in the same culture
- They shared the same prenatal environment
- Most studied pairs spent a certain amount of time together in the same family environment, were aware of each other's existence when studied, and often had regular contact over long periods of time

Source: Adapted from Joseph, 2004, p. 126.

literature published until 1980, Farber found that only 3 of the 121 reported MZA pairs were separated during the first year of life, were reared with no knowledge that they had a twin, and were studied at the time of their first meeting. ''Of the 121 cases reported in the last fifty years,'' she wrote, ''only three are 'twins reared apart' in the classical sense'' (Farber, 1981, p. 60). The ''most accurate description of this sample,'' she concluded, is ''MZ twins *partially* reared apart'' (p. 273, italics added). And there is every reason to believe that the ''separation'' of Bouchard and colleagues' Minnesota MZA pairs followed a similar pattern (Joseph, 2004).

In the Swedish study (SATSA), the investigators classified twin pairs as having been ''reared apart'' if they had been separated before age *11* (Pedersen, Plomin, Nesselroade, & McClearn, 1992, p. 347). The average SATSA age at separation was 2.8 years (Pedersen, McClearn, Plomin, & Nesselroade, 1992, p. 257), and about 75% had some degree of contact after separation. According to the SATSA data, MZAs (average age: 65.6 years) were ''separated'' for an average of only 10.9 years at the time of testing (Pedersen, Plomin, et al., 1992, p. 347; at least one pair had less than one year of separation). Twins supplied information by mail, and many were not investigated personally. In the Finnish study, 12 of the 30 MZA pairs were separated after the age of five (the mean age at separation for the entire MZA sample was 4.3 years), and the degree of post-separation contact was not stated (Langinvainio, Koskenvuo, Kaprio, & Sistonen, 1984).

Cohort effects It is critically important to understand that the behavioral resemblance of reared-apart MZ twin pairs is influenced by *cohort effects*, which account for similarities in people's behaviors and preferences that arise from the characteristics of the historical periods and cultural milieu in which they experience stages of life at the same time. In other words, we would expect two genetically-unrelated people of the same gender, who are born at the same time, to resemble each other more for psychological traits and behaviors than would two randomly selected members of the population.

Thus, for reasons having nothing to do with heredity, we should expect to find a much higher "video game playing behavior" correlation in the United States among pairs of randomly selected genetically-unrelated 15-year-old boys than we would expect to find among randomly selected pairs drawn from the entire 15–100-year-old male and female population of the United States. This example illustrates the central fallacy of TRA studies.

As Farber (1981, p. 77) astutely observed, MZAs are "not so much similar to each other as they are similar to people of their eras and SES [socioeconomic statuses]." According to behavior geneticist Richard Rose,

> A colleague suggests that we cannot know [the importance of MZA resemblance] without necessary control data on similarities found in pairs of age-matched strangers . . . Were one to capitalize on cohort effects by sampling unrelated but age-matched pairs, born, say, over a half-century period, the observed similarities in interests, habits, and attitudes might, indeed, be "astonishing." (Rose, 1982, p. 960)

In most cases, MZAs share at least seven different cultural influences with MZTs: national, regional, ethnic, religious, economic class, birth cohort, and gender cohort. As Rose suggested, comparing MZAs to "age-matched strangers" might reveal "astonishing" resemblances accounted for not by common genes, but by common cultural influences.

For example, a 1981 study on the relationship between age effects and personality, among biologically unrelated people, found that the mean correlation between age and California Psychological Inventory (CPI) scale scores was .28 across all 18 scales, with 10 scales showing a correlation of .35 or higher (Martin, Blair, Dannenmaier, Jones, & Asako, 1981; the CPI purports to measure normal aspects of personality, called "folk concepts," and was used to assess personality in the MISTRA studies). If these findings reflect age effects in the general population, the influence of common age, which represents only one of many environmental similarities shared by MZAs (see Box 18.1), accounts for more than half of the reported MZA personality correlations.

One could therefore conclude that, in addition to methodological issues and biases, a critical common flaw in the twins reared-apart (TRA) studies published to date is that the investigators mistakenly compared reared-apart MZ twin pairs

(MZAs) to *reared-together* MZ pairs (MZTs), thereby failing to control for the fact that both MZAs and MZTs share several important *non-familial* environmental influences. (The MISTRA researchers (McGue & Bouchard, 1984) attempted to correct MZA correlations for age and sex effects, but these adjustments were inadequate (see Joseph, 2004).)

Potential testing bias Another issue is that researchers do not directly compare twins' psychological test scores to each other. Rather, they compare each twin's total score with a norm group established by the test developer, after which twins' scores versus the norm group are compared to each other. Theoretically, members of a twin pair could answer very differently on individual questions, or perform differently on individual tasks, yet their *total* scores could appear to be "highly correlated." If both twins endorse a similar *number* of items on a personality scale, it does not necessarily mean that they endorsed the *same* items. As personality researcher Paul Kline (1993) noted in relation to "empirically keyed" personality tests such as the MMPI and the CPI, "if two subjects have the same score on the scale, the scores are not necessarily psychologically equivalent" (p. 129). Thus, it is possible for two people (e.g., MZAs) to answer many questions on a personality test differently, yet still be recorded as having highly correlated test scores.

What a valid TRA study might look like In Figure 18.5, I have outlined what a scientifically acceptable TRA study might look like. Such a study would compare the psychological trait resemblance of a group consisting of MZAs unknown to each other and reared apart from birth, versus the resemblance of a control group consisting not of MZTs, but, as Rose suggested, of *biologically unrelated pairs of strangers* sharing the non-genetic characteristics and influences also shared by MZAs. Thus, they should be the same age, they should be the same sex, they should share the same ethnicity, they should have been raised in comparable cultural and socioeconomic conditions, and they should be similar in appearance. Moreover, they should have no contact with each other until after the completion of evaluation and testing.

After concluding such a study, we might find that the biologically unrelated pairs correlate similarly to MZAs on psychological traits, which would suggest that MZA correlations are mainly, if not entirely, the result of environmental influences. Unfortunately, to my knowledge no study of this type has ever been attempted.

The Five Publications on TRA Studies

Bouchard and McGue (2003) did not discuss TRA study methodological issues, although Bouchard had previously defended his studies in several publications (see Bouchard, 1987, 1993a, 1997b). Faraone et al. (1999) did not address TRA studies, but Faraone and Tsuang (1995, p. 51) had written previously, "Since MZ twins

Reared-Apart MZ Pairs (MZAs)

- Share 100% genetic similarity
- Reared apart from birth
- Unbiased ascertainment methods used
- Unknown to each other
- Can have no contact with each other until after the completion of testing and assessment
- Raw MZA data to be stored in a central registry and made available for inspection to qualified researchers (Farber, 1981)

vs.

Biologically Unrelated Pairs

- Genetically unrelated
- Selected from the general population
- Unknown to each other
- Matched with MZAs on the basis of common sex, common age, common appearance, common culture, common ethnicity, and common socioeconomic status (see Box 18.1)
- Can have no contact with each other until after the completion of testing and assessment

Method

Compare the IQ and personality resemblance of the MZA pairs versus the resemblance of the Biologically Unrelated pairs

Genetic theory predicts that the MZA group will resemble each other more than the Biologically Unrelated group

Environmental theories predict that there will be little or no difference between the MZA and Biologically Unrelated groups

Figure 18.5. What a valid twins reared apart (TRA) study might look like.

reared apart do not share a common environment, any phenotypic similarity must be due to genetic factors. We cannot invoke shared environment as a cause of phenotypic concordance.'' In a similar manner, Plomin et al. (2008, p. 383) wrote, ''Because MZ twins reared apart are genetically identical but do not share any environmental influences, the correlation directly estimates heritability.'' We have seen, however, that MZA pairs do indeed share many important environmental influences. Behavior geneticists' denial of obvious environmental influences in TRA studies is paralleled by their denial that the twin method in contaminated by environmental factors.

Rutter (2006, pp. 52–53) took a more critical approach toward TRA studies. He wrote that the ''basic idea'' of these studies ''is a good one,'' and that ''At first sight, the inference that these similarities [of MZAs] reflect genetic influences seems incontrovertible. However, the design has rather more problems than are usually acknowledged.'' He noted that it is very unusual to separate twins at birth, and that published reports indicate that ''actual separation took place at a rather late age, leaving open the possibility of shared rearing influences.'' Rutter made reference to

previous critics' point about the similarity bias of the samples: ''There are inevitable question marks over the influences that led separated twins to volunteer to participate in the research.'' He also agreed with previous critics that MZA pairs were often placed in ''somewhat similar'' homes, and raised questions about the ''rather limited reporting so far of quantitative findings presented in scientific papers that have been subject to peer review.'' Rutter concluded that, although he believed that TRA studies lend support to genetic theories of behavioral differences, ''there are too many queries for it to be reasonable to place great reliance on the findings.''

Conclusion

The results of TRA studies have been widely cited in scientific and popular works as supplying definitive evidence that personality traits and cognitive ability are strongly influenced by genetic factors. However, these studies contain important problems, which include the questionable ''separation'' of twins, the similarity bias of the samples, the failure to publish or share raw data and life history information for the twins under study (MISTRA), and the researchers' bias in favor of genetic explanations. Finally, both TRA researchers and the popularizers of their work have failed to recognize that reared-apart MZ twin pairs share several important environmental influences that researchers did not control for.

Thus, for environmental reasons alone, we would expect MZA pairs to correlate well above zero on IQ and personality measures. It follows that we can draw no valid conclusions in support of genetic influences on psychological trait variation from TRA studies published to date (see Joseph 2001b, 2004). As Gottesman (1982, p. 351) concluded long ago, ''After a quarter century of experience with twins reared together and twins reared apart, it is my conviction that twins reared apart are a wonderful source of hypothesis generation, but not a useful source for hypothesis testing.''

Adoption Studies

Background

The decision to perform psychiatric adoption studies was based on their authors' conviction that both family studies and the twin method are potentially confounded by environmental factors. In the opinion of schizophrenia adoption researchers Seymour Kety, David Rosenthal, and their colleagues, the evidence from family and twin studies is ''inconclusive . . . in that it fails to remove the influence of certain environmental factors . . . In the case of monozygotic twins it

has been pointed out that such individuals usually share a disproportionate segment of environmental and interpersonal factors in addition to their genetic identity'' (Kety, Rosenthal, Wender, & Schulsinger, 1968, p. 345). They believed that adoption studies would finally provide a means of clearly disentangling environmental and genetic influences on schizophrenia. Adoption studies have also been used to assess the role of genes and environment for general intelligence (presumably measured by standardized IQ tests), and personality traits.

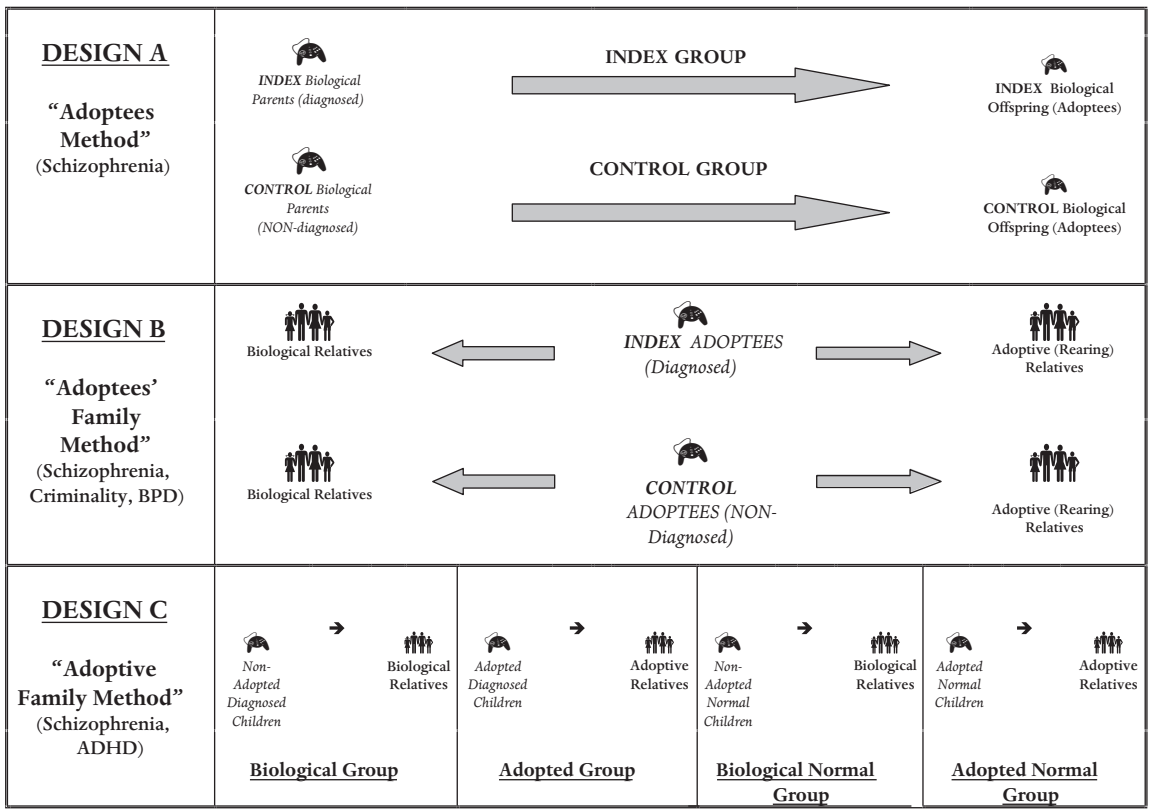
The underlying principle of an adoption study is its assumed ability to make a clean separation of genetic and environmental influences, since adoptees inherit the genes of their biological (birth) parents, but are reared in the environment of another (adoptive) family with whom they share no genetic relationship. After briefly describing how these studies are performed, however, I will attempt to show that adoption studies, like family and twin studies, are subject to major biases and environmental confounds.

The goal of the earliest adoption studies was to assess the role of genetic and environmental influences on IQ (e.g., Burks, 1928; Leahy, 1935; Skodak & Skeels, 1949). These were followed a few decades later by a new group of IQ studies, which included the Minnesota Adoption Study (MAS; Scarr & Weinberg, 1978), the Texas Adoption Project (TAP; Horn, Loehlin, & Willerman, 1979; Loehlin, Horn, & Willerman, 1989), and the Colorado Adoption Project (CAP; Plomin & DeFries, 1985; Plomin, Fulker, Corley, & DeFries, 1997).

In the 1960s, psychiatric genetic researchers extended the adoption study method to assess the role of genetic influences on psychiatric disorders. The first studies looked at schizophrenia (Heston, 1966; Kety et al., 1968; Kety, Rosenthal, Wender, Schulsinger, & Jacobsen, 1975; Kety et al., 1994; Rosenthal, Wender, Kety, Welner, & Schulsinger, 1971; Wender, Rosenthal, Kety, Schulsinger & Welner, 1974). Other psychiatric adoption studies include a schizophrenia investigation carried out in Finland (Tienari et al., 1987; Tienari et al., 2003), two adoption studies of bipolar disorder (Mendlewicz & Rainer, 1977; Wender et al., 1986), six ADHD studies (e.g., Cantwell, 1975; Sprich, Biederman, Crawford, Mundy, & Faraone, 2000), and several studies of criminality and antisocial behavior (e.g., Bohman, Cloninger, Sigvardsson, & von Knorring, 1982; Mednick, Gabrielli, & Hutchings, 1984).

Methods

The two most frequently used adoption study models in psychiatric genetics are the *Adoptees* method (Figure 18.6, Design A), and the *Adoptees' Family* method (Figure 18.6, Design B). The Adoptees method (e.g., Rosenthal et al., 1971; Tienari et al., 2003) begins with parents (usually mothers) diagnosed with the disorder in question. The researchers then determine the prevalence of this disorder among their adopted-away biological offspring (index group). The prevalence of the



Notes : BPD = Bipolar Disorder. ADHD = Attention Deficit/Hyperactivity Disorder. *Italics* indicate the first identified relatives (cases; called "probands" in the psychiatric genetics literature). *Source*: Adapted from Joseph, 2006, p.44.

Figure 18.6. Three psychiatric adoption study designs.

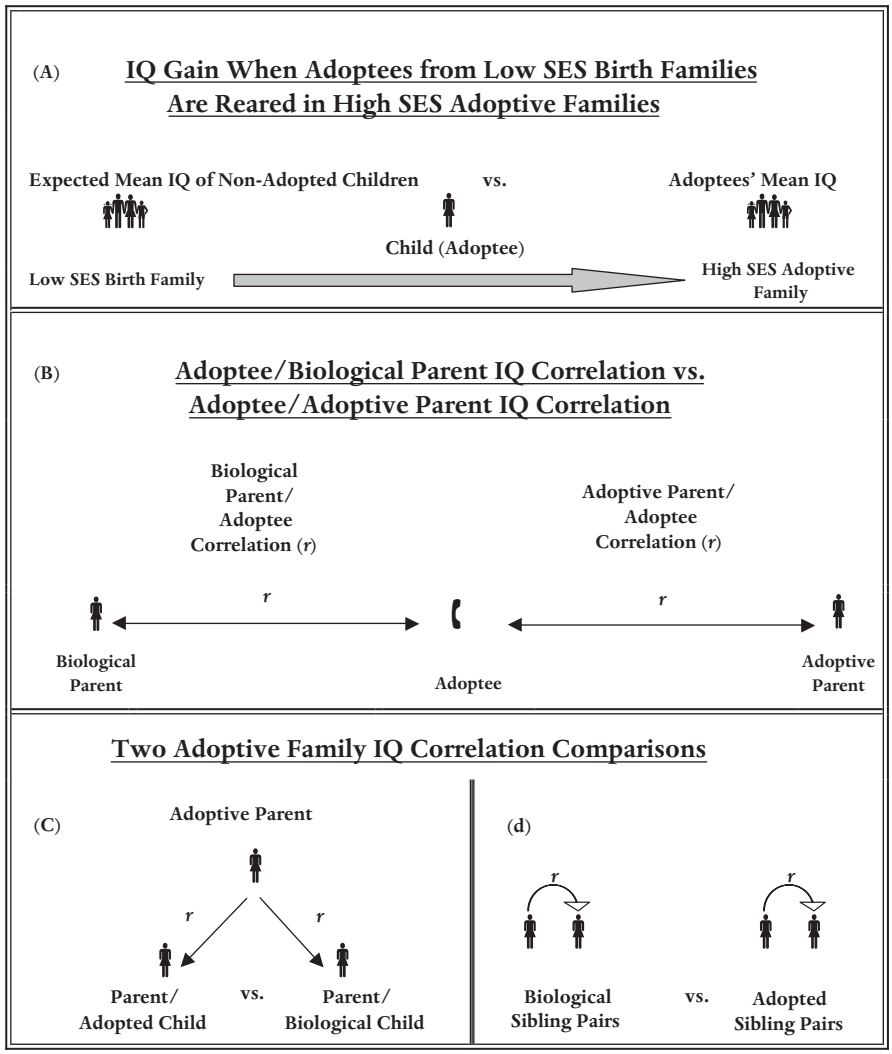
disorder is then compared with that of a control group consisting of the adopted-away biological offspring of parents *not* diagnosed with the disorder. The researchers conclude that a statistically significant higher rate of the disorder among index versus control adoptees suggests a role for genetic factors in causing the disorder.

The Adoptees' Family method (e.g., Kety et al., 1968; Kety et al., 1975) begins with adoptees diagnosed with the disorder in question. A control group of non-diagnosed adoptees is also established. The investigators then attempt to identify and diagnose the biological and adoptive relatives in each group, and make statistical comparisons between these groups. Although Kety and colleagues (1968, 1975) based their conclusions on the rate of ''schizophrenia spectrum disorders'' among their index versus control biological relatives, Faraone and Tsuang wrote that, for the purposes of assessing genetic influences, researchers should compare the diagnostic rates of the biological versus adoptive relatives of the index adoptee group: ''If the biologic relatives of ill adoptees have higher rates of illness than the adoptive relatives of ill adoptees, then a genetic hypothesis is supported. In contrast, if the adoptive relatives show higher rates of illness, then an environmental hypothesis gains support'' (Faraone & Tsuang, 1995, p. 92)

The *Adoptive Family* method (Wender, Rosenthal, & Kety, 1968; see Figure 18.6, Design C) has been used mainly in ADHD research. This method suffers from its inability to make a comparison between the biological and adoptive relatives of the same index adoptee (Joseph, 2000, 2006). A less utilized technique is the *Cross-fostering* method (Wender, Rosenthal, Kety, Schulsinger, & Welner, 1974), which investigates the adopted-away children of biological parents not diagnosed with the disorder in question, who are raised by an adoptive parent eventually diagnosed with this disorder.

Correlational adoption studies of cognitive ability calculate IQ test score correlations between adoptees and various biological and non-biological relatives. A frequently cited comparison is the correlation of adoptees and their biological parents, versus the correlation of the same adoptees and their adoptive parents (see Figure 18.7, Design B). Other studies assess the IQ scores of adoptees raised in adoptive family environments in a higher socioeconomic bracket than that of their biological parents (Figure 18.7, Design A). The aim of these studies is to determine whether improved socioeconomic environments contribute to higher IQ scores.

In addition to calculating parent-adoptee correlations, some IQ and personality studies recorded correlations among different combinations of biological and adoptive relatives. For example, the TAP researchers (Loehlin, Horn, & Willerman, 1990) compared the personality test score correlations of adopted sibling pairs versus biological sibling pairs (Figure 18.7, Design D), and a 2004 CAP IQ study used a similar design (Petrill et al., 2004). These studies sometimes find a higher correlation among biological siblings when compared to adopted siblings. Another method compares the IQ correlations of adoptive parents and their biological



Note: SES = Socioeconomic status.

Figure 18.7. Four IQ adoption study designs.

children, versus the correlation of these adoptive parents with their adopted children (see Figure 18.7, Design C).

Results

The authors of most psychiatric and psychological adoption studies concluded in favor of genetic influences on the trait under study. IQ studies have found that the correlation of adoptees and their biological (birth) parents is greater than the

correlation of adoptees and their adoptive (rearing) parents (see Horn et al., 1979; Plomin et al., 1997; Scarr & Weinberg, 1978). Thus, the investigators concluded that genetic heritage is a more important factor in determining IQ than is the rearing environment. A study performed in France (Schiff, Duyme, Dumaret, & Tomkiewicz, 1982) found that the adopted-away children of unskilled workers, who were reared in the homes of adoptive families in the upper 13% of the socio-professional scale, scored 14 points higher on IQ tests when compared with children who were reared in the homes of their unskilled worker biological parents (Figure 18.7, Design A). This finding suggests that socioeconomic environments are an important factor in determining IQ scores.

Looking at normal variation in personality, although the authors of most studies found evidence that they believed pointed in the genetic direction, a CAP personality study found no significant correlation between the personality scores of adoptees and their biological parents (Adoptive family N = 245; Plomin, Corley, Caspi, Fulker, & DeFries, 1998). Rather than conclude that their carefully performed study found no evidence in support of genetic influences on personality, however, Plomin et al. utilized the ''converging evidence'' argument and linked their results to those of twin studies. This enabled these leading behavioral genetic researchers to conclude in favor of genetics: ''Although several factors might contribute to the discrepancy between twin and adoption results, we suggest that nonadditive genetic influence, which can be detected by twin studies but not by adoption studies, is a likely culprit'' (Plomin et al., 1998, p. 211).[1]

Critical Issues in Adoption Research

The Danish-American schizophrenia adoption studies The Danish-American schizophrenia adoption studies were initiated in the early 1960s by American psychiatric genetic researchers and their Danish colleagues. These studies are widely seen as having definitively established schizophrenia as a genetic disorder, and helped support the view that most other psychiatric disorders have an important genetic component as well. A pair of behavior geneticists looked back on these studies in the late 1990s, writing, ''When a single theory is monolithic in a field, contrary findings can break paradigms ... It is just this role, we believe, that the first adoption studies of schizophrenia played in the 1960s'' (Rowe & Jacobson, 1999, p. 14). However, the following problems, detailed by critics since the 1970s, are among those calling such claims into question (please refer to Figure 18.6, Designs A & B):

- The investigators decided to expand the definition of schizophrenia to include non-psychotic ''schizophrenia spectrum disorders,'' and they would not have found statistically significant results without such an expansion (Joseph, 2004, 2006). In fact, the Kety et al. 1968 study found *zero* cases of chronic

schizophrenia among the 65 identified first-degree biological relatives of adoptees diagnosed with a schizophrenia spectrum disorder, and Rosenthal et al. (1968) found that only 1 of the 76 adopted-away biological offspring of a parent diagnosed with a spectrum disorder had received a hospital diagnosis of schizophrenia.

- In Kety et al.'s famous 1968 study (see Design B), there is evidence suggesting that the researchers decided to change the design of their study after the initial relative group comparisons failed to obtain statistically significant results in the genetic direction (Joseph, 2004, pp. 220–222).
- The researchers failed to adequately define schizophrenia and ''schizophrenia spectrum disorders.''
- In Rosenthal's study (Rosenthal et al., 1971; see Design A), the researchers counted manic depression (bipolar disorder) as a ''schizophrenia spectrum disorder'' despite their insistence elsewhere that this diagnosis is genetically *un*related to schizophrenia. For example, Kety, Rosenthal, Wender, & Schulsinger wrote, ''manic-depressive illness was *never* thought to be in the schizophrenia spectrum by us'' (Kety et al., 1976, p. 417, italics added; see also Rosenthal, 1971). Without these manic-depressive subjects, Rosenthal would not have been able to claim statistically significant results in the genetic direction (see Lidz, Blatt, & Cook, 1981).
- In the Kety et al. studies using interviews to make diagnoses, there were inconsistencies in the way that the researchers decided to count and diagnose dead or unavailable relatives (Lewontin et al., 1984; Lidz & Blatt, 1983).
- The researchers failed to provide case history information on adoptees or relatives, and failed to study important environmental variables.
- As an earlier critic noted, in Kety's Adoptees' Family study (Design B), the ''procedure of counting up all the possible relatives of each index case and pooling them as if they were independent samples ... would allow some families to disproportionately affect the results'' (Benjamin, 1976, p. 1130). Thus, the investigators' decision to emphasize the diagnostic rate among individual *relatives*, as opposed to individual *families*, violated the assumption of independent observations underlying the statistical comparisons they used.
- In the Kety studies, the researchers decided to count first- and second-degree relatives with equal weighting.
- The researchers decided to include many late-separated and late-placed adoptees in their samples. This meant that, during sensitive developmental periods, these adoptees: (a) were reared for a certain period of time by their biological parents; (b) suffered a disruption of attachment bonds with their biological parents; and/or (c) were placed in unstable environments between separation and adoption.
- The investigators used substandard interviews to make diagnoses. In the Kety et al. studies, many of these ''interviews'' never took place, and were simply *fabricated* by the investigators on the basis of hospital records (Kendler &

Gruenberg, 1984; Lewontin et al., 1984). In the raw data Kety and colleagues called them ''pseudointerviews,'' but no mention of them appeared in any of the Danish-American investigators' publications. Of the interviews that *were* conducted, the researchers believed that a five-minute doorstep conversation was sufficient to diagnose someone with schizophrenia (Paikin et al., 1974, pp. 308–310).

- The genetic bias of the investigators appeared to influence how they decided to count relatives, how they decided to define schizophrenia, the types of comparisons they decided to make, and the conclusions they reached (Joseph, 2004).

Selective Placement

A critical issue in psychiatric adoption research is the ''no selective placement assumption.'' Researchers must assume that factors relating to the adoption process (including the policies of adoption agencies) did not lead to the placement of certain groups of adoptees into environments contributing to a higher rate of the disorder in question. They must assume that children were placed into homes uncorrelated with the socioeconomic or presumed genetic status of their biological family. In many psychiatric adoption studies, however, the evidence suggests that index adoptees did experience more psychologically harmful rearing environments than those experienced by control adoptees (Joseph, 2004, 2006; Lewontin et al., 1984). This suggests that children whose biological family had a history of mental disorders were seen as inferior potential adoptees, and were therefore more likely to be placed into more chaotic and harmful adoptive families. Thus, adoption studies' theoretical ability to disentangle genetic and environmental influences may not have occurred in these studies.

Adoptees subsequently becoming the subjects of schizophrenia adoption research were placed in the early-to-middle part of the 20th century in three countries: Denmark, the United States (Oregon), and Finland. However, all three countries had laws permitting the compulsory eugenic sterilization of people diagnosed with schizophrenia and other mental disorders (Broberg & Roll-Hansen, 1996; Joseph, 2004). These laws were passed on the basis of the widespread belief in these countries, in that era, that people diagnosed with schizophrenia or ''insanity'' were the dangerous carriers of ''hereditary taint.''

Thus, if we look at schizophrenia adoption research in the context of the social and political environments in which it was performed, it is clear that the great majority of studied adoptees were given up for adoption in an era in which the compulsory sterilization of ''schizophrenics'' for eugenic purposes was permitted by law in the country or state in which their adoptions took place (Denmark, Finland, Oregon). Leaving aside all other problems, the likelihood that a violation of the ''no selective placement assumption'' occurred in these studies is reason enough to reject conclusions in favor of genetics.

Selective placement is also a potentially confounding factor in adoption studies of IQ, since adoption agencies often attempt to place children they perceive as "bright" (an assessment they make on the basis of the perceived intelligence of the children's birthmother) into better adoptive homes. According to adoption researcher and behavior geneticist Harry Munsinger,

> A . . . possible source of bias in adoption studies is the selective placement of adopted children in adopting homes that are similar to their biological parents' social and educational backgrounds. "Fitting the home to the child" has been the standard practice in most adoption agencies, and this selective placement can confound genetic endowment with environmental influence to invalidate the basic logic of an adoptive study. (Munsinger, 1975, p. 627)

Naturally, agencies "fitting the home to the child" is a far cry from random placement, which a valid adoption study would seem to require. Elsewhere, Kamin concluded, "selective placement accounts for a considerable portion of the [IQ] correlation between unmarried [birth] mothers and their relinquished offspring" (Kamin, in Eysenck vs. Kamin, 1981, p. 123).

Range Restriction

Another problem adoption researchers must address is *range restriction*. Although IQ adoption studies frequently assess the correlation of adoptees and their adoptive parents, this correlation may have little meaning because adoptive parents represent a specially selected, and therefore non-representative, population.

Kamin used boxing to illustrate the problems posed by range restriction in adoption studies (Kamin, in Eysenck vs. Kamin, 1981). He pointed out that if boxing authorities decided to abolish weight divisions, we would observe high correlations between boxers' weights and their won-loss records. "To avoid such a correlation," wrote Kamin,

> definite weight divisions have been established by boxing authorities. Fights can only take place between boxers of reasonably similar weight, and the correlation between weight and boxing success is consequently very low. We are suggesting that in terms of the environments provided for their children almost all adoptive parents – unlike biological parents – are in the heavyweight division. That would account for the lower parent-child IQ correlation observed in adoptive families. The correlation would presumably be much higher if parents who would provide poor environments wanted to, and were allowed to, adopt more often. (Kamin, in Eysenck vs. Kamin, 1981, p. 117)

Lacking an understanding of the sport of boxing and the purpose of weight divisions, one could erroneously conclude that there is no inherent relationship

between weight and boxing success. The same error in interpretation could be occurring in researchers' finding that adoptees and their adoptive parents do not correlate highly on IQ tests.

Behavior geneticist Mike Stoolmiller (1998, 1999) argued forcefully that range restriction constitutes a major bias in adoption research. He showed that studied adoptive parents represent only 37% of the ''environmental quality distribution of the full population of families'' (Stoolmiller, 1998, p. 429). The main sources of this bias, according to Stoolmiller, are: (a) the selection of families who want to adopt a child; (b) the criteria adoption agencies use in allowing a family to adopt; and (c) the decision of adoptive families to volunteer to be part of an adoption study. He suggested that range restriction was a major confounding factor in American adoption studies of personality and IQ, such as the CAP and the TAP (e.g., Loehlin et al., 1989), and that it is also a factor influencing the MZA correlations reported in twins reared-apart (TRA) studies.

According to psychologists Ken Richardson and Sarah Norgate, ''The effect of restricted socio-demographic factors in adoptive families, and their reflection in test score variances, is to reduce adoptive parents-adopted children correlations but not biological mothers-adopted children correlations'' (2006, p. 327; see Figure 18.7, Design B). They further observed that IQ adoption studies' assumption ''that the adoption situation approximates a randomized-effects design'' is not supported by the evidence (p. 319). They called for a ''radical re-appraisal of the [genetic] interpretations and conclusions'' found in IQ adoption study publications (p. 322).

Representativeness

Another critical assumption in adoption research is that samples of adoptees, biological parents, and adoptive parents are representative of their respective populations. However, this is rarely the case (see below). Thus, for example, the already greatly flawed and limited ADHD adoption studies (for a critique, see Joseph, 2000, 2006, 2009) are further flawed by the finding that adoptees are more likely than non-adoptees to receive an ADHD diagnosis (Deutsch, 1989; Deutsch et al., 1982; Tully, Iacono, & McGue, 2008).

Attachment Disturbance

Another issue in adoption research is the potential impact of attachment disturbance on the psychological well being of the adoptees under study. In fact, a team of critics preferred to designate this body of research ''Studies of abandoned children'' (Cassou, Schiff, & Stewart, 1980). Although attachment disturbance may not be an issue in studies that use children adopted away at birth, it becomes

another potentially confounding factor in cases where children are separated from their birthparent months or years after birth. According to Faraone et al., ''If a child has lived with a parent for even a short period of time prior to adoption, the biological relationship will have been 'contaminated' by the environment created by the child's biologic parents'' (1999, p. 42).

Furthermore, research performed over the past two decades suggests that disturbed parent-child attachment patterns can influence brain development during critical developmental periods (Siegel, 1999, 2001; Shore, 2001). This body of research raises the possibility that there are environmentally-caused biological differences between the brains of some adoptees and the brains of securely-attached non-adoptees, which leads to even more questions about the generalizability of adoption research to the non-adoptee population.

Correlation vs. Mean Differences in IQ Test Scores

Potential environmental confounds aside, we have seen that IQ adoption studies have found that the IQs of adopted-away children correlate more with their biological parents than with their adoptive parents. On the other hand, studies using Design A in Figure 18.7 have shown that the biological children of poor or working-class parents show a substantial IQ increase when raised in the homes of families in the upper ranges of the socioeconomic scale (Scarr & Weinberg, 1976; Schiff et al., 1982). Generally speaking, genetically-oriented commentators focus on the correlational data, whereas environmentally-oriented commentators stress the large gains made by poor or working-class children adopted into professional or upper-class families.

In assessing this ''correlation versus test score rise'' issue, we should keep in mind that a correlation coefficient does not measure similarity, but only how traits vary together. As IQ hereditarian theorist Arthur Jensen correctly observed, the finding of higher adoptee-biological parent vs. adoptee-adoptive parent correlations ''should not be misinterpreted as meaning that adopted children's level of IQ is, on average, closer to that of their biological mothers than to that of their adoptive mothers'' (Jensen, 1998, p. 339). He continued,

> In assessing the malleability of IQ . . . one must take account of the mean difference between the biological mother and her adopted child and compare this difference with the mother-child difference in IQ for mothers of the same IQ and socio-economic level who did not put their child up for adoption. (Jensen, 1998, p. 339)

Schiff and Lewontin (1986) presented a table of hypothetical yet ''plausible'' IQ data suggesting the possibility that ''adopted children, even though they may correlate *individually* with their biological parents more than with their adoptive parents, are, in fact, more similar as *a group* to the adoptive parents than to their

biological ones'' (p. 179, italics in original). In Schiff and Lewontin's example, seen in Table 18.1, the IQs of adoptees and their biological parents are perfectly correlated, whereas there is virtually no correlation between adoptees and their adoptive parents. However, adoptees *as a group* had the same mean IQ as adoptive parents as a group, and differed from their biological parents' mean group IQ by seven points.

Schiff and Lewontin's example shows that statistically significant adoptee-biological parent correlations are compatible with adoptees' large IQ gains when reared in enriched adoptive family environments. And we have seen that there are several potentially confounding factors that call into question behavioral genetic researchers' interpretations of these correlations. Thus, focusing on parent-child correlations at the expense of evaluating group mean IQ differences, as behavioral geneticists frequently do, can paint a misleading picture of the potential roles of genetic and environmental influences on intelligence (see also Walker & Emory, 1985).

The Authors of the Five Publications on Adoption Research

The authors of four of the five publications pointed to several problems in adoption research discussed above. Faraone et al. (1999) wrote that, although adoption studies ''can disentangle genetic and environmental contributions to the familial aggregation of disorders'' (p. 44), these studies ''must be viewed with some caution due to potential methodologic problems that cloud their unambiguous interpretations'' (p. 42). They regarded the ''greatest limitation'' of these studies to be ''the fact that adoptees and their families are not representative of the general

Table 18.1. Schiff and Lewontin's hypothetical IQ adoption study data

IQ scores				
Biological parents		*Children (adoptees)*		*Adoptive parents*
93		100		108
94		101		100
95		102		106
96	$r=1.0$	103	$r=0.03$	112
97	$\longleftrightarrow$	104	$\longleftrightarrow$	101
99		106		104
101		108		111
103		110		102
104		111		110
105		112		103
Mean = 98.7		*Mean* = 105.7		*Mean* = 105.7

Source: Adapted from Schiff & Lewontin, 1986, p. 180.

population.'' Therefore, ''one cannot be sure that results from adoption studies will generalize to the broader population of nonadoptees'' (p. 42). They noted further that adoptees as a population are at greater risk for being diagnosed with a psychiatric disorder than are members of the population of non-adoptees, and pointed to problems related to ''contamination'' (p. 43) of these studies by the late separation of adoptees from their biological parents. Similar to their evaluation of twin studies, however, Faraone and colleagues attempted to validate adoption research as constituting one component of the ''converging evidence'' in favor of genetics.

Rutter (2006) noted that, although behavioral geneticists frequently claim that the parent groups in their studies are comparable, ''it is obvious that they are not'' (p. 54). Biological (birth) mothers are often unmarried teenagers who are much more likely to have manifested ''antisocial behavior,'' and whose offspring received ''sub-optimal obstetric care.'' Conversely, Rutter observed that ''adopting parents tend to differ systematically from other parents in being better educated, more socially advantaged, and in having low rates of psychopathology'' (p. 54). Rutter also discussed problems such as the fact that prospective adopting parents are screened by agencies for psychopathology, that the adoptee population has a higher rate of psychopathology than the non-adoptee population, and selective placement, which critics see as a ''built-in confound between genetic risk and environmental risk'' (p. 55). Regarding selective placement, Rutter saw this problem as ''far less important'' than that posed by the ''non-representativeness of adoptees,'' and the ''major differences between the biological and adoptive parents'' (p. 56).

Plomin et al. (2008) also raised the issue of representativeness: ''If biological parents, adoptive parents, or adopted children are not representative of the rest of the population, the generalizability of adoption results could be affected'' (p. 76). They pointed to the potential problems of range restriction, adoptees and birth mothers sharing prenatal environments, and selective placement, which ''could cloud the separation of nature and nurture by placing adopted-apart 'genetic' relatives into correlated environments'' (p. 77).

According to Bouchard and McGue (2003), the adoptive homes into which agencies place adoptees ''may be overly homogeneous,'' which could lead to ''an underestimation of shared environmental effects'' (p. 10). They also wrote, in explicit agreement with Stoolmiller's position on range restriction, ''if adopted children were only placed in high-income families and never reared in poverty, then environmental effects associated with family income and poverty would never be revealed in an adoption study'' (p. 10).

As an example of how unaccounted-for or misunderstood bias can lead to false conclusions, we turn to the National Football League (NFL). Although most games are played on Sunday, there is a game played each Monday night (Monday Night Football). Football analysts have long suggested that teams playing on Monday night are at a competitive disadvantage for the following Sunday's game because,

compared to their upcoming opponent, they have roughly 30 fewer hours to recover from injuries and to prepare for their next opponent.

Suppose a team of researchers decides to test this ''competitive disadvantage hypothesis'' by examining the won-loss records of teams playing Sunday games the week after they play on Monday night. The records go back to 1970, so they would have several decades of data at hand. The researchers write that the null hypothesis states that there is no competitive disadvantage, and that Monday night teams would win 50% of their games the following Sunday. Suppose the researchers find that teams playing on Monday night have a 49% winning record on the following Sunday, a result not significantly different from 50%. They would probably conclude that playing on Monday night does not lead to a competitive disadvantage in games played the following Sunday.

But the researchers would be mistaken, because teams chosen to appear on Monday Night Football are not randomly selected from the pool of all 32 NFL teams. Rather, the NFL and the television network select teams that did well in the previous season (especially Super Bowl winners) to appear two or three times on Monday night, whereas teams that did poorly appear one time, or not at all. Because teams are not chosen randomly, and because teams with the best win-loss record appear more often, it might be more realistic for the null hypothesis to expect these teams to win *70%* of their games the following Sunday. With the expectation adjusted to account for the lack of random assignment, the researchers would likely arrive at a completely different conclusion, namely, that their research suggests that playing on Monday night does indeed place teams at a competitive disadvantage (49% winning percentage vs. the expected winning percentage of 70%). (Another factor that might affect these results is that the NFL gives Super Bowl participants more difficult schedules the following season.)

Analogous types of confounding biases appear to be operating in adoption research, as most of the authors of the five publications concede. This suggests that results, appearing at first glance to be clear-cut, are not so clear-cut upon further investigation. And we have already seen that family and twin studies contain their own set of potentially confounding biases.

Conclusion

Adoption studies provide another example of an area of kinship research where, although well-performed studies might initially seem to provide a clear separation of nature and nurture (and many were not well-performed), further analysis shows that it is very difficult to achieve such separation. The authoritative authors of the five publications were able to identify several important problem areas in adoption research. At the same time, we have seen them defend genetic interpretations of the twin method, even though its ability to disentangle potential environmental and genetic factors is even more questionable than in adoption studies.

Molecular Genetic Research

> It is no secret that our field has published thousands of candidate gene association studies but few replicated findings. (Psychiatric genetic researchers Faraone, Smoller, Pato, Sullivan, & Tsuang, 2008, p. 1)

> Mental disorders take a staggering health and economic toll . . . Yet progress in understanding the underlying causes of these conditions seems to be moving at a crawl . . . decades of futile hunting have made it painfully clear that the contribution of any single gene to disease is probably minuscule. (Editorial in *Nature*, 7/10/2008; cited as Anonymous, 2008, p. 137)

> Despite progress in risk gene identification for several complex diseases, few disorders have proven as resistant to robust gene finding as psychiatric illnesses. The slow rate of progress in psychiatry and behavioral sciences partly reflects a still-evolving classification system, absence of valid pathognomonic diagnostic markers, and lack of well-defined etiologic pathways. Although these disorders have long been assumed to result from some combination of genetic vulnerability and environmental exposure, direct evidence from a specific example has not been forthcoming. Few if any of the genes identified in candidate gene association studies of psychiatric disorders have withstood the test of replication. (Molecular genetic researcher Neil Risch et al., 2009, p. 2363)

> No patient, not a single one, has ever benefited from genetic research into mental illness, although many have been indirectly harmed by it (because it has discouraged the development of adequate services for patients and, during one shameful period, was used to justify their slaughter). No effective treatments have so far been devised on the basis of genetic information and, given what we now know, it seems very unlikely that further research into the genetics of psychosis will lead to important therapeutic advances in the future. Indeed, from the point of view of patients, there can be few other areas of medical research that have yielded such a dismal return for effort expended. (Psychologist Richard Bentall, 2009, p. 145)

The ongoing search for the genes believed to underlie psychiatric disorders and psychological traits is based on the consensus opinion that family, twin, and adoption studies have conclusively established an important role for hereditary factors. For example, a team of schizophrenia molecular genetic researchers justified their work on the grounds that ''family, twin, and adoption studies have demonstrated that schizophrenia is predominantly a genetic disorder with a high heritability'' (Brzustowicz et al., 2004, p. 1057). According to Kendler (2005a, p. 1248), kinship studies have provided ''convincing evidence'' that ''genes that affect risk for [psychiatric] disorders must exist somewhere in the human genome.'' (The genome is defined as the total genetic material of an organism or species.) And Plomin and his team of IQ molecular genetic researchers wrote in 1995, ''Family, twin, and adoption studies constantly converge on the conclusion that general

cognitive ability ('*g*'), often indexed by scores on intelligence (IQ) tests, is one of the most highly heritable behavioral traits'' (Plomin et al., 1995, p. 107).

However, the evidence from kinship studies of families, twins, and adoptees is, as we have seen, very far from convincing. In the following pages we will examine psychiatric and psychological molecular genetic research in the context of the ongoing fruitless search for genes. In the process, we will consider some possible explanations for these failures.

Research Methods

Molecular genetic investigators use several types of research methods. In a *linkage* study, they attempt to identify genetic markers associated with a presumed disease gene among blood relatives. Findings are often represented as a logarithm of odds (LOD) score, which expresses the probability that the linkage occurred by chance. Although valid linkage results identify areas of the genome where relevant genes might be located, they are unable to identify specific genes. A *genome scan* analyzes the complete genome of an individual against a set of markers whose positions on the chromosomes are known, and then looks for common patterns of inheritance between these markers and the disease characteristics. *Association studies* compare the frequency of genetic markers among unrelated affected individuals and a control group, and are performed with population-based case-control, or family-based samples. A genetic marker is defined as a segment of DNA with an identifiable physical location on a chromosome, whose inheritance can be followed.

A more recently developed method, upon which genetic researchers have pinned much hope, is the *genome-wide association study* (GWAS; Hirschhorn & Daly, 2005; Psychiatric GWAS Consortium Steering Committee, 2009). Previously, many leading psychiatric genetic researchers had pinned their hopes on the completion of the Human Genome Project (see Goldsmith, Gottesman, & Lemry, 1997; Hyman, 1999, 2000; Potash & DePaulo, 2000). Some psychiatric genetic researchers have concluded that the ''linkage era'' has been a failure: ''The linkage era (1980–2005) for psychiatric disorders failed to identify any single locus that was unequivocally replicated across multiple independent samples'' (Burmeister, McInnis, & Zöllner, 2008, p. 528). Thus, we have entered the ''era of the genome-wide association study'' (Maher, Riley, & Kendler, 2008, p. 1042). A description of how a GWAS is performed can be found at a US *National Institutes of Health* website:

> To carry out a genome-wide association study, researchers use two groups of participants: people with the disease being studied and similar people without the disease. Researchers obtain DNA from each participant, usually by drawing a blood sample or by rubbing a cotton swab along the inside of the mouth to harvest cells. Each person's complete set of DNA, or genome, is then purified from the blood or

> cells, placed on tiny chips and scanned on automated laboratory machines. The machines quickly survey each participant's genome for strategically selected markers of genetic variation, which are called single nucleotide polymorphisms, or SNPs. If certain genetic variations are found to be significantly more frequent in people with the disease compared to people without disease, the variations are said to be ''associated'' with the disease. The associated genetic variations can serve as powerful pointers to the region of the human genome where the disease-causing problem resides. However, the associated variants themselves may not directly cause the disease. They may just be ''tagging along'' with the actual causal variants. For this reason, researchers often need to take additional steps, such as sequencing DNA base pairs in that particular region of the genome, to identify the exact genetic change involved in the disease. (National Institutes of Health, 2008)

Like previous linkage and association studies, a GWAS is susceptible to the production of false positive results (Pearson & Manolio, 2008). To date, GWAS gene finding efforts in psychology and psychiatry have failed to produce consistently replicated results (Akil et al. 2010; Plomin et al., 2008; Risch et al., 2009).

Types of Theorized Genetic Transmission

Genetic researchers postulate two main types of genetic transmission for the disorders they study. The first is *Mendelian inheritance*, in which a disease or trait is passed from parents to offspring by a single dominant, recessive, or sex-linked gene. Medical disorders such as Huntington's disease and PKU are caused by a person inheriting a single disease gene. Genetic researchers now believe that it is very unlikely that any psychiatric disorder is caused by a single gene.

The second type of genetic transmission is *polygenic inheritance*, meaning that many genes of varying effect sizes contribute to the appearance of a disorder. This means that investigators look for several genes, or individual genes thought to have a large effect size. Most researchers believe that environmental factors or triggers are necessary to bring about disorders in people presumed to be susceptible on the basis of polygenic inheritance. Psychiatric genetic and behavioral genetic researchers believe that most of the traits and disorders they study are caused by the actions of many genes (possibly hundreds) in combination with environmental factors. Researchers believe that these ''multifactorial complex'' traits and disorders are the result of ''a complex interacting admixture of multiple genes and multiple environmental risk factors'' (Rutter, 2001, p. 227).

The Fruitless Search for Genes

The work carried out by molecular genetic researchers in psychiatry and psychology is characterized by the stunning failure to identify genes, even as countless

media reports, often based on overly optimistic or premature claims by the original researchers, continue to misleadingly suggest otherwise. (Internet searches for topics such as ''schizophrenia gene discovery'' or ''autism gene discovery'' provide many such examples.) A 2006 edition of the *Wall Street Journal* found science writer Sharon Begley conveying the following misinformation:

> As tough as neuroscientists have been on Freud – replacing his quaint notions of ego and id with neurotransmitters and brain circuits – geneticists have struck the unkindest blow, linking depression, neuroticism, impulsivity, sexual orientation and more to people's 25,000 or so genes. The complicated tapestry of the mind woven by Freud, a respected neuroscientist in his day, has been reduced to a four-letter genetic code. (Begley, 2006)

Psychiatric genetic researchers of the 1980s believed that they would identify genes for the major psychiatric disorders by the end of that decade (McInnis & Potash, 2004; Propping, 2005). As we saw Faraone et al. acknowledge in 2008, however, ''It is no secret that our field has published thousands of candidate gene association studies but few replicated findings'' (Faraone, Smoller, et al., 2008, p. 1). One could go further and argue that the psychiatric genetics field has produced *no* consistently replicated findings (see Akil et al., 2010). And even in cases where such associations are claimed, we must keep in mind the maxim that correlation (association) does not imply cause.

Indeed, sustained worldwide research over the past few decades has *failed* to identify genes presumed to underlie conditions or traits such as *addictions* (Buckland, 2008), *ADHD* (Faraone, Doyle, et al., 2008; Waldman & Gizer, 2006), *anxiety disorders* (Smoller, Gardner-Schuster, & Covino, 2008), *autism* (Akil et al., 2010; Burmeister et al., 2008; Losh, Sullivan, Trembath, & Piven, 2008), *bipolar disorder* (Plomin et al., 2008; Craddock & Sklar, 2009), *major depressive disorder* (Risch et al., 2009), *obsessive-compulsive disorder* (Pauls, 2008), *personality disorders* (Reichborn-Kjennerud, 2008), and *schizophrenia* (Akil et al., 2010; Bergen et al., 2010).

Turning to the search for the genes believed to underlie general cognitive ability (which researchers theorize as ''quantitative trait loci,'' or ''QTL''), Plomin et al. recognized that, after the initial failures of the mid-1990s, ''Dozens of studies have subsequently explored other candidate gene associations with *g* [general cognitive ability] but none have shown consistent results'' (Plomin et al., 2008, p. 170). Molecular genetic investigations into personality trait variation have suffered a similar fate. As Plomin and colleagues acknowledged, the ''replication of [personality] associations has been difficult.'' They proposed ''employing powerful strategies using mouse models'' (Plomin et al., 2008, p. 263).

The following is a partial list of major problem areas in psychological and psychiatric molecular genetic research (for more details, see Joseph, 2006, Chapter 11):

- The field is massively plagued by false positive results (Abbott, 2008; Faraone, Smoller, et al., 2008). Clearly, some type of systematic error is common to many or most of these studies (see Ioannidis, 2005; Wacholder, Chanock, Garcia-Closas, El ghormli, & Rothman, 2004).
- Researchers (usually mistakenly) assume that previous family, twin, and adoption studies have definitively established the genetic basis of the trait or disorder under study. Few have subjected this body of research to critical analysis.
- Researchers frequently interpret negative results as evidence that the trait is more complex than they originally believed. Proponents of genetic theories have rhetorically transformed years, if not decades, of fruitless gene finding efforts into evidence of the ''complex genetic nature'' of psychiatric disorders and psychological trait variation. It seems the more failures that are recorded, the more ''genetically complex'' these traits and disorders become.
- The validity and reliability of ''continuously distributed'' psychological traits such as IQ and personality, the establishment of which is a prerequisite for performing genetic research in psychology, is questionable.
- The validity and reliability of psychiatric disorders, the establishment of which is a prerequisite for performing genetic research in psychiatry, is questionable (see Kirk & Kutchins, 1992).
- Some methods and accompanying statistical calculations *assume* that some type of genetic transmission is occurring, although it is possible that, in reality, *no* genetic transmission is occurring.
- The association (correlation) of a gene and a trait does not mean that the gene *causes* the trait, and a basic principle of statistics is that ''correlation does not imply cause.'' There are, in fact, several non-causal explanations for gene-trait correlations (see Page, Varghese, Go, Page, & Allison, 2003).
- Even if a gene is a ''necessary component'' of a trait, it does not necessarily mean that the gene *causes* the trait (Ratner, 2004).
- Researchers rarely consider the possibility that the genes they are searching for do not exist.
- It is often assumed that the discovery of genes would be an important achievement. However, focusing research and money on environmental interventions might be a far better course, even if genes actually are involved.

The Four Stages of Molecular Genetic Research in Psychiatry and Psychology

The justification for conducting molecular genetic research in psychiatry and psychology has followed a series of stages, which are described below.

Stage one Researchers and the authors of influential secondary sources argue, usually ignoring or dismissing the publications of critics, that previous kinship research (family, twin, and in some cases adoption studies) has established the

genetic basis of the trait or disorder in question. For most traits, genetic researchers believe (a) that genes exist and await discovery, and (b) that finding genes would aid in the understanding, treatment, or prevention of the trait. Researchers then proceed to search for these presumed genes at the molecular level. They sometimes place gene-finding efforts on the same level as the search for the cure of a deadly disease, or the virus causing an epidemic. The unfounded assumption is that we cannot understand or prevent mental disorders until we know their underlying genetic structure.

Stage two This stage involves speculation about what type of genetic transmission might be occurring. Researchers usually reject single gene theories after the initial failures to find such genes, and then put forward theories about polygenic inheritance (the actions of several genes of various effect sizes). This sometimes involves further speculation about how many genes may be involved, and on which chromosomes they might be located. Meanwhile, failed gene finding efforts continue to pile up.

Stage three This has been called the rhetoric stage (Joseph, 2006). At this point molecular genetic researchers and their supporters choose not to emphasize the unexpected failure to find genes for psychological traits and psychiatric disorders. Instead, they argue that the task of finding genes for ''multifactorial complex disorders'' is more difficult than they first imagined. They often predict that discoveries in the 21st century ''post-genomic era'' are coming soon, and speculate on the direction their research will take after they find genes. For example, psychiatric geneticists Maher et al. (2008, p. 1043), instead of emphasizing decades of *failure*, instead emphasized *optimism*:

> The search for the genetic causes of schizophrenia has been a focus of both psychiatry and genetics for nearly a century. Evidence is beginning to emerge for the involvement of two different underlying mechanisms: genomic and genetic variation. Although it is premature to declare a new dawn, some rays of light are providing new directions for research.

These researchers speak of ''evidence . . . beginning to emerge,'' and ''rays of light . . . providing new directions,'' instead of the more obvious conclusion that three decades of schizophrenia gene finding efforts have uncovered zero schizophrenia genes.

At other times, prominent researchers simply proclaim that genes have been discovered. An example is C. Robert Cloninger's subsequently unsubstantiated 2002 claim, where he wrote in a leading scientific journal of a ''watershed event'' in psychiatry, where for ''the first time, specific genes have been discovered that influence susceptibility to schizophrenia'' (Cloninger, 2002, p. 13365). Four years earlier, Plomin and Rutter had written, ''Genes associated with behavioral

dimensions and disorders are beginning to be identified'' (Plomin & Rutter, 1998, p. 1223). And ten years before that, in their 1988 *Annual Review of Psychology* contribution, Loehlin, Willerman, and Horn wrote, ''We are witnessing major breakthroughs in identifying genes coding for some mental disorders'' (Loehlin et al., 1988, p. 124).

Researchers continue to view psychiatric disorders such as ADHD, bipolar disorder, autism, and schizophrenia as multifactorial complex disorders even after the initial gene finding failures, and then view subsequent failures as additional evidence of the ''complex'' nature of the ''disorder.'' A pair of prominent autism genetic researchers displayed such reasoning when they wrote that the ''current lack of success in finding genes for autism is similar to that of complex diseases'' (Volkmar & Pauls, 2003, p. 1136). In fact, the ''lack of success'' in finding genes is currently a *defining feature* of ''multifactorial complex'' traits and disorders in psychiatry and psychology (Joseph, 2006).

Stage four This ''throw in the towel'' stage (Joseph, 2006) has yet to occur in psychiatric and psychological molecular genetic research. However, fruitless gene finding efforts cannot go on forever, and, assuming that future searches continue to come up empty, funding and support will eventually run out. Of course, the failure to discover genes does not prove that they do not exist. What is striking, however, is that researchers rarely entertain the mere *possibility* that the genes they are searching for do not exist.

For example, in a 2010 ''Policy Forum'' article published in *Science* (Akil et al., 2010), three Nobel prize winning researchers and their colleagues, while recognizing a ''frustrating lack of progress'' (p. 1580) in understanding the genetics of mental disorders, asked for one billion US dollars in genome research money over ten years. They saw this as ''a very small price to pay to reduce or eliminate the awful misery and burden to society caused by mental illness'' (p. 1581). (Some researchers have raised the possibility that the genes they are searching for do not exist; see DeLisi, 2008; Hardy, Low, & Singleton, 2008; Sullivan et al., 2008).

An alternative explanation holds that the failure to find genes can be explained on the grounds that the basic premise of molecular genetic research – namely, the assumption that family, twin, and adoption studies have provided definitive evidence that sought-after genes actually exist – is wrong. In 2009, a leading group of psychiatric genetic researchers acknowledged, ''it is unlikely but formally possible'' that current interpretations of these studies ''are substantially incorrect'' (Psychiatric GWAS Consortium Steering Committee, 2009, p. 15). If the basic argument I have laid out in this chapter is correct, which is that these studies do in fact provide ''substantially incorrect'' evidence, then it is inevitable that the fields of psychology and psychiatry will be compelled to undertake a massive re-examination of the methodology and assumptions of these kinship studies. Such re-examinations are often the basis of rejecting outmoded and unsupported paradigms, and lead to the creation of new paradigms (Kuhn, 1996).

Conclusion

Media reports and some researchers' claims notwithstanding, molecular genetic researchers have failed to uncover the genes that they believe underlie psychiatric disorders and psychological trait variation. We have seen that the field is characterized by ''decades of futile hunting.'' In some cases, such as cognitive ability, autism, and ADHD, the search has been going on for over a decade. In other cases, such as schizophrenia and bipolar disorder, it has been going on since the 1970s. (Over three decades ago, a pair of bipolar adoption study researchers wrote, ''A genetic vulnerability to manic-depressive disorder has been demonstrated by family, twin, and linkage studies''; Mendlewicz & Rainer, 1977, p. 327.) Researchers and their backers should revisit the question of whether this research continues to be worth the cost and resources it requires, in light of the very real possibility that the genes they have been searching for do not exist.

Summary and Conclusions

In this chapter I have described and analyzed the most frequently used and cited genetic research methods in psychiatry and psychology. We have seen that family, twin, and adoption studies contain numerous flaws and questionable underlying theoretical assumptions. While most researchers view the ''converging evidence'' from kinship research as showing conclusively that genes play an important role, a plausible alternative hypothesis holds that these studies fail to provide scientifically acceptable evidence in support of genetic influences on psychiatric disorders and psychological trait variation. The ongoing failure to discover the genes believed to underlie these disorders and traits provides additional support for this position.

A few years ago, I published (Joseph, 2005b) a brief critique of Kendler's (2005c) attempt to reconcile the failure of ''gene finding methods'' with the results of family, twin, and adoption studies, which Kendler (p. 6) viewed as demonstrating the importance of ''genetic risk factors . . . for nearly all psychiatric and drug abuse disorders examined to date.'' In his response to my critique, Kendler wrote,

> It is one thing to criticize the methodology of specific studies. It is quite another to suggest, as Dr. Joseph does, that we reject the results of an entire field of scientific inquiry. This might have been warranted for some pseudoscientific systems, such as astrology, alchemy, and the Ptolemaic astronomic system. It is highly unlikely that modern psychiatric genetics will be judged by future historians of science to be in such company. (Kendler, 2005b, p. 1986)

Although it may be premature to ''reject the results of an entire field of scientific inquiry,'' an extensive critical review of these results by the fields of psychiatry and

psychology is long overdue. After this review is complete, it may become more likely than Kendler believed in 2005 that future historians will view his discipline in a similar light as historians now view the pseudosciences of previous eras.

Genetic research is usually performed by people who believe strongly that genetic factors play an important role. It is reasonable to expect this bias to influence their research and their conclusions. Over a generation ago, psychologist George Albee concluded that his early belief that social scientists discover facts in order to build theories was wrong, and that:

> it is more accurate to say that people, and particularly social scientists, select theories that are consistent with their personal values attitudes, and prejudices, and then go out into the world, or into the laboratory, to seek facts that validate their beliefs about the world and about human nature, neglecting or denying observations that contradict their personal prejudices. (Albee, 1982, p. 5)

The understanding that researchers' conclusions are influenced by their belief systems, and their personal and professional interests, simply means that critical analysis of research should become the default mode in science in general, and in human genetic research in particular (Joseph & Baldwin, 2000). This implies that a shift from confirmation to falsification is in order.

But is it really possible that a scientific method supported by experts for decades could turn out to be bad science? The case of ''comparative bullet-lead analysis'' provides a recent example. Beginning in the early 1960s, the FBI provided expert testimony in support of this technique, which ''used chemistry to link crime-scene bullets to [unused] ones possessed by suspects on the theory that each batch of lead had a unique elemental makeup'' (Solomon, 2007). However, by 2004 the US National Academy of Sciences concluded that comparative bullet-lead analysis is ''unreliable and potentially misleading'' (Solomon, 2007), and the method was no longer admissible as evidence. Perhaps a thorough investigation into the claim that twin and adoption studies provide conclusive evidence in favor of genetics would also find such claims to be ''unreliable and potentially misleading.''

David Rosenthal (1968, p. 414) once astutely observed that ''hereditarians'' like to look at ''numbers,'' whereas ''environmentalists'' like to look at ''patients'' (people). The behavioral and psychiatric genetic literature tends to emphasize numbers and statistics at the expense of understanding the complexity of people's histories and life circumstances, in the context of an increasingly complicated world.

As a practicing clinical psychologist, I was not very familiar with the writings of Gilbert Gottlieb when I was invited to contribute to this book. Having now read some of his work (e.g., Gottlieb, 1998), and what others have written about it (Greenberg, 2007), I understand what prompted the invitation. Although I do not use the terminology Gottlieb employed (e.g., probabilistic epigenesis, bidirectionality), I am in agreement with him on the meaning of it and of its implications for

behavioral development. Like Gottlieb, I am critical of the central dogma of molecular biology. I believe that environmental factors play a crucial role in human development and behavior. On the other hand, genetic researchers frequently argue that genes play a predominant role. However, we have seen that the research supporting this position is greatly flawed by factors that Gottlieb also discussed, not the least of which is the bias of researchers and interest groups seeking to promote the primacy of genetics. Obviously, the development of every organism is influenced by heredity and environment, but this was understood long before behavioral genetic and psychiatric genetic research came onto the scene.

It is clear that theories of human psychological trait variation and psychopathology that emphasize genetics are harmful in that they serve to divert society's attention from the need to change the environment. My work is not really about fusing nature and nurture, as it was for Gottlieb, but is mainly about illuminating the fatal flaws of behavioral genetic and psychiatric genetic research, regardless of the role that genes might play in the development of particular traits. The current emphasis on genetics helps absolve society from the responsibility of making the social and political changes necessary to improve the human condition. I trust that readers will see the relationship between my ideas and those Gottlieb put forward.

Regardless of possible genetic influences, it is clear that factors such as culture, family, birth cohort, political policies, access to health care, nutrition, religion, education, the mass media, and oppression on the basis of race, sex, sexual orientation, or social class, play a crucial and dominant role in shaping who we are and how we function and behave. Unfortunately, genetic research often diverts society's attention from identifying and mitigating critical environmental factors that cause human distress and disease, and impede human growth (Chaufan, 2007). It contributes to putting off the day when society decides to undertake a serious effort to implement the necessary environmental interventions to alleviate and prevent human suffering, and to promote human growth to the fullest.

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Notes

1. Frank Miele (2005), a supporter of behavioral genetics, quoted Plomin et al.'s 1998 *conclusions* in his rejoinder to my (2005a) description of these researchers' negative

results. Although Miele strongly implied that Plomin et al.'s conclusions trumped their data, he actually lent support to critics' longtime contention that genetically-oriented researchers' beliefs and biases strongly influence their research, and strongly influence the conclusions they draw from their research. Apparently, Plomin and his colleagues were committed to conclude in favor of genetics regardless of what their findings showed.

References

Abbott, A. (2008). The brains of the family: Does the difficulty in finding the genes responsible for mental illness reflect the complexity of the genetics or the poor definitions of psychiatric disorders? *Nature*, **454**, 154–157.

Ainslie, R. C. (1985). *The psychology of twinship*. Lincoln: University of Nebraska Press.

Akil, H., Brenner, S., Kandel, E., Kendler, K. S., King, M., Scolnick, E., Watson, J. D., et al. (2010). The future of psychiatric research: Genomes and neural circuits. *Science*, **327**, 1580–1581.

Albee, G. W. (1982). The politics of nature and nurture. *American Journal of Community Psychology*, **10**, 4–36.

Alford, J. R., Funk, C. L., & Hibbing, J. R. (2005). Are political orientations genetically transmitted? *American Political Science Review*, **99**, 153–167.

American Psychiatric Association (2000). *Diagnostic and statistical manual of mental disorders* (4th ed., text revision). Washington, DC: Author.

Anonymous. (2008). An unnecessary battle: Neuroscientists and geneticists don't need to be at loggerheads over the biology of mental disorders. *Nature*, **454**, 137–138.

Begley, S. (2006, February 24). Parents can counteract "environments" created by children's genes. *Wall Street Journal*, p. B1.

Benjamin, L. S. (1976). A reconsideration of the Kety and associates study of genetic factors in the transmission of schizophrenia. *American Journal of Psychiatry*, **133**, 1129–1133.

Bentall, R. P. (2003). *Madness explained: Psychosis and human nature*. London: Allen Lane.

Bentall, R. P. (2009). *Doctoring the mind: Is our current treatment of mental illness really any good?* New York: New York University Press.

Bergen, S. E., Fanous, A. H., Kuo, P.-H., Wormley, B. K., O'Neill, F. A., Walsh, D., et al. (2010). No association of dysbindin with symptom factors of schizophrenia in an Irish case-controls sample. *American Journal of Medical Genetics (Part B)*, **153B**, 700–705.

Biederman, J., Faraone, S. V., Keenan, K., Knee, D., & Tsuang, M. T. (1990). Family-genetic and psychosocial risk factors in DSM-III attention deficit disorder. *Journal of the American Academy of Child and Adolescent Psychiatry*, **29**, 526–533.

Black, E. (2003). *War against the weak: Eugenics and America's campaign to create a master race*. New York: Four Walls Eight Windows.

Bleuler, M. (1978). *The schizophrenic disorders: Long-term patient and family disorders*. New Haven: Yale University Press.

Block, N. J. (1995). How heritability misleads about race. *Cognition*, **56**, 99–128.

Bohman, M., Cloninger, C. R., Sigvardsson, S., & von Knorring, A. (1982). Predisposition to petty criminality in Swedish adoptees. *Archives of General Psychiatry*, **39**, 1233–1241.

Borkenau, P., Riemann, R., Angleitner, A., & Spinath, F. M. (2002). Similarity of childhood experiences and personality resemblance in monozygotic and dizygotic twins: A test of the equal environments assumption. *Personality and Individual Differences*, **33**, 261–269.

Bouchard, T. J., Jr. (1987). The hereditarian research program: Triumphs and tribulations. In S. Modgil & C. Modgil (Eds.), *Arthur Jensen: Consensus and controversy* (pp. 55–75). New York: Falmer Press.

Bouchard, T. J., Jr. (1993a). The genetic architecture of human intelligence. In P. Vernon (Ed.), *Biological approaches to the study of human intelligence* (pp. 33–93). Norwood, NJ: Ablex Publishing Corporation.

Bouchard, T. J., Jr. (1993b). Genetic and environmental influences on adult personality: Evaluating the evidence. In J. Hettema & I. Deary (Eds.), *Basic issues in personality* (pp. 15–44). Dordrecht, The Netherlands: Kluwer Academic Publishers.

Bouchard, T. J., Jr., (1997a). The genetics of personality. In K. Blum & E. Noble (Eds.), *Handbook of psychiatric genetics* (pp. 273–296). Boca Raton, FL: CRC Press.

Bouchard, T. J., Jr. (1997b). IQ similarity in twins reared apart: Findings and responses to critics. In R. Sternberg & E. Grigorenko (Eds.), *Intelligence, heredity, and environment* (pp. 126–160). New York: Cambridge University Press.

Bouchard, T. J., Jr., Lykken, D. T., McGue, M., Segal, N. L., & Tellegen, A. (1990). Sources of human psychological differences: The Minnesota Study of twins reared apart. *Science*, **250**, 223–228.

Bouchard, T. J., Jr., & McGue, M. (1981). Familial studies of intelligence: A review. *Science*, **212**, 1055–1059.

Bouchard, T. J., Jr., & McGue, M. (2003). Genetic and environmental influences on human psychological differences. *Journal of Neurobiology*, **54**, 4–45.

Boyle, M. (1990). *Schizophrenia: A scientific delusion?* New York: Routledge.

Boyle, M. (2002). *Schizophrenia: A scientific delusion?* (2nd ed.). Hove, UK: Routledge.

Breggin, P. R. (1991). *Toxic psychiatry*. New York: St. Martin's Press.

Broberg, G., & Roll-Hansen, N. (Eds.). (1996). *Eugenics and the welfare state: Sterilization policy in Denmark, Sweden, Norway, and Finland*. East Lansing, MI: Michigan State University Press.

Brzustowicz, L. M., Simone, J., Mohseni, P., Hayter, J. E., Hodgkinson, K. A., Chow, E. W. C., et al. (2004). Linkage disequilibrium mapping of schizophrenia susceptibility to the CAPON region of chromosome 1q22. *American Journal of Human Genetics*, **74**, 1057–1053.

Buckland, P. R. (2008). Will we ever find the genes for addiction? *Addiction*, **103**, 1768–1776. (Advanced online publication. Published online 8/14/2008).

Burks, B. S. (1928) The relative influence of nature and nurture upon mental development; A comparative study of foster parent-foster child resemblance and true parent-true child resemblance. *27th Yearbook of the National Society for the Study of Education* (Part 1, pp. 219–316). Bloomington, IL: Public School Publishing Co.

Burmeister, M., McInnis, M. G., & Zöllner, S. (2008). Psychiatric genetics: Progress amid controversy. *Nature Reviews Genetics*, **9**, 527–540.

Burt, C. (1966). The genetic determination of differences in intelligence: A study of monozygotic twins reared together and apart. *British Journal of Psychology*, **57**, 137–153.

Cantwell, D. P. (1975). Genetic studies of hyperactive children: Psychiatric illness in biologic and adopting parents. In R. Fieve, D. Rosenthal, & H. Brill (Eds.), *Genetic research in psychiatry* (pp. 273–280). Baltimore: The Johns Hopkins Press.

Carter, H. D. (1940). Ten years of research on twins: Contributions to the nature-nurture problem. *National Society for the Study of Education Yearbook*, **39**, 235–255.

Cassou, B., Schiff, M., & Stewart, J. (1980). Génétique et schizophrénie: Réévaluation d'un consensus [Genetics and schizophrenia: Reevaluation of a consensus]. *Psychiatrie de l'Enfant*, **23**, 87–201.

Charney, E. (2008). Genes and Ideologies. *Perspectives on Politics*, **6**, 292–319.

Chaufan, C. (2007). How much can a large population study on genes, environments, their interactions and common diseases contribute to the health of the American people? *Social Science and Medicine*, **65**, 1730–1741.

Chaufan, C. (2008). Unpacking the heritability of diabetes: The problem of attempting to quantify the relative contributions of nature and nurture. *DataCrítica: International Journal of Critical Statistics*, **2**, 23–38.

Cloninger, C. R. (2002). The discovery of susceptibility genes for mental disorders. *Proceedings of the National Academy of Sciences*, **99**, 13365–13367.

Craddock, N., & Sklar, P. (2009). Genetics of bipolar disorder: Successful start to a long journey. *Trends in Genetics*, **25**, 99–105.

Davenport, C. B. (1911). *Heredity in relation to eugenics*. New York: Henry Holt and Company.

DeLisi, L. E. (2008). Reviewing the ''facts about schizophrenia'': A possible or impossible task? *Schizophrenia Research*, **102**, 19–20.

Deutsch, C. K. (1989). Adoption and attention deficit disorder. In L. Bloomingdale & J. Swanson (Eds.), *Attention deficit disorder, current concepts and emerging trends in attentional and behavioral disorders of childhood* (Vol. **IV**, pp. 67–79). New York: Pergamon Press.

Deutsch, C. K., Swanson, J. M., Bruell, J. H., Cantwell, D. P., Weinberg, F., & Baren, M. (1982). Overrepresentation of adoptees in children with the attention deficit disorder. *Behavior Genetics*, **12**, 231–238.

Essen-Möller, E. (1963). Twin research in psychiatry. *Acta Psychiatrica Scandinavica*, **39**, 65–77.

Eysenck, H. J.,vs. Kamin, L. J. (1981). *The intelligence controversy*. New York: John Wiley & Sons.

Faraone, S. V., & Biederman, J. (2000). Nature, nurture, and attention deficit hyperactivity disorder. *Developmental Review*, **20**, 568–581.

Faraone, S. V., Doyle, A. E., Lasky-Su, J., Sklar, P. B., D'Angelo, E., Gonzalez-Heydrich, J., et al. (2008). Linkage analysis of attention deficit hyperactivity disorder. *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics)*, **147B**, 1387–1391.

Faraone, S. V., Smoller, J. W., Pato, C. N., Sullivan, P., & Tsuang, M. T. (2008). The new neuropsychiatric genetics. *American Journal of Medical Genetics (Part B, Neuropsychiatric Genetics)*, **147B**, 1–2.

Faraone, S. V., & Tsuang, M. T. (1995). Methods in psychiatric genetics. In M. Tsuang, M. Tohen, & G. Zahner (Eds.), *Textbook in psychiatric epidemiology* (pp. 81–134). New York: Wiley-Liss.

Faraone, S. V., Tsuang, M. T., & Tsuang, D. W. (1999). *Genetics of mental disorders*. New York: The Guilford Press.

Farber, S. L. (1981). *Identical twins reared apart: A reanalysis*. New York: Basic Books.

Feldman, M. W., & Lewontin, R. C. (1975). The heritability hang-up. *Science*, **190**, 1163–1168.

Fischer, M. (1971). Psychoses in the offspring of schizophrenic monozygotic twins and their normal co-twins. *British Journal of Psychiatry*, **118**, 43–51.

Goldsmith, H. H., Gottesman, I. I., & Lemry, K. S. (1997). Epigenetic approaches to developmental psychopathology. *Development and Psychopathology*, **9**, 365–387.

Gottesman, I. I. (1982). Identical twins reared apart: A reanalysis [Book review]. *American Journal of Psychology*, **95**, 350–352.

Gottesman, I. I. (1991). *Schizophrenia genesis*. New York: W. H. Freeman & Company.

Gottesman, I. I., & Bertelsen, A. (1989). Confirming unexpressed genotypes for schizophrenia. *Archives of General Psychiatry*, **46**, 867–872.

Gottesman, I. I., & Shields, J. (1966). Contributions of twin studies to perspectives on schizophrenia. In B. Maher (Ed.), *Progress in experimental personality research* (Vol. **3**, pp. 1–84). New York: Academic Press.

Gottesman, I. I., & Shields, J. (1972). *Schizophrenia and genetics: A twin study vantage point*. New York: Academic Press.

Gottlieb, G. (1998). Normally occurring environmental and behavioral influences on gene activity: From central dogma to probabilistic epigenesis. *Psychological Review*, **105**, 792–802.

Greenberg, G. (2005). The limitations of behavior-genetic analyses: Comment on McGue, Elkins, Walden, and Iacono. *Developmental Psychology*, **41**, 989–992.

Greenberg, G. (2007). Why psychology is not a biological science: Gilbert Gottlieb and probabilistic epigenesis. *European Journal of Developmental Science*, **1**, 111–121.

Hardy, J., Low, N., & Singleton, A. (2008). Whole genome association studies: Deciding when persistence becomes perseveration. *American Journal of Medical Genetics (Part B, Neuropsychiatric Genetics)*, **147B**, 131–133.

Harris, J. R. (1998). *The nurture assumption: Why children turn out the way they do*. New York: The Free Press.

Hay, D. A., McStephen, M., & Levy, F. (2001). Introduction to the genetic analysis of attentional disorders. In F. Levy & D. Hay (Eds.), *Attention, genes, and ADHD* (pp. 7–34). East Sussex, UK: Brunner-Routledge.

Hearnshaw, L. S. (1979). *Cyril Burt: Psychologist*. Ithaca, NY: Cornell University Press.

Herrell, R., Goldberg, J., True, W. R., Ramakrishnan, V., Lyons, M., Eisen, S., et al. (1999). Sexual orientation and suicidality: A co-twin control study in adult men. *Archives of General Psychiatry*, **56**, 867–874.

Heston, L. L. (1966). Psychiatric disorders in foster home reared children of schizophrenic mothers. *British Journal of Psychiatry*, **112**, 819–825.

Hill, D. (1983). *The politics of schizophrenia*. Lanham, MD: University Press of America.

Hirsch, J. (1997). Some history of heredity-vs-environment, genetic inferiority at Harvard (?), and The (incredible) Bell Curve. *Genetica*, **99**, 207–224.

Hirsch, J. (2004). Uniqueness, diversity, similarity, repeatability, and heritability. In C. Coll, E. Bearer, & R. Lerner (Eds.), *Nature and nurture: The complex interplay of genetic and environmental influences on human behavior and development* (pp. 127–138). Mahwah, NJ: Erlbaum.

Hirschhorn, J. N., & Daly, M. J. (2005). Genome-wide association studies for common diseases and complex traits. *Nature Reviews Genetics*, **6**, 95–108.

Hoffman, L. W. (1985). The changing genetics/socialization balance. *Journal of Social Issues*, **41**, 127–148.

Hoffman, L. W. (1991). The influence of the family environment on personality: Accounting for sibling differences. *Psychological Bulletin*, **110**, 187–203.

Horn, J. M., Loehlin, J. C., & Willerman, L. (1979). Intellectual resemblance among adoptive and biological relatives: The Texas Adoption Project. *Behavior Genetics*, **9**, 177–207.

Hyman, S. E. (1999). Introduction to the complex genetics of mental disorders. *Biological Psychiatry*, **45**, 518–521.

Hyman, S. E. (2000). Goals for research on bipolar disorder: The view from NIMH. *Biological Psychiatry*, **48**, 436–441.

Ioannidis, J. (2005). Why most published research findings are false. *PLoS Medicine*, **2**, 696–701.

Jackson, D. D. (1960). A critique of the literature on the genetics of schizophrenia. In D. Jackson (Ed.), *The etiology of schizophrenia* (pp. 37–87). New York: Basic Books.

Jensen, A. R. (1998). *The g factor*. Westport, CT: Praeger.

Joseph, J. (1998). The equal environment assumption of the classical twin method: A critical analysis. *Journal of Mind and Behavior*, **19**, 325–358.

Joseph, J. (2000). Not in their genes: A critical view of the genetics of attention-deficit hyperactivity disorder. *Developmental Review*, **20**, 539–567.

Joseph, J. (2001a). Don Jackson's "A Critique of the Literature on the Genetics of Schizophrenia" – A reappraisal after 40 years. *Genetic, Social, and General Psychology Monographs*, **127**, 27–57.

Joseph, J. (2001b). Separated twins and the genetics of personality differences: A critique. *American Journal of Psychology*, **114**, 1–30.

Joseph, J. (2002). Twin studies in psychiatry and psychology: Science or pseudoscience? *Psychiatric Quarterly*, **73**, 71–82.

Joseph, J. (2004). *The gene illusion: Genetic research in psychiatry and psychology under the microscope*. New York: Algora. (2003 United Kingdom Edition by PCCS Books)

Joseph, J. (2005a). Comments about Frank Miele's article "The revival of human nature ≠ The denial of human nature" [Letter to the editor]. *Skeptic*, **11**(3), 24–26.

Joseph, J. (2005b). Research paradigms of psychiatric genetics [Letter to the editor]. *American Journal of Psychiatry*, **162**, 1985.

Joseph, J. (2006). *The missing gene: Psychiatry, heredity, and the fruitless search for genes*. New York: Algora.

Joseph, J. (2009). ADHD and genetics: A consensus reconsidered. In S. Timimi & J. Leo (Eds.), *Rethinking ADHD* (pp. 58–91). London: Palgrave Macmillan.

Joseph, J., & Baldwin, S. (2000). Four editorial proposals to improve social sciences research and publication. *International Journal of Risk and Safety in Medicine*, **13**, 117–127.

Joseph, J., & Leo, J. (2006). Genetic relatedness and the lifetime risk for being diagnosed with schizophrenia: Gottesman's 1991 Figure 10 reconsidered. *Journal of Mind and Behavior*, **27**, 73–89.

Juel-Nielsen, N. (1965/1980). *Individual and environment: Monozygotic twins reared apart* (rev ed). New York: International Universities Press.

Kallmann, F. J. (1938). *The genetics of schizophrenia: A study of heredity and reproduction in the families of 1,087 schizophrenics*. New York: J. J. Augustin.

Kamin, L. J. (1974). *The science and politics of I.Q*. Potomac, MD: Erlbaum.

Kamin, L. J., & Goldberger, A. S. (2002). Twin studies in behavioral research: A skeptical view. *Theoretical Population Biology*, **61**, 83–95.

Kaprio, J., Koskenvuo, M., & Rose, R. J. (1990). Change in cohabitation and intrapair similarity of monozygotic (MZ) cotwins for alcohol use, extraversion, and neuroticism. *Behavior Genetics*, **20**, 265–276.

Kendler, K. S. (1983). Overview: A current perspective on twin studies of schizophrenia. *American Journal of Psychiatry*, **140**, 1413–1425.

Kendler, K. S. (1993). Twin studies of psychiatric illness: Current status and future directions. *Archives of General Psychiatry*, **50**, 905–915.

Kendler, K. S. (2000). Schizophrenia: Genetics. In B. Sadock & V. Sadock (Eds.), *Kaplan & Sadock's comprehensive textbook of psychiatry* (7th ed., Vol. **1**, pp. 1147–1158). Philadelphia: Lippincott, Williams, & Wilkins.

Kendler, K. S. (2005a). "A gene for . . .": The nature of gene actions in psychiatric disorders. *American Journal of Psychiatry*, **162**, 1243–1252.

Kendler, K. S. (2005b). Dr. Kendler responds [Letter to the editor]. *American Journal of Psychiatry*, **162**, 1985–1986.

Kendler, K. S. (2005c). Psychiatric genetics: A methodologic critique. *American Journal of Psychiatry*, **162**, 3–11.

Kendler, K. S., & Gardner, C. O. (1998). Twin studies of adult psychiatric and substance dependent disorders: Are they biased by differences in the environmental experiences of monozygotic and dizygotic twins in childhood and adolescence? *Psychological Medicine*, **28**, 625–633.

Kendler, K. S., & Gruenberg, A. M. (1984). An independent analysis of the Danish adoption study of schizophrenia. *Archives of General Psychiatry*, **41**, 555–564.

Kendler, K. S., & Prescott, C. A. (2006). *Genes, environment, and psychopathology*. New York: Guilford.

Kety, S. S., Rosenthal, D., Wender, P. H., & Schulsinger, F. (1968). The types and prevalence of mental illness in the biological and adoptive families of adopted

schizophrenics. In D. Rosenthal & S. Kety (Eds.), *The transmission of schizophrenia* (pp. 345–362). New York: Pergamon Press.

Kety, S. S., Rosenthal, D., Wender, P. H., & Schulsinger, F. (1976). Studies based on a total sample of adopted individuals and their relatives: Why they were necessary, what they demonstrated and failed to demonstrate. *Schizophrenia Bulletin*, **2**, 413–427.

Kety, S. S., Rosenthal, D., Wender, P. H., Schulsinger, F., & Jacobsen, B. (1975). Mental illness in the biological and adoptive families of adopted individuals who have become schizophrenic: A preliminary report based on psychiatric interviews. In R. Fieve, D. Rosenthal, & H. Brill (Eds.), *Genetic research in psychiatry* (pp. 147–165). Baltimore: The Johns Hopkins Press.

Kety, S. S., Wender, P. H., Jacobsen, B., Ingraham, L. J., Jansson, L., Faber, B., et al. (1994). Mental illness in the biological and adoptive relatives of schizophrenic adoptees: Replication of the Copenhagen study to the rest of Denmark. *Archives of General Psychiatry*, **51**, 442–455.

Kirk, S. A., & Kutchins, H. (1992). *The selling of DSM: The rhetoric of science in psychiatry*. New York: Aldine De Gruyter.

Kline, P. (1993). *The handbook of psychological testing*. London: Routledge.

Kringlen, E. (1967). *Heredity and environment in the functional psychoses: An epidemiological-clinical study*. Oslo: Universitetsforlaget.

Kringlen, E., & Cramer, G. (1989). Offspring of monozygotic twins discordant for schizophrenia. *Archives of General Psychiatry*, **46**, 873–877.

Kuhn, T. S. (1996). *The structure of scientific revolutions* (3rd ed.). Chicago: The University of Chicago Press. (Originally published in 1962).

Kutchins, H., & Kirk, S. A. (1997). *Making us crazy: DSM, the psychiatric bible and the creation of mental disorders*. New York: The Free Press.

LaBuda, M. C., Svikis, D. S., & Pickens, R. V. (1997). Twin closeness and co-twin risk for substance use disorders: Assessing the impact of the equal environment assumption. *Psychiatry Research*, **70**, 155–164.

Laing, R. D. (1981). A critique of Kallmann's and Slater's genetic theory of schizophrenia. In R. Evans, *Dialogue with R. D. Laing* (pp. 97–156). New York: Praeger.

Langinvainio, H., Kaprio, J., Koskenvuo, M., & Lönnqvist, J. (1984). Finnish twins reared apart III: Personality factors. *Acta Geneticae Medicae et Gemellologiae*, **33**, 259–264.

Langinvainio, H., Koskenvuo, M., Kaprio, J., & Sistonen, P. (1984). Finnish twins reared apart II: Validation of zygosity, environmental dissimilarity and weight and height. *Acta Geneticae Medicae et Gemellologiae*, **33**, 251–258.

Leahy, A. M. (1935). Nature-nurture and intelligence. *Genetic Psychological Monographs*, **17**, 237–308.

Lerner, R. M. (1986). *Concepts and theories of human development* (2nd ed.). New York: Random House.

Lewontin, R. C. (1987). The irrelevance of heritability. *Science for the People*, **19**, 23, 32.

Lewontin, R. C., Rose, S., & Kamin, L. J. (1984). *Not in our genes*. New York: Pantheon.

Lidz, T., & Blatt, S. (1983). Critique of the Danish-American studies of the biological and adoptive relatives of adoptees who became schizophrenic. *American Journal of Psychiatry*, **140**, 426–435.

Lidz, T., Blatt, S., & Cook, B. (1981). Critique of the Danish-American studies of the adopted-away offspring of schizophrenic parents. *American Journal of Psychiatry*, **138**, 1063–1068.

Lilienfeld, S. O., Lynn, S. J., & Lohr, J. M. (2003). Science and pseudoscience in clinical psychology: Initial thoughts, reflections, and considerations. In S. Lilienfeld, S. Lynn, & J. Lohr (Eds.), *Science and pseudoscience in clinical psychology* (pp. 1–14). New York: Guilford.

Loehlin, J. C., Horn, J. M., & Willerman, L. (1989). Modeling IQ change: Evidence from the Texas Adoption Project. *Child Development*, **60**, 993–1004.

Loehlin, J. C., Horn, J. M., & Willerman, L. (1990). Heredity, environment, and personality change: Evidence from the Texas Adoption Project. *Journal of Personality*, **58**, 221–243.

Loehlin, J. C., Willerman, L., & Horn, J. M. (1988). Human behavior genetics. *Annual Review of Psychology*, **39**, 101–133.

Losh, M., Sullivan, P. F., Trembath, D., & Piven, J. (2008). Current developments in the genetics of autism: From phenome to genome. *Journal of Neuropathology and Experimental Neurology*, **67**, 829–837.

Lykken, D. T., McGue, M., Bouchard, T. J., Jr., & Tellegen, A. (1990). Does contact lead to similarity or similarity to contact? *Behavior Genetics*, **20**, 547–561.

Lyons, M. J., Kendler, K. S., Provet, A., & Tsuang, M. T. (1991). The genetics of schizophrenia. In M. Tsuang, K. Kendler, & M. Lyons (Eds.), *Genetic issues in psychosocial epidemiology* (pp. 119–152). New Brunswick, NJ: Rutgers University Press.

Lytton, H. (1977). Do parents create, or respond to, differences in twins? *Developmental Psychology*, **13**, 456–459.

Maher, B. S., Riley, B. P., & Kendler, K. S. (2008). Psychiatric genetics gets a boost. *Nature Genetics*, **40**, 1042–1044.

Marshall, R. (1990). The genetics of schizophrenia: Axiom or hypothesis? In R. Bentall (Ed.), *Restructuring schizophrenia* (pp. 89–117). London: Routledge.

Martin, J. D., Blair, G. E., Dannenmaier, W. D., Jones, P. C., & Asako, M. (1981). Relationship of scores on the California Psychological Inventory to age. *Psychological Reports*, **49**, 151–154.

McGue, M., & Bouchard, T. J., Jr. (1984). Adjustment of twin data for the effects of age and sex. *Behavior Genetics*, **14**, 325–343.

McInnis, M. G., & Potash, J. B. (2004). Psychiatric genetics: Into the 21st century. *International Review of Psychiatry*, **16**, 301–310.

Mednick, S. A., Gabrielli, W. F., & Hutchings, B. (1984). Genetic influences in criminal convictions: Evidence from an adoption cohort. *Science*, **224**, 891–894.

Mendlewicz, J., & Rainer, J. D. (1977). Adoption study supporting genetic transmission in manic-depressive illness. *Nature*, **268**, 327–329.

Mensh, E., & Mensh, H. (1991). *The IQ mythology*. Carbondale, IL: Southern Illinois Press.

Miele, F. (2005). Nature and nurture: Putting all the pieces together – Miele replies to Joseph and Schlinger. *Skeptic*, **11**(3), 26–30.

Moore, D. S. (2001). *The dependent gene: The fallacy of "nature vs. nurture"*. New York: Times Books.

Morris-Yates, A., Andrews, G., Howie, P., & Henderson, S. (1990). Twins: A test of the equal environments assumption. *Acta Psychiatrica Scandinavica*, **81**, 322–326.

Mosher, L. R., Pollin, W., & Stabenau, J. R. (1971). Families with identical twins discordant for schizophrenia: Some relationships between identification, thinking styles, psychopathology and dominance-submissiveness. *British Journal of Psychiatry*, **118**, 29–42.

Munsinger, H. (1975). The adopted child's IQ: A critical review. *Psychological Bulletin*, **82**, 623–659.

National Institutes of Health. (2008). Genome-wide association studies. Retrieved on August 31, 2008, from http://www.genome.gov/20019523.

Neel, J. V., & Schull, W. J. (1954). *Human heredity*. Chicago: University of Chicago Press.

Neisser, U., Boodoo, G., Bouchard, T. J., Jr., Boykin, A. W., Brody, N., Ceci, S. J., et al. (1996). Intelligence: Knowns and unknowns. *American Psychologist*, **51**, 77–101.

Newman, H. H., Freeman, F. N., & Holzinger, K. J. (1937). *Twins: A study of heredity and environment*. Chicago: The University of Chicago Press.

Page, G. P., Varghese, G., Go, R. C., Page, P. Z., & Allison, D. B. (2003). "Are we there yet?": Deciding when one has demonstrated specific genetic causation in complex diseases and quantitative traits. *American Journal of Human Genetics*, **73**, 711–719.

Paikin, H., Jacobsen, B., Schulsinger, F., Godtfredsen, K., Rosenthal, D., Wender, P. H., et al. (1974). Characteristics of people who refused to participate in a social and psychopathological study. In S. Mednick, F. Schulsinger, J. Higgins, & B. Bell (Eds.), *Genetics, environment and psychopathology* (pp. 293–322). New York: American Elsevier Publishing Company.

Pam, A. (1995). Biological psychiatry: Science or pseudoscience? In C. Ross & A. Pam (Eds.), *Pseudoscience in biological psychiatry: Blaming the body* (pp. 7–84). New York: Wiley.

Pam, A., Kemker, S. S., Ross, C. A., & Golden, R. (1996). The "equal environment assumption" in MZ-DZ comparisons: An untenable premise of psychiatric genetics? *Acta Geneticae Medicae et Gemellologiae*, **45**, 349–360.

Pauls, D. L. (2008). The genetics of obsessive compulsive disorder: A review of the evidence. *American Journal of Medical Genetics (Part C, Semin Med Genet)*, **148C**, 133–139.

Pearson, T. A., & Manolio, T. A. (2008). How to interpret a genome-wide association study. *JAMA*, **299**, 1335–1344.

Pedersen, N. L., McClearn, G. E., Plomin, R., & Nesselroade, J. R. (1992). Effects of early rearing environment on twin similarity in the last half of the life span. *British Journal of Developmental Psychology*, **10**, 255–267.

Pedersen, N. L., Plomin, R., McClearn, G. E., & Friberg, L. (1988). Neuroticism, extraversion, and related traits in adult twins reared apart and reared together. *Journal of Personality and Social Psychology*, **55**, 950–957.

Pedersen, N. L., Plomin, R., Nesselroade, J. R., & McClearn, G. E. (1992). A quantitative genetic analysis of cognitive abilities during the second half of the life span. *Psychological Science*, **3**, 346–353.

Petrill, S. A., Lipton, P. A., Hewitt, J. K., Plomin, R., Cherny, S. S., Corely, R., et al. (2004). Genetic and environmental contributions to general cognitive ability through the first 16 years of life. *Developmental Psychology*, **40**, 805–812.

Phillips, D. I. W. (1993). Twin studies in medical research: Can they tell us whether diseases are genetically determined? *Lancet*, **341**, 1008–1009.

Pinker, S. (2002). *The blank slate*. New York: Viking.

Plomin, R., Corley, R., Caspi, A., Fulker, D. W., & DeFries, J. C. (1998). Adoption results for self-reported personality: Evidence for nonadditive genetic effects? *Journal of Personality and Social Psychology*, **75**, 211–218.

Plomin, R., & DeFries, J. C. (1985). *Origins of individual differences in infancy: The Colorado Adoption Project*. Orlando, FL: Academic Press.

Plomin, R., DeFries, J. C., McClearn, G. E., & McGuffin, P. (2008). *Behavioral genetics* (5th ed.). New York: Worth Publishers.

Plomin, R., Fulker, D. W., Corley, R., & DeFries, J. C. (1997). Nature, nurture and cognitive development from 1–16 years: A parent-offspring adoption study. *Psychological Science*, **6**, 442–447.

Plomin, R., McClearn, G. E., Smith, D. L., Vignetti, S., Chorney, M. J., Chorney, K., et al. (1995). DNA markers associated with high versus low IQ: The IQ quantitative trait loci (QTL) project. *Behavior Genetics*, **24**, 107–118.

Plomin, R., & Rutter, M. (1998). Child development, molecular genetics, and what to do with genes once they are found. *Child Development*, **69**, 1223–1242.

Potash, J. B., & DePaulo, J. R., Jr. (2000). Searching high and low: A review of the genetics of bipolar disorder. *Bipolar Disorders*, **2**, 8–26.

Proctor, R. N. (1988). *Racial hygiene: Medicine under the Nazis*. Cambridge, MA: Harvard University Press.

Propping, P. (2005). The biography of psychiatric genetics: From early achievements to historical burden, from an anxious society to critical geneticists. *American Journal of Medical Genetics (Part B, Neuropsychiatric Genetics)*, **136B** (1), 2–7.

Psychiatric GWAS Consortium Steering Committee. (2009). A framework for interpreting genome-wide association studies of psychiatric disorders. *Molecular Psychiatry*, **14**, 10–17.

Ratner, C. (2004). Genes and psychology in the news. *New Ideas in Psychology*, **22**, 29–47.

Read, J., Fink, P. J., Rudegeair, T., Felitti, V., & Whitfield, C. L. (2008). Child maltreatment and psychosis: A return to a genuinely integrated bio-psycho-social model. *Clinical Schizophrenia and Related Psychoses*, **2**, 235–254.

Read, J., Mosher, L., & Bentall, R. (Eds.). (2004). *Models of madness: Psychological, social and biological approaches to schizophrenia*. Andover, UK: Taylor & Francis.

Reichborn-Kjennerud, T. (2008). Genetics of personality disorders. *Psychiatric Clinics of North America*, **31**, 421–40.

Richardson, K. (1998). *The origins of human potential*. London: Routledge.

Richardson, K. (2000). *The making of intelligence*. New York: Columbia University Press.

Richardson, K., & Norgate, S. (2005). The equal environment assumption of classical twin studies may not hold. *British Journal of Educational Psychology*, **75**, 1–13.

Richardson, K., & Norgate, S. (2006). A critical analysis of IQ studies of adopted children. *Human Development*, **49**, 319–335.

Ridley, M. (2003). *The agile gene: How nature turns on nurture* [Originally published as Nature via nurture]. Perennial. New York:

Risch, N., Herrell, R., Lehner, T., Liang, K., Eaves, L., Hoh, J., et al. (2009). Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression. *Journal of the American Medical Association*, **301**, 2462–2471.

Rose, R. J. (1982). Separated twins: Data and their limits [Review of the book *Identical twins reared apart: A reanalysis*]. *Science*, **215**, 959–960.

Rosenthal, D. (1960). Confusion of identity and the frequency of schizophrenia in twins. *Archives of General Psychiatry*, **3**, 297–304.

Rosenthal, D. (1961). Sex distribution and the severity of illness among samples of schizophrenic twins. *Journal of Psychiatric Research*, **1**, 26–36.

Rosenthal, D. (1962a). Familial concordance by sex with respect to schizophrenia. *Psychological Bulletin*, **59**, 401–421.

Rosenthal, D. (1962b). Problems of sampling and diagnosis in the major twin studies of schizophrenia. *Journal of Psychiatric Research*, **1**, 116–134.

Rosenthal, D. (1968). The heredity-environment issue in schizophrenia: Summary of the conference and present status of our knowledge. In D. Rosenthal & S. Kety (Eds.), *The transmission of schizophrenia* (pp. 413–427). New York: Pergamon Press.

Rosenthal, D. (1971). *Genetics of psychopathology*. New York: McGraw-Hill.

Rosenthal, D. (1979). Genetic factors in behavioural disorders. In M. Roth & V. Cowie (Eds.), *Psychiatry, genetics and pathography: A tribute to Eliot Slater* (pp. 22–33). London: Oxford University Press.

Rosenthal, D., Wender, P. H., Kety, S. S., Schulsinger, F., Welner, J., & Østergaard, L. (1968). Schizophrenics' offspring reared in adoptive homes. In D. Rosenthal & S. Kety (Eds.), *The transmission of schizophrenia* (pp. 377–391). New York: Pergamon Press.

Rosenthal, D., Wender, P. H., Kety, S. S., Welner, J., & Schulsinger, F. (1971). The adopted-away offspring of schizophrenics. *American Journal of Psychiatry*, **128**, 307–311.

Rowe, D. C. (1994). *The limits of family influence: Genes, experience, and behavior*. New York: The Guilford Press.

Rowe, D. C., & Jacobson, K. C. (1999). In the mainstream. In R. Carson & M. Rothstein (Eds.), *Behavioral genetics: The clash of culture and biology* (pp. 12–34). Baltimore: The Johns Hopkins University Press.

Rüdin, E. (1916). *Zur Vererbung und Neuentstehung der Dementia Praecox* [On the heredity and new development of dementia praecox]. Berlin: Springer Verlag OHG.

Rutter, M. (2001). Child psychiatry in the era following sequencing the genome. In F. Levy & D. Hay (Eds.), *Attention, genes, and ADHD* (pp. 225–248). East Sussex, UK: Brunner-Routledge.

Rutter, M. (2006). *Genes and behavior: Nature-nurture interplay explained*. Malden, MA: Blackwell.

Scarr, S. (1968). Environmental bias in twin studies. *Eugenics Quarterly*, **15**, 34–40.

Scarr, S., & Carter-Saltzman, L. (1979). Twin method: Defense of a critical assumption. *Behavior Genetics*, **9**, 527–542.

Scarr, S., & Weinberg, R. A. (1976). IQ test performance of Black children adopted by White families. *American Psychologist*, **31**, 726–739.

Scarr, S., & Weinberg, R. A. (1978). The influence of "family background" on intellectual attainment. *American Sociological Review*, **43**, 674–692.

Schiff, M., Duyme, M., Dumaret, A., & Tomkiewicz, S. (1982). How much *could* we boost scholastic achievement and IQ scores? A direct answer from a French adoption study. *Cognition*, **12**, 165–196.

Schiff, M., & Lewontin, R. C. (1986). *Education and class: The irrelevance of IQ genetic studies*. Oxford: Clarendon Press.

Schönemann, P. H. (1997). On models and muddles of heritability. *Genetica*, **99**, 97–108.

Shields, J. (1954). Personality differences and neurotic traits in normal twin schoolchildren. *Eugenics Review*, **45**, 213–246.

Shields, J. (1962). *Monozygotic twins brought up apart and brought up together*. London: Oxford University Press.

Shore, A. N. (2001). Effects of a secure attachment relationship on right brain development, affect regulation, and infant mental health. *Infant Mental Health Journal*, **22**, 7–66.

Siegel, D. J. (1999). *The developing mind: Toward a neurobiology of interpersonal experience*. New York: Guilford.

Siegel, D. J. (2001). Toward an interpersonal neurobiology of the developing mind: Attachment relationships, "mindsight," and neural integration. *Infant Mental Health Journal*, **22**, 67–94.

Skodak, M., & Skeels, H. M. (1949). A final follow-up study of one hundred adopted children. *Journal of Genetic Psychology*, **75**, 85–125.

Smoller, J. W., Gardner-Schuster, E., & Covino, J. (2008). The genetic basis of panic and phobic anxiety disorders. *American Journal of Medical Genetics (Part C, Semin Med Genet)*, **148C**, 118–126.

Solomon, J. (2007, November 18). FBI's forensic test full of holes. *Washington Post*, A01.

Sprich, S., Biederman, J., Crawford, M. H., Mundy, E., & Faraone, S. V. (2000). Adoptive and biological families of children and adolescents with ADHD. *Journal of the American Academy of Child and Adolescent Psychiatry*, **39**, 1432–1437.

Stoltenberg, S. F. (1997). Coming to terms with heritability. *Genetica*, **99**, 89–96.

Stoolmiller, M. (1998). Correcting estimates of shared environmental variance for range restriction in adoption studies using a truncated multivariate normal model. *Behavior Genetics*, **28**, 429–441.

Stoolmiller, M. (1999). Implications of the restricted range of family environments for estimates of heritability and nonshared environment in behavior-genetic adoption studies. *Psychological Bulletin*, **125**, 392–409.

Sullivan, P. F., Lin, D., Tzeng, J. Y., van den Oord, E., Perkins, D., Stroup, T. S., et al. (2008). Genomewide association for schizophrenia in the CATIE study: Results of stage 1. *Molecular Psychiatry*, **13**, 570–584.

Taylor, H. F. (1980). *The IQ game: A methodological inquiry into the heredity-environment controversy*. New Brunswick, NJ: Rutgers University Press.

Tellegen, A., Lykken, D. T., Bouchard, T. J., Jr., Wilcox, K. J., Segal, N. L., & Rich, S. (1988). Personality similarity in twins reared apart and together. *Journal of Personality and Social Psychology*, **54**, 1031–1039.

Tienari, P., Sorri, A., Lahti, I., Naarala, M., Wahlberg, K., Moring, J., et al. (1987). Genetic and psychosocial factors in schizophrenia: The Finnish adoptive family study. *Schizophrenia Bulletin*, **13**, 477–484.

Tienari, P., Wynne, L. C., Läksy, K., Moring, J., Nieminen, P., Sorri, A., et al. (2003). Genetic boundaries of the schizophrenia spectrum: Evidence from the Finnish Adoptive Family Study. *American Journal of Psychiatry*, **160**, 1587–1594.

Torrey, E. F. (1990). Offspring of twins with schizophrenia [Letter to the editor]. *Archives of General Psychiatry*, **47**, 976–977.

Torrey, E. F., Bowler, A. E., Taylor, E. H., & Gottesman, I. I. (1994). *Schizophrenia and manic–depressive disorder: The biological roots of mental illness as revealed by the landmark study of identical twins*. New York: Basic Books.

Tully, E. C., Iacono, W. G., & McGue, M. (2008). An adoption study of parental depression as an environmental liability for adolescent depression and childhood disruptive disorders. *American Journal of Psychiatry*, **165**, 1148–1154.

Volkmar, F. R., & Pauls, D. (2003). Autism. *Lancet*, **362**, 1133–1141.

Wacholder, S., Chanock, S., Garcia-Closas, M., El ghormli, L., & Rothman, N. (2004). Assessing the probability that a positive report is false: An approach for molecular epidemiology studies. *Journal of the National Cancer Institute*, **96**, 434–442.

Wahlsten, D. (1990). Insensitivity of the analysis of variance to heredity-environment interaction. *Behavioral and Brain Sciences*, **13**, 109–120.

Wahlsten, D. (1994). The intelligence of heritability. *Canadian Psychology*, **35**, 244–259.

Waldman, I. D., & Gizer, I. A. (2006). The genetics of attention deficit hyperactivity disorder. *Clinical Psychology Review*, **26**, 396–432.

Walker, E., Downey, G., & Caspi, A. (1991). Twin studies of psychopathology: Why do concordance rates vary? *Schizophrenia Research*, **5**, 211–221.

Walker, E., & Emory, E. (1985). Commentary: Interpretive bias and behavioral genetic research. *Child Development*, **56**, 775–778.

Weissman, M. M., Merikangas, K. R., John, P., Wickramaratne, P., Prusoff, B. A., & Kidd, K. K. (1986). Family-genetic studies of psychiatric disorders. *Archives of General Psychiatry*, **43**, 1104–1116.

Wender, P. H., Kety, S. S., Rosenthal, D., Schulsinger, F., Ortmann, J., & Lunde, I. (1986). Psychiatric disorders in the biological and adoptive families of adopted individuals with affective disorders. *Archives of General Psychiatry*, **43**, 923–929.

Wender, P. H., Rosenthal, D., & Kety, S. S. (1968). A psychiatric assessment of the adoptive parents of schizophrenics. In D. Rosenthal & S. Kety (Eds.), *The transmission of schizophrenia* (pp. 235–250). New York: Pergamon Press.

Wender, P. H., Rosenthal, D., Kety, S. S., Schulsinger, F., & Welner, J. (1974). Crossfostering: A research strategy for clarifying the role of genetic and experiential factors in the etiology of schizophrenia. *Archives of General Psychiatry*, **30**, 121–128.

World Health Organization. (1966). The use of twins in epidemiological studies. *Acta Geneticae Medicae et Gemellologiae*, **15**, 109–128.

Wright, L. (1997). *Twins: And what they tell us about who we are*. New York: Wiley.

Wright, W. (1998). *Born that way*. New York: Alfred A. Knopf.

Zerbin-Rüdin, E. (1972). Genetic research and the theory of schizophrenia. *International Journal of Mental Health*, **1**, 42–62.

19

On the Limits of Standard Quantitative Genetic Modeling of Inter-Individual Variation

Extensions, ergodic conditions and a new genetic factor model of intra-individual variation

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Introduction

This chapter has two distinct purposes. First, a review is given of work exploring the boundaries of the standard genetic factor model underlying much of applied quantitative genetic modeling. This review includes aspects of longitudinal genetic modeling, testing for interactions between genetic and environmental factors, and genetic decomposition of mean differences. It summarizes innovative work in which I have been involved before my perspective on standard quantitative genetic modeling changed substantially when I discovered the fundamental implications of a general mathematical theorem about stochastic processes – the so-called individual ergodic theorem of Birkhoff (1931). These implications invalidate the quantitative genetic analysis based on inter-individual variation of a large class of phenotypic processes, the so-called non-ergodic processes. Instead it is necessary to base quantitative genetic modeling of non-ergodic phenotypic processes on intra-individual variation (time series analysis). In the second part of this chapter the implications of the individual ergodic theorem will be explained at some length, including an excursion to relevant results of mathematical modeling of nonlinear epigenetic processes and possible solutions to arrive at valid quantitative genetic

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analyses of non-ergodic processes. The discussion of these solutions will touch upon an entirely innovative approach to carry out quantitative genetic modeling at the level of individual twin pairs.

Aspects of the Standard Genetic Factor Model

Perhaps one of the most widely used quantitative genetic models for the analysis of multivariate phenotypic data obtained with MZ and DZ twins is the standard genetic factor model proposed by Martin & Eaves (1977). In its elementary form this model consists of three common factors: an additive genetic factor, a common environmental factor and a specific environmental factor. This particular interpretation of the factors is guaranteed by the *a priori* fixed pattern of factor correlations.

In what follows the following notation will be used: lower-case bold face letters denote (column) vectors, upper-case bold-face letters denote matrices, Greek letters denote model parameters. Let y_{ijkm} denote the observed phenotypic score at the mth observed variable ($m = 1,2, \ldots, M$) for the jth member ($j = 1,2$) of the ith twin pair of type k ($k = 1$ for MZ and $k = 2$ for DZ). Martin & Eaves' (1977) genetic factor model is defined as:

$$y_{ijkm} = \alpha_m A_{ijk} + \delta_m \Delta_{ijk} + \phi_m \Phi_{ijk} + \varepsilon_{ijkm} \qquad (19.1)$$

In (19.1) A_{ijk} is the additive genetic factor score of the jth member of the ith twin pair of type k; α_m is the factor loading of the mth phenotypic variable on A_{ijk}; Δ_{ijk} is the common environmental factor score of the jth member of the ith twin pair of type k; δ_m is the factor loading of the mth phenotypic variable on Δ_{ijk}; $\mathbf{\Phi}_{ijk}$ is the specific environmental factor score of the jth member of the ith twin pair of type k; ϕ_m is the factor loading of the mth phenotypic variable on $\mathbf{\Phi}_{ijk}$; ε_{ijkm} is the measurement error associated with the mth phenotypic score of the jth member of the ith twin pair of type k.

Model (19.1) is completed with the following constraints. For MZ twins ($k = 1$): $\text{cor}[A_{i11}, A_{i21}] = 1$; $\text{cor}[\Delta_{i11}, \Delta_{i21}] = 1$; $\text{cor}[\mathbf{\Phi}_{i11}, \mathbf{\Phi}_{i21}] = 0$. For DZ twins ($k = 2$): the average correlation between the additive genetic factor scores of the two members of DZ twin pairs is .5, that is, $\text{cor}[A_{i12}, A_{i22}] = .5$. In addition, $\text{cor}[\Delta_{i12}, \Delta_{i22}] = 1$; $\text{cor}[\mathbf{\Phi}_{i12}, \mathbf{\Phi}_{i22}] = 0$. Finally, for different phenotypic variables m and n, the correlation of measurement errors always equals zero: $\text{cor}[\varepsilon_{ijkm}, \varepsilon_{ijkn}] = 0$.

The (2M,2M)-dimensional covariance matrices $\sum_k$ for MZ ($k = 1$) and DZ ($k = 2$) twins associated with (19.1) and (19.2) is:

$$\mathbf{\Sigma}_k = \mathbf{\Lambda \Psi}_k \mathbf{\Lambda} + \mathbf{\Theta} \qquad (19.2)$$

In (19.2) Λ is the (M,6)-dimensional matrix of factor loadings with the following block structure: $\Lambda = \{\Lambda_{ab}, a,b = 1,2\}$, where $\Lambda_{11} = \Lambda_{22}$ are identical (M,3)-dimensional sub-matrices and $\Lambda_{12} = \Lambda_{12}$ are (M,3)-dimensional sub-matrices with zero entries. The first M-variate column of Λ_{11} and Λ_{22} consists of the M additive genetic loadings α_m, $m = 1,2,\ldots,M$; the second M-variate column of Λ_{11} and Λ_{22} consists of the M common environmental loadings δ_m, $m = 1,2,\ldots,M$; and the third M-variate column of Λ_{11} and Λ_{22} consists of the M specific environmental loadings ϕ_m, $m = 1,2,\ldots,M$. Θ is the (2M,2M)-dimensional covariance matrix of the measurement errors ε_{ijkm}. Only the diagonal elements of Θ, that is, the variances of the measurement errors, are nonzero; the mth variance being equal to the $(m + M)$th variance, $m = 1,2,\ldots,M$. Finally, the diagonal elements of the (6,6)-dimensional covariance matrices Ψ_k, $k = 1,2$, are all equal to 1. For MZ twins ($k = 1$), the only nonzero off-diagonal elements of Ψ_1 are: $\psi_1(4,1) = \text{cor}[A_{i11}, A_{i21}] = 1$ and $\psi_1(5,2) = \text{cor}[\Delta_{i11}, \Delta_{i21}] = 1$ (notice that Ψ_1 is a symmetrical matrix). For DZ twins ($k = 2$) the only nonzero off-diagonal elements of Ψ_2 are: $\psi_2(4,1) = \text{cor}[A_{i12}, A_{i22}] = .5$ and $\psi_2(5,2) = \text{cor}[\Delta_{i12}, \Delta_{i22}] = 1$ (notice that Ψ_2 is a symmetrical matrix).

Fitting (19.2) to Phenotypic Data

Evidently, (19.2) defines a structural equation model. Boomsma & Molenaar (1986) were the first to fit (19.2) to multivariate phenotypic twin data by means of standard structural equation modeling software. Since the excellent computer program Mx (Neale, Walters, Eaves, Maes, & Kendler, 1994) became available, it has rightly become the preferred tool to fit models like (19.2) to multivariate phenotypic data.

Genetic and Environmental Factor Scores

To reiterate, (19.2) is a structural equation model, more specifically a multi-group confirmatory oblique factor model. The only feature which distinguishes the genetic factor model from other factor models is that the correlations between the factors are known *a priori*. The correlation between an additive genetic factor and a common environmental factor equals zero, which also is the case for the correlation between an additive genetic factor and a specific environmental factor and the correlation between a common environmental factor and a specific environmental factor. The correlation between the additive genetic factors within MZ twin pairs equals 1 and within DZ twins pairs equals 0.5; the correlation between the common environmental factors within MZ and DZ twin pairs equals 1; the correlation between the specific environmental factors within MZ an DZ twin pairs equals zero. This fixed pattern of factor correlations ensures that the factors have unique interpretations, but does not affect the status of (19.2) as a factor model. Because it is possible to estimate the factor scores for each individual subject based on any factor

model, this also holds for (19.2). Boomsma, Molenaar & Orlebeke (1990) were the first to exploit this possibility and estimate the additive genetic, common environmental and specific environmental factor scores for individual subjects.

Longitudinal Genetic Modeling

Each factor model defined at a single measurement occasion can be extended into a longitudinal factor model defined at consecutive measurement occasions. Consequently, (19.1)-(19.2) also can be extended into a longitudinal genetic factor model defined at multiple measurement occasions. Boomsma & Molenaar (1987) were the first to accomplish this for univariate phenotypes repeatedly measured at a limited number of occasions; Molenaar & Boomsma (1987a) were the first to extend this to time series data measured at numerous occasions. An alternative longitudinal genetic factor model was proposed by Hewitt, Eaves, Neale, & Meyer (1988), but was shown to be nested under the Boomsma & Molenaar (1987) model by Rovine & Molenaar (2005).

Genetic Decomposition of Mean Differences

Conceived of as structural equation models, genetic factor models explaining individual phenotypic differences can be extended in order to also explain mean differences between different groups (e.g., males and females). Dolan, Molenaar & Boomsma (1989, 1992, 1994) were the first to accomplish this for cross-sectional multivariate twin data as well as longitudinal twin data (Dolan, Molenaar, & Boomsma, 1991).

Interactions between Latent Genetic and Environmental Factors

The final innovative extension of the genetic factor model (19.1)-(19.2) in which I was involved concerned detection of the presence of interactions of the types $A \times \Delta$ (interaction between additive genetic and common environmental factors), $A \times \Phi$ (interaction between additive genetic and specific environmental factors), and $\Delta \times \Phi$ (interaction between common and specific environmental factors). In Molenaar & Boomsma (1987b) nonlinear factor analysis was used to accomplish this. In Molenaar, Boomsma, Neeleman, & Dolan (1990) a new test was derived based on: a) application of the standard genetic factor model (19.2) to multivariate phenotypic twin data; b) estimation of the additive genetic, common environmental and specific environmental factors scores as indicated in the *Genetic and environmental factor scores* section; and c) application of new statistical tests based on the kurtosis of factor scores to determine to which extent interactions are present.

Problems with the Standard Genetic Factor Model

Interpretation of the standard genetic factor model as a specific instance of a structural equation model leads in a natural way to several important extensions which were reviewed in the previous sections. This interpretation in terms of structural equation modeling conceives of the standard genetic factor model as a particular statistical model, neglecting the specific interpretations of its latent factors. The interpretations of the latent factors in (19.2) derive from the *a priori* fixed pattern of factor correlations which have been specified in the *Genetic and environmental factor scores* section. Notice that this way of assigning interpretations to latent factors in a factor model is nonstandard. The standard way of interpreting latent factors is based on the pattern of factor loadings in the matrix Λ. That is, each factor is assigned an interpretation based on the contents of observed variables which have large positive or negative loadings on this factor, while having small loadings on the remaining factors. If this particular pattern of factor loadings obtains, the loadings are said to display simple structure. Factor rotation is a standard *a posteriori* option in exploratory factor analysis which is aimed at establishing simple structure, thus enabling unambiguous interpretation of the factors.

Given the nonstandard way to ascertain the interpretation of latent factors in (19.2) as being additive genetic, common and specific environmental, the question arises whether these interpretations indeed are warranted. It is obvious that the *a priori* fixed pattern of correlations underlying the nonstandard way to interpret the factors in (19.2) is beyond criticism. The within-twins correlations of additive genetic factors are a direct consequence of Mendel's laws and Fisher's (1918) groundbreaking paper. The within-twins correlations of common environmental factors and specific environmental factors are self-evident. Consequently the *a priori* fixed pattern of correlations in (19.2) cannot be improved upon and would seem to warrant the interpretation of the latent factors.

But we already encountered problems regarding the correct interpretation of latent factors in (19.2) at a different level. Earlier, reference was made to the possibility that, for instance, an additive genetic factor in (19.2) in reality is a combination of an additive genetic factor and an interaction between the additive genetic factor and the common environmental factor. In this case we have the following scenario. An instance of (19.2) is fitted to the multivariate phenotypic data and yields an acceptable fit (it was shown in Molenaar et al. (1990), that the presence of interactions between the latent factors in (19.2) does not seriously affect the multivariate normality of the phenotypic data). The usual procedure then is to determine *a posteriori* the narrow-sense heritability of each of the M univariate phenotypes as follows:

$$(h_m)^2 = (\alpha_m)^2/[(\alpha_m)^2 + (\delta_m)^2 + (\phi_m)^2],\ m = 1,\ 2, \ldots,\ M \qquad (19.3)$$

Similar indices can be determined for the effects of common environmental and specific environmental factors. This would conclude the genetic analysis based on the standard genetic factor model.

We supposed, however, that the additive genetic factor A identified by applying the standard genetic factor (19.1)-(19.2) consists in reality of the following composition: $\pi A + (1 - \pi)(A \times \Delta)$, where A and Δ denote, respectively, the additive genetic factor and the common environmental factor and where π is an unknown proportion $0 \leq \pi \leq 1$. It was shown in Molenaar et al. (1990) how to estimate π using the fourth-order central moments of the factor scores. If it is found that $\pi > 0$, then the usual interpretation that A is a (pure) additive genetic factor is incorrect. Moreover, the determination of the narrow-sense heritability according to (19.3) will be biased in the sense that the true narrow-sense heritability will be lower. This bias is a monotonic increasing function of π. Similar remarks apply to the cases in which $A \times \Phi$ and/or $\Delta \times \Phi$ interactions are present.

Consequently, a satisfactorily fitting instance of (19.2) does not guarantee that the factors thus determined have their nominal interpretations (additive genetic, common environmental and specific environmental). It may be the case that interactions between genetic and environmental factors are present, making up unknown components of the nominal genetic and environmental factors. The standard likelihood ratio tests of goodness-of-fit appear to be insensitive to the presence of such interactions (cf. Molenaar et al., 1990). Special *a posteriori* tests are required to estimate the degrees to which the nominal factors are confounded by these interactions and thus enable the determination of purified genetic and environmental factors.

What are Specific Environmental Influences?

Problems with determining the correct interpretation of the factors in the standard genetic factor model are not confined to the confounding effects of interactions. In particular the specific environmental factors give rise to additional interpretational problems of a profound nature. For many, if not most, phenotypic variables it is found that specific environmental influences explain a substantial proportion of the inter-individual variation, often exceeding 50% of the total variation. Hence specific environmental factors are dominant causes of inter-individual variation. Yet, sustained efforts to identify the nature of these environmental factors (cf. Plomin & Daniels, 1987) have not been successful. Candidate causes such as birth order explain at most only a few percent of the variation. Hence, despite their importance in explaining inter-individual differences, the nature of specific environmental influences appears to be an enigma.

Why is it so difficult to determine the identity of those environmental influences which (to paraphrase the title of the important paper by Plomin & Daniels, 1987) make children within the same family different from each other? The answer put

forward by Molenaar, Boomsma, & Dolan (1993) is that the alleged specific environmental influences causing these differences may not be environmental at all, but instead pertain to the actions of nonlinear developmental processes. They refer to converging evidence obtained in inbreeding studies, which indicates that the influences concerned constitute a third source of variation, in addition to genetic and environmental influences. For instance in Sewall Wright's (1920) first published path diagram, more than 91% of the total variation in fur coloration of an inbred line of guinea pigs is explained by what is called "developmental irregularity". Also, Mather & Jinks (1977, p. 6) report that 91% of the total variation in the number of chaetae between the left and right side of the thorax of an inbred line of *Drosophila melanogaster* is caused by what is called "the vagaries of development." Another impressive result is presented by Gaertner (1990) who reports the failure of 30 years of concentrated attempts to standardize inbred strains of mice, employing highly standardized husbandry to effectively eliminate environmental variability. He concludes that at least 80% of the range of body weight in inbred mice is due to a third source, in addition to genetic and environmental components.

While in inbreeding studies it is possible to identify the cause of phenotypic variation as being associated to development, independent of genetic and environmental influences, this is impossible in quantitative genetic analyses of human phenotypes. In applications of the standard genetic factor model (19.2) to human phenotypic twin data, the variation caused by "developmental irregularity" or "the vagaries of development" is misallocated as being caused by specific environmental factors. Hence this variation, caused by a third source in addition to genetic and environmental influences, constitutes a confounding factor for specific environmental influences. And it has to be considered a strongly confounding factor given that it explains large proportions of the total variance in inbreeding studies.

General Problems with Analyses of Inter-Individual Variation

Quantitative genetic modeling, in particular standard genetic factor modeling using (19.2), only applies to inter-individual variation, that is, individual differences in a presumably homogeneous population of human subjects. This is generally considered to be the essential kind of variation for Darwinian evolution to occur. Analysis of inter-individual variation is the dominant approach to data analysis in psychology. For instance, analysis of variance, regression analysis, factor analysis, latent growth curve modeling, mixture analysis, etc., are all focused on inter-individual differences in (sub-)populations of subjects. A random sample of subjects is drawn from a presumably homogeneous population of interest and consecutively the variation between these subjects is employed to carry out statistical tests about the true state of affairs in the population (generalization from sample

to population of subjects). The focus may be on mean differences between (sub-)populations (like in analysis of variance) or on a description of the structure of individual differences in a population (like in factor analysis). But it is always the state of affairs at the population level that is of prime interest in standard psychological data analysis and the source of information for such states of affairs is inter-individual variation. This information is obtained by pooling the data of sampled subjects. Pooling across subjects is the hallmark of analysis of inter-individual variation.

Variation in itself, considered quite independently from issues of modeling and statistical analysis, exists in two different forms: inter-individual variation and intra-individual variation. The latter form, intra-individual variation, will be understood as pertaining to time-dependent differences occurring along a single subject's life trajectory. Hence intra-individual variation is associated with single-subject time series and implies a dynamic process perspective. Intra-individual variation thus defined is of interest to Lamarckian evolution (cf. Jablonka & Lamb, 1995, 2005) and to the study of interactions between evolution and development (evo-devo; cf. Robert, 2004).

Until recently, analysis of intra-individual variation (time series analysis) did not constitute a prominent approach in psychology. There do not exist principled reasons for this neglect in psychology of one of the two forms of variation, only historic contingencies (cf. Danziger, 1990; Lamiell, 2003). It was assumed without much further reflection that states of affairs at the level of a population of subjects would generalize to equivalent states of affairs at the level of a single subject's life trajectory in case that subject belonged to the population concerned. That is, it was assumed that the structure of inter-individual variation in a homogeneous population of subjects is equivalent to the analogous structure of intra-individual variation at the level of each individual subject belonging to that population. Given these assumptions (homogeneity of subjects and equivalence of the structure of variation at the population and individual levels), it would seem to follow that it is sufficient to focus on the structure of inter-individual variation and generalize the results thus obtained to the level of intra-individual variation characterizing individual subjects. For instance, if inter-individual differences in personality obey a five-factor structure at the population level, and the population is homogeneous (i.e., subjects are exchangeable), then the structure of intra-individual variation characterizing each individual subject also would seem to have to obey the same five-factor structure.

As will be explained shortly, the assumption about the equivalence of a structure of inter-individual variation at the population level and the analogous structure of intra-individual variation at the individual level is referred to as the *ergodic* assumption. It is reiterated that the ergodic assumption underlies all standard statistical analyses techniques in psychology. These techniques only use information provided by inter-individual variation. As soon as results thus obtained are generalized to the level of individual subjects (e.g., in individual assessment or

prediction), it is assumed that the ergodic assumption holds. Almost always the ergodic assumption is not stated or tested explicitly, but that does not mean that it is not required to make generalizations from states of affairs obtaining at the population level to the level of single subjects.

The denotation "ergodic" is inspired by the so-called classical ergodic theorems (cf. Krengel, 1985). These theorems, in particular Birkhoff's individual ergodic theorem (Birkhoff, 1931), imply general mathematical conditions that have to be met by any measurable dynamic process in order to guarantee that the population structure of inter-individual variation can be validly generalized to the level of intra-individual variation, and vice versa. To ease the presentation, attention will be restricted to Gaussian (normally distributed) dynamic processes. Any ergodic Gaussian process has to obey the following two conditions (Molenaar, 2004):

- (19.4a) The Gaussian process has to be *stationary*. This implies: a) that the mean of the process has to be constant in time; b) that the variance of the process has to be constant in time; and c) that the sequential dependencies characterizing the process only depend upon the relative distance (lag) between time points.
- (19.4b) The Gaussian process also has to be *invariant across subjects*. This implies that each subject in the population has to obey the same dynamic model.

Please notice that the two conditions are independent of each other. That is, a process may obey (19.4a) without obeying (19.4b), or vice versa. In both of these cases the process is non-ergodic, as it has to obey both conditions in order to be ergodic.

The requirement that ergodic processes have to be stationary immediately rules out developmental processes from being ergodic. Almost by definition developmental processes have time-varying means and/or time-varying sequential dependencies and therefore are non-ergodic. This implies that for developmental processes the structure of inter-individual variation at the population level is not equivalent to the structure of intra-individual variation at the level of individual subjects. Consequently, the proper level required to obtain valid information about developmental processes is the level of intra-individual variation within single subjects.

The latter conclusion that the proper level to investigate development is not the population level (inter-individual variation) but the level of individual subjects (intra-individual variation) corroborates an old insight within developmental theory. For example, Wohlwill's monograph on the study of behavioral development (Wohlwill, 1973) emphasizes the use of individual developmental functions while criticizing individual difference approaches. Similar remarks apply to Gottlieb's treatise on individual development and the genesis of novel behavior (Gottlieb, 1992) and to Ford & Lerner's seminal text on developmental systems theory (Ford & Lerner, 1992). Hence the conclusion itself is not new, only

the rationale of its defense is. My rationale is based on the individual ergodic theorem, a general mathematical theorem which pertains to all measurable processes, including psychological and biological processes. The theorem implies that it is necessary to study non-ergodic processes such as developmental processes at the level of intra-individual variation, that is, time series analysis, thus providing a formal proof of the validity of the insights of Wohlwill, Gottlieb, Ford, and Lerner.

When are Developmental Genetic Processes Ergodic?

Evidently, criterion (19.4a) has immediate consequences for the validity of developmental behavior genetics. For those phenotypic developmental processes which are non-stationary, standard analyses of inter-individual variation based on the longitudinal version of the standard genetic factor model will yield invalid results. And almost all developmental processes are non-stationary. Hence their analysis should not be based on inter-individual variation but intra-individual variation. This conclusion is in complete agreement with Gottlieb's important discussion of developmental behavioral genetics (Gottlieb, 2003). The remainder of this section is somewhat more technical and can be skipped on first reading without loss of continuity.

Perhaps the best way to convey the severity of criterion (19.4a) is to specify the special case of a standard longitudinal genetic factor model which is ergodic.

Let y_{ijkmt} denote the observed phenotypic score at the mth observed variable ($m = 1,2,\ldots,M$) for the jth member ($j = 1,2$) of the ith twin pair of type k ($k = 1$ for MZ and $k = 2$ for DZ) at the tth measurement occasion t ($t = 1,2,\ldots,T$). Then the longitudinal version of Martin & Eaves' (1977) genetic factor model is defined as:

$$y_{ijkmt} = \alpha_{mt}A_{ijkt} + \delta_{mt}\Delta_{ijkt} + \phi_{mt}\Phi_{ijkt} + \varepsilon_{ijkmt} \qquad (19.5a)$$

In (19.5a) A_{ijkt} is the additive genetic factor score of the jth member of the ith twin pair of type k at measurement occasion t; α_{mt} is the factor loading at measurement occasion t of the mth phenotypic variable on A_{ijkt}; Δ_{ijkt} is the common environmental factor score of the jth member of the ith twin pair of type k at measurement occasion t; δ_{mt} is the factor loading at measurement occasion t of the mth phenotypic variable on Δ_{ijkt}; Φ_{ijkt} is the specific environmental factor score of the jth member of the ith twin pair of type k at measurement occasion t; ϕ_{mt} is the factor loading at measurement occasion t of the mth phenotypic variable on Φ_{ijkt}; ε_{ijkmt} is the measurement error at occasion t associated with the mth phenotypic score of the jth member of the ith twin pair of type k.

(19.5a) defines the longitudinal version of (19.1) *at* each measurement occasion $t = 1,2,\ldots,T$. It has to be complemented with a set of equations describing

the relationships *between* consecutive measurement occasions. For $t = 2,\ldots,T$ these are:

$$\begin{aligned} A_{ijkt} &= \beta_{t,t-1} A_{ijkt-1} + \zeta_{ijkt} \\ \Delta_{ijkt} &= \gamma_{t,t-1} \Delta_{ijkt-1} + \xi_{ijkt} \\ \Phi_{ijkt} &= \nu_{t,t-1} \Phi_{ijkt-1} + \sigma_{ijkt} \end{aligned} \tag{19.5b}$$

In (19.5b) the first equation describes the relationship of the additive genetic factor score A_{ijkt} of the *j*th member of the *i*th twin pair of type *k* at measurement occasion *t* with this additive genetic factor score A_{ijkt-1} at the previous measurement occasion *t-1*; $\beta_{t,t-1}$ is the regression coefficient in this relationship; ζ_{ijkt} is the residual part of A_{ijkt} which cannot be predicted by A_{ijkt-1}. The second equation describes the relationship of the common environmental factor score Δ_{ijkt} of the *j*th member of the *i*th twin pair of type *k* at measurement occasion *t* with this common environmental factor score Δ_{ijkt-1} at the previous measurement occasion *t-1*; $\gamma_{t,t-1}$ is the regression coefficient in this relationship; ξ_{ijkt} is the residual part of Δ_{ijkt} which cannot be predicted by Δ_{ijkt-1}. The third equation describes the relationship of the specific environmental factor score Φ_{ijkt} of the *j*th member of the *i*th twin pair of type *k* at measurement occasion *t* with this specific environmental factor score Φ_{ijkt-1} at the previous measurement occasion *t-1*; $\nu_{t,t-1}$ is the regression coefficient in this relationship; σ_{ijkt} is the residual part of Φ_{ijkt} which cannot be predicted by Φ_{ijkt-1}.

According to criterion (19.4a) the standard longitudinal genetic factor (19.5a) should obey at least all of the following constraints in order not to be non-ergodic:

$$\begin{aligned} &\alpha_{mt} = \alpha_m \text{ for all } t = 1, 2, \ldots, T \\ &\delta_{mt} = \delta_m \text{ for all } t = 1, 2, \ldots, T \\ &\phi_{mt} = \phi_m \text{ for all } t = 1, 2, \ldots, T \\ &\mathrm{Var}[\varepsilon_{ijkmt}] \text{ is constant for each } m = 1, 2, \ldots, M \text{ across } t = 1, 2, \ldots, T \\ &\beta_{t,t-1} = \beta \text{ for } t = 2, \ldots, T \\ &\gamma_{t,t-1} = \gamma \text{ for } t = 2, \ldots, T \\ &\nu_{t,t-1} = \nu \text{ for } t = 2, \ldots, T \\ &\mathrm{Var}[\zeta_{ijkt}] \text{ is constant across } t = 2, \ldots, T \\ &\mathrm{Var}[\zeta_{ijk1}] = \mathrm{Var}[\zeta_{ijk2}]/(1 - \beta^2) \\ &\mathrm{Var}[\xi_{ijkt}] \text{ is constant across } t = 2, \ldots, T \\ &\mathrm{Var}[\xi_{ijk1}] = \mathrm{Var}[\xi_{ijk2}]/(1 - \gamma^2) \\ &\mathrm{Var}[\sigma_{ijkt}] \text{ is constant across } t = 2, \ldots, T \\ &\mathrm{Var}[\sigma_{ijk1}] = \mathrm{Var}[\sigma_{ijk2}]/(1 - \nu^2) \end{aligned} \tag{19.6}$$

It is evident that the list of constraints given by (19.6) is long and very restrictive. All factor loadings have to be invariant in time, all measurement error variances have

to be invariant in time, all relationships of factor scores between consecutive measurement occasions have to be invariant in time. The *a priori* chances are slim that these constraints will be met in applications to real data. If one or more of these constraints do not hold, then the phenotypic developmental process is non-ergodic. If the constraints hold, then the standard longitudinal factor model reduces to a model for phenotypic processes in steady state which normally would not be called developmental.

Heterogeneous Populations

The second ergodicity condition is (19.4b). This condition stipulates that the population of subjects has to be homogeneous. Stated more specifically, it is required that all subjects in the population have to obey exactly the same dynamic model. With respect to the standard longitudinal genetic factor model (19.5a) this implies that the same set of numerical values for the model parameters (factor loadings, regression coefficients, measurement error variances, etc.) applies for each subject in the population. This is also the implication of (19.4b) for the standard genetic factor model (19.1), which can be conceived of as the special case of (19.5a) restricted to a single measurement occasion.

In standard genetic analysis of inter-individual variation this criterion is called the homogeneity assumption: populations (or, in mixture modeling, the sub-populations) have to be homogeneous in that each subject obeys exactly the same statistical model with the same numerical values for the fixed model parameters. It is the homogeneity assumption which underlies pooling the data of sampled subjects which is typical of analyses of inter-individual variation. In particular, the homogeneity assumption is critical for the derivation of estimators for the parameters in the genetic factor models (19.1)-(19.2) and (19.5a). These estimators (e.g., maximum likelihood estimators) are derived on the explicit assumption that the population of subjects is homogeneous. Consequently, if this assumption is violated, that is, if the population is heterogeneous, one would expect that this gives rise to a bad fit of models (19.1)-(19.2) and (19.5a). It may come as a surprise (it certainly was a surprise for me when I first discovered it) that this is not at all the case.

In a series of simulation studies (e.g., Molenaar, 1997, 1999, 2007) heterogeneous data were generated according to several versions of factor models (including longitudinal and genetic factor models), where each "subject" had its own factor model. That is, the values of factor loadings, measurement error variances, etc., were subject-specific. This constitutes a severe violation of the homogeneity assumption which stipulates that these model parameters should be invariant across subjects. Notwithstanding these violations of the homogeneity assumption, standard factor analysis of the simulated heterogeneous data yielded excellently fitting models. Hence it turns out that standard factor analysis, including standard genetic factor analysis, is insensitive to violations of the homogeneity assumption.

Or, stated otherwise, standard factor analysis of inter-individual variation is insensitive to the presence of large-scale heterogeneity in the population. A formal proof of this result is presented in Kelderman & Molenaar (2007).

It is concluded that standard (longitudinal) genetic factor analysis of inter-individual phenotypic variation has very low power to detect the presence of heterogeneity in the population. A satisfactorily fitting standard genetic factor model is no guarantee that the data obey the homogeneity assumption underlying the model. It may be the case that the model fits the data very well while in reality the homogeneity assumption is severely violated. If the latter is the case, then evidently standard genetic factor analysis yields results which may not be valid for any individual subject in the population.

Nonlinear Epigenesis Creates Heterogeneity

The appropriate empirical approach to determine whether a population violates condition (19.4b) is to carry out single-subject and replicated time series analyses of intra-individual variation (cf. Hamaker, Dolan, & Molenaar, 2005). In addition there exist theoretical reasons why human populations should be expected to be heterogeneous. Of particular importance from a theoretical perspective are the neural networks underlying phenotypic psychological processes. The growth of neural networks is governed by nonlinear epigenetic processes. The mathematical-biological models explaining these epigenetic processes create endogenous variation that is neither due to genetic or environmental influences, but is caused by the nonlinear growth process itself. Hence these models explain the importance of third source variation introduced in the section *What are specific environmental influences?*

McLachlan (1999) presents eight types of influences, not directly specified by the genome, each inducing individual variation in individual developmental morphologies. These influences involve physical constraints such as minimum energy considerations, extra-chromosomal inheritance, environmental influences such as those determining polarity, self-organizing mechanisms (see below), influences of parental genotype, tissue self-assembly, tissue interaction, and specification by use. McLachlan concludes that due to such influences not directly specified by the genome, the morphology of each individual is different from all other individuals. With respect to the mechanisms underlying these influences he specifies:

> This is more than the interaction of the environment with the genotype, or reaction norm. Such variation comes from the nature of the developmental program, which includes mechanisms of high determinacy, but also some with a high degree of indeterminacy. In consequence, development of the individual is stochastic, and contingent rather than deterministic, and the operation of identical environmental factors on identical developmental programs may lead to different morphologies. (McLachlan, 1999, p. 167)

The influences referred to by McLachlan create endogenous variation, that is, variation that is neither of genetic, environmental, nor of gene-environmental interactive origin. Instead this variation is created by what McLachlan calls stochastic developmental programs. In mathematical biology a class of very successful models has been developed: the class of nonlinear reaction-diffusion models of biological pattern formation (e.g., Meinhardt, 1982). The key characteristic of nonlinear reaction-diffusion models is self-organization. See Molenaar (2007) for further discussion of reaction-diffusion models and many references to the relevant literature.

The self-organizing nonlinear epigenetic processes represented by reaction-diffusion models of biological pattern formation also underlie the growth of neural networks (cf. Molenaar, 2007). Hence it can be expected that at the micro level human brain architecture will be quite heterogeneous. Insofar as human behavior and information processing is dependent upon neural models or networks, this heterogeneity will be reflected in psychological measurements, thus causing violations of condition (19.4b). This conclusion has been corroborated in simulation studies of neural network growth (Molenaar & Raijmakers, 1999; Kan et al., 2009).

Genetic Decomposition of Intra-Individual Variation

The foregoing discussion of problems with standard genetic factor analysis has touched upon various issues, only one of which will be further considered in this section. It is the issue of heterogeneity, that is, violation of condition (19.4b) for a Gaussian process to be ergodic. Before proceeding, however, some general remarks are in order.

Genetic influences, environmental influences as well as (self-organizing) growth and developmental processes act in real time at the level of individual subjects. To study these influences and processes at the population level therefore is to study them at a derived level. Each subject constitutes a high-dimensional dynamic system, the state variables of which evolve in time. The natural time scale of some state variables is very fast; for instance the fluctuations of electric field potentials in the brain vary in the order of milliseconds. Other state variables have much slower natural time scales, such as fluctuations in behavioral activity levels or circadian rhythms. Conceiving of the fluctuations of all measurable state variables of a subject as function of continuous time yields a high-dimensional ensemble of trajectories which spans up the behavior space of this subject.

The concept of high-dimensional ensemble of life trajectories spanning up each subject's behavior space has direct implications for the way in which the phenotypic data obtained in a typical twin study should be conceived. Taking the standard longitudinal genetic factor model (19.5a) as example, the data of the first member ($j = 1$) of the first MZ twin pair ($i = 1$ and $k = 1$) consists of an M-variate time series

of phenotypic scores y_{111mt} where $m = 1,2,\ldots,M$ and $t = 1,2,\ldots,T$. It is evident that the phenotypic data set $\{y_{111mt};\ m = 1,2,\ldots,M;\ t = 1,2,\ldots,T\}$ constitutes a cross-section of the behavior space of this subject, restricted to the M phenotypic state variables and the T measurement occasions. Using more pictorial language, $\{y_{111mt};\ m = 1,2,\ldots,M;\ t = 1,2,\ldots,T\}$ constitutes a partial snapshot of the high-dimensional ensemble of life trajectories which evolve in continuous time. The snapshot is partial because only a selection of M state variables is measured, and these state variables are measured at only a selection of T discrete measurement occasions. Similar remarks apply to all other subjects in a longitudinal twin design.

Any application of the longitudinal genetic factor model (19.5a) is based on analysis of inter-individual variation. The M-variate phenotypic data obtained at T measurement occasions of each twin pair are stacked in a 2MT-dimensional supervector. Next the (2MT, 2MT)-dimensional covariance matrices for MZ and DZ twin pairs are estimated by pooling across the 2MT-dimensional supervectors of, respectively, the MZ and DZ twin pairs in the sample. Pooling across subjects – in this case twin pairs – is the hallmark of analysis of inter-individual variation.

Statement of the Problem

As indicated earlier, standard genetic factor analysis of phenotypic inter-individual data is insensitive to violation of criterion (19.4b), that is, violation of the homogeneity condition. The best and perhaps only way to detect heterogeneity in the sense of criterion (19.4b) is to carry out genetic factor analysis of phenotypic intra-individual variation. To accomplish that, we need to develop genetic factor analysis of multivariate phenotypic time series for individual twin pairs. This new genetic factor analysis of multivariate time series should yield a decomposition of intra-individual variation into additive genetic, common environmental, and specific environmental factors which is equivalent to the decomposition of inter-individual variation by means of the standard longitudinal genetic factor analysis.

Reiterating, criteria (19.4a) and (19.4b) are independent of each other. Whether or not a psychological process obeys or violates criterion (19.4a) is independent of whether or not this process obeys or violates criterion (19.4b). Consequently, to ease the presentation, we will consider detection of violations of (19.4b) while assuming that the phenotypic process obeys criterion (19.4a), in other words, that the process is stationary.

A Genetic Factor Model for Intra-Individual Variation

Our starting point is the standard longitudinal factor model. Instead of the general model (19.5a), it is assumed that the phenotypic M-variate process obeys the stationarity criterion (19.4a). That is, it is assumed that the standard longitudinal

factor model (19.5a) obeys the list of constraints given by (19.6). The specific stationary instance of (19.5a) thus obtained is:

$$\begin{aligned} y_{ijkmt} &= \alpha_m A_{ijkt} + \delta_m \Delta_{ijkt} + \phi_m \Phi_{ijkt} + \varepsilon_{ijkmt} \\ A_{ijkt} &= \beta A_{ijkt-1} + \zeta_{ijkt} \\ \Delta_{ijkt} &= \gamma \Delta_{ijkt-1} + \xi_{ijkt} \\ \Phi_{ijkt} &= \nu \Phi_{ijkt-1} + \sigma_{ijkt} \end{aligned} \tag{19.7}$$

Please note that (19.7) is a standard longitudinal genetic factor model. Hence all variables in (19.7) have exactly the same interpretations as in (19.5a). The only difference with (19.5a) is that the model parameters in (19.7) (including the factor loadings and regression coefficients) are all invariant in time. Hence (19.7) obeys the stationarity criterion (19.4a).

Let's go back again to the way in which (19.7) (or any other standard genetic model) is applied. A sample of N_1 of MZ twin pairs and N_2 DZ twin pairs is available. Each subject in the sample of $2N_1 + 2N_2$ subjects is measured at T consecutive measurement occasions. Almost always T is very small, for example, $T = 2$ or $T = 3$. At each measurement occasion each subject yields M phenotypic scores. Hence at T measurement occasions each subject yields MT phenotypic scores. The MT-dimensional vectors of phenotypic scores for the two subjects within each twin pair are collected in a 2MT-dimensional supervector of phenotypic scores and the (2MT, 2MT)-dimensional covariance matrix for the MZ and DZ twins is estimated by pooling across the N_1 MZ pairs and the N_2 DZ twin pairs, respectively.

From the perspective of behavior space introduced above, the data of each subject in an application of (19.7) constitutes an M-variate cross-section at T measurement occasions, where T is small. Or, stated otherwise, the data of each subject constitutes a short stretch of observations of an M-variate phenotypic time series at T measurement occasions. This perspective shows how to define genetic factor analysis of phenotypic time series of intra-individual variation. We simply have to increase the number T of measurement occasions (stretch the cross-section taken from behavior space along the time dimension). If T is large enough then the covariance matrix to which (19.7) has to be fitted can be estimated by pooling across time (intra-individual variation) instead of pooling across subjects (inter-individual variation).

These considerations lead to the following definition of the genetic factor model for intra-individual variation for a *single* MZ twin pair ($k = 1$) or a *single* DZ twin pair ($k = 2$):

$$\begin{aligned} y_{jkmt} &= \alpha_{jkm} A_{jkt} + \delta_{jkm} \Delta_{jkt} + \phi_{jkm} \Phi_{jkt} + \varepsilon_{jkmt} \\ A_{jkt} &= \beta_{jk} A_{jkt-1} + \zeta_{jkt} \\ \Delta_{jkt} &= \gamma_{jk} \Delta_{jkt-1} + \xi_{jkt} \\ \Phi_{jkt} &= \nu_{jk} \Phi_{jkt-1} + \sigma_{jkt} \end{aligned} \tag{19.8}$$

It is evident that the structural equations making up (19.8) have the same form as those of (19.7). But the indices of the variables in (19.8) differ in important ways from the indices of variables in (19.7). One difference only is typographical: in (19.8) the subscript i has been deleted. This subscript i indicates the ith twin pair. It can be deleted because (19.8) applies to a given single twin pair. The remaining differences of indices in (19.7) and (19.8), however, are substantial.

The important differences between the stationary longitudinal genetic factor model (19.7) and the genetic factor model for intra-individual variation (19.8) pertain to the factor loadings and regression coefficients. In conformance with the homogeneity condition (19.4b), all factor loadings and regression coefficients in (19.7) are assumed to be invariant within and between MZ and DZ twin pairs. In contrast, in (19.8) these factor loadings and regression coefficients are allowed to be subject-specific, in violation of the homogeneity condition (19.4b). For instance, α_{jkm} in (19.8) is the factor loading of the mth phenotypic variable on A_{ijkt}, the additive genetic factor score of the jth member of the given twin pair of type k at measurement occasion t. α_{jkm} depends upon j, that is, its value can differ within the given twin pair if $j = 1$ or $j = 2$. α_{jkm} also depends upon k, that is, its value can differ whether the given twin pair is MZ ($k = 1$) or DZ ($k = 2$). The remaining factor loadings δ_{jkm} and ϕ_{jkm} as well as the regression coefficients β_{jk}, γ_{jk} and ν_{jk} in (19.8) also depend upon j and k and hence their values can be subject-specific.

Given that the factor loadings and regression coefficients in (19.8) can be subject-specific, it follows that the measurement error variances, $\mathrm{var}[\varepsilon_{jkmt}]$, and the variances of the residuals, $\mathrm{var}[\zeta_{jkt}]$, $\mathrm{var}[\xi_{jkt}]$ and $\mathrm{var}[\sigma_{jkt}]$ also can be subject-specific. In contrast, if the given twin pair is MZ then the pattern of correlations among the additive genetic, common and environmental factors is exactly the same as for (19.7). If the given twin pair is DZ then the pattern of correlations among the additive genetic, common and environmental factors is exactly the same as for (19.7), save for one exception. In (19.7) the correlation between the additive genetic factor scores within twin pairs is on average 0.5; $\mathrm{cor}[A_{12t}, A_{22t}] = 0.5$. However, for a given DZ twin pair the correlation of the additive genetic factor scores within this twin pair can range between zero and one: $0 \leq \mathrm{cor}[A_{12t}, A_{22t}] \leq 1$. Hence in each application of (19.8) to a given DZ twin pair the correlation of the additive genetic factor scores has to be estimated.

iFACE

Suppose the genetic factor model for intra-individual variation (19.8) is applied twice, once to a single DZ twin pair and once again to another single DZ twin pair (the choice of DZ twins is immaterial for the argument to follow). Then whatever the patterns of subject-specific factor loadings which are thus obtained, there is no ambiguity about the interpretation of the factors obtained in both analyses. For

each of the four subjects in the two analyses in the example the additive genetic factor A is qualitatively the same factor as the A factors for the other subjects. The same remarks apply to the common and specific environmental factors. This is because the interpretations of the factors in (19.8) do not depend on the factor loadings but on the pattern of correlations within a twin pair.

Recently Nesselroade, Gerstorf, Hardy, & Ram et al. (2007) presented a new measurement theory in which the interpretation of each factor is based on this factor's invariant pattern of correlations with other factors, while factor loadings can be subject-specific. From the perspective of this new measurement theory, what is invariant across subjects are the interpretations of the factors based on fixed patterns of factor correlations, while the ways in which factors manifest themselves in observed variables can differ between subjects. Nesselroade et al. (2007) apply multi-subject factor analysis of multivariate time series obtained in a replicated time series design to estimate the possibly subject-specific values of factor loadings while constraining the pattern of correlations among the factors to be invariant across subjects. They refer to this application of factor models with subject-specific factor loadings as idiographic filtering.

The genetic factor model for intra-individual variation (19.8) can be conceived of as a combination of the idiographic filter (iF) and the standard genetic factor model. The commonly used acronym for the latter model is ACE, based on the interpretations of the factors (Additive genetic, Common environmental, specific Environmental). Hence (19.8) is referred to as the iFACE model.

Illustrative Application of iFACE

The genetic factor model for intra-individual variation (19.8) will be applied to simulated phenotypic data of a single DZ twin pair. The simulation model (i.e., the "true" model) is a specific instance of (19.8) with the following specifications: $M = 4$, $T = 900$, and the values for the model parameters shown in Table 19.1.

Table 19.1.

Twin 1	*Twin 2*
$\alpha_{121} = 1.73$	$\alpha_{221} = 2.61$
$\alpha_{122} = 3.64$	$\alpha_{222} = 1.03$
$\alpha_{123} = 1.30$	$\alpha_{223} = 1.68$
$\alpha_{124} = 2.65$	$\alpha_{224} = 1.57$
$\phi_{121} = 2.27$	$\phi_{221} = 1.20$
$\phi_{122} = 1.10$	$\phi_{222} = 1.79$
$\phi_{123} = 1.46$	$\phi_{223} = 1.17$
$\phi_{124} = 2.08$	$\phi_{224} = 1.22$

The loadings on the common environmental factors were invariant across the two subjects and all equal to 1. The regression coefficients β, γ and ν were invariant across the two subjects, equaling 0.7. The variances of all measurement errors ε and residuals ζ, ξ and σ were invariant across the two subjects, equaling 1. The correlation between the genetic factor scores of the two subjects equaled 0.5.

A single 4-variate time series of length 900 was generated for DZ twin 1 and twin 2. The iFACE model was fitted to these data using the block-Toeplitz approach described in Molenaar (1985). In this approach (8,8)-dimensional covariance matrices are estimated by pooling the observations across time points – the hallmark of analysis of intra-individual variation. There are two (8,8)-dimensional covariance matrices, one for contemporaneous relationships between the phenotypic time series and another one for the phenotypic time series at t and t-1, $t = 1,2,\ldots,899$. These two covariance matrices constitute the blocks in a (16,16)-dimensional block-Toeplitz matrix. The iFACE model was fitted to this block-Toeplitz matrix by means of commercially available structural equation modeling software (e.g., Lisrel, Jőreskog & Sőrbom, 1993).

It is emphasized that in fitting (19.8) to the data, all parameters are allowed to be subject-specific save for one exception. In the true model underlying the simulated data several parameters were assigned values which are invariant across the two subjects. However, in the model fitted to the data thus simulated, all parameters save for one exception are allowed to vary between the two subjects. It also is emphasized that the correlation between the additive genetic factor scores of DZ twin 1 and twin 2 is a freely estimated parameter. The only exception is the set of loadings on the common environmental factor: these were constrained to be equal across the two subjects, but could freely vary across the four phenotypic time series.

The iFACE yields an excellent fit to the data (chi-square = 78.5, df = 97, P = 0.91; standardized root mean square residual = 0.02; non-normed fit index = 1; comparative fit index = 1). Perhaps the best and most concise way to convey this is to report the estimated heritability coefficients determined by application of (19.4) to the "true" and estimated factor loadings for each of the four univariate phenotypic time series (see Table 19.2).

Table 19.2.

Twin 1		*Twin 2*	
true $(h_1)^2 = .34$	est $(h_1)^2 = .36$	true $(h_1)^2 = .73$	est $(h_1)^2 = .62$
true $(h_2)^2 = .86$	est $(h_2)^2 = .86$	true $(h_2)^2 = .20$	est $(h_2)^2 = .15$
true $(h_3)^2 = .35$	est $(h_3)^2 = .36$	true $(h_3)^2 = .54$	est $(h_3)^2 = .45$
true $(h_4)^2 = .57$	est $(h_4)^2 = .60$	true $(h_4)^2 = .49$	est $(h_4)^2 = .40$

Discussion

The standard genetic factor model and the standard longitudinal genetic factor model are models for the genetic decomposition of multivariate phenotypic inter-individual variation. Inter-individual variation is a derived type of variation, namely variation occurring at the population level. In contrast, the basic type of variation occurs at the level of individual subjects evolving in time: intra-individual variation. Consequently the question arises what is the relationship between the structure of intra-individual variation and inter-individual variation. The definite answer to this question is provided by Birkhoff's individual ergodic theorem: there only exists an equivalence relationship between the structures of intra- and inter-individual variation if the process under consideration is stationary and all subjects in a population obey the same statistical model. If either of these conditions is not met, the process is non-ergodic and its analysis should proceed at the basic level of intra-individual variation.

Developmental processes almost always have time-varying characteristics, hence are non-stationary and therefore non-ergodic. To model such non-stationary processes at the appropriate level of intra-individual variation, special time series models with arbitrarily time-varying parameters are required. Such models have become available only recently (Molenaar, 2009; Molenaar, Sinclair, Rovine, Ram, & Corneal, 2009).

To detect whether all subjects in a population obey the same statistical model for a given process – the second condition which processes have to obey in order to be ergodic – again dedicated analysis of intra-individual variation is required. In this chapter the standard longitudinal genetic model for the analysis of inter-individual variation has been transformed into an equivalent genetic factor model for the analysis of intra-individual variation: iFACE. The latter model allows for detection of violations of the homogeneity condition required for processes to be ergodic. It was shown in a first illustrative application to simulated data that the new iFACE model appears to perform quite satisfactorily.

The iFACE model is a new model to detect and quantify heterogeneity in a population of subjects. Its application requires multivariate phenotypic time series of sufficient length. Large scale Monte Carlo studies have to be carried out to determine the fidelity of iFACE in recovering genetic and environmental influences as a function of number of repeated observations, dimension of the phenotypic time series, etc. In addition, alpha-numeric derivations have to be carried out to determine the limits of identifiability of iFACE model parameters. For instance, the reason why the factor loadings on the common environmental factor are constrained to be invariant across both members of the DZ twin pair in the illustrative application is to guarantee unique parameter identifiability. These issues require further elaboration in future research, including application of iFACE to multi-lead EEG time series obtained with DZ and MZ twins (Molenaar, Boomsma, & Nesselroade, in preparation).

References

Birkhoff, G. (1931). Proof of the ergodic theorem. *Proceedings of the National Academy of Sciences, USA*, **17**, 656–660.

Boomsma, D. I., & Molenaar, P. C. M. (1986). Using Lisrel to analyze genetic and environmental covariance structure. *Behavior Genetics*, **16**, 237–250.

Boomsma, D. I., & Molenaar, P. C. M. (1987). The genetic analysis of repeated measures I: Simplex models. *Behavior Genetics*, **17**, 111–123.

Boomsma, D. I., Molenaar, P. C. M., & Orlebeke, J. F. (1990). Estimation of individual genetic and environmental factor scores. *Genetic Epidemiology*, **7**, 83–92.

Danziger, K. (1990). *Constructing the subject: Historical origins of psychological research*. Cambridge: Cambridge University Press.

Dolan, C. V., Molenaar, P. C. M., & Boomsma, D. I. (1989). Lisrel analysis of twin data with structured means. *Behavior Genetics*, **19**, 51–62.

Dolan, C. V., Molenaar, P. C. M., & Boomsma, D. I. (1991). Simultaneous genetic analysis of longitudinal means and covariance structure in the simplex model using twin data. *Behavior Genetics*, **21**, 49–65.

Dolan, C. V., Molenaar, P. C. M., & Boomsma, D. I. (1992). Decomposition of multivariate phenotypic means in multi-group genetic covariance structure analysis. *Behavior Genetics*, **22**, 319–335.

Dolan, C. V., Molenaar, P. C. M., & Boomsma, D. I. (1994). Simultaneous genetic analysis of means and covariance structure: Pearson-Lawley selection rules. *Behavior Genetics*, **24**, 17–24.

Fisher, R. A. (1918). The correlation between relatives on the supposition of Mendelian inheritance. *Philosophical Transactions of the Royal Society, Edinburgh*, **52**, 399–433.

Ford, D. H., & Lerner, R. M. (1992). *Developmental systems theory: An integrative approach*. Thousand Oaks, CA: Sage.

Gaertner, K. (1990). A third component causing variability beside environment and genotype: A reason for the limited success of a 30 year long effort to standardize laboratory animals? *Laboratory Animals*, **24**, 71–77.

Gottlieb, G. (1992). *Individual development and evolution: The genesis of novel behavior*. New York: Oxford University Press.

Gottlieb, G. (2003). On making behavioral genetics truly developmental. *Human Development*, **46**, 337–355.

Hamaker, E. L., Dolan, C. V., & Molenaar, P. C. M. (2005). Statistical modeling of the individual: Rationale and application of multivariate stationary time series analysis. *Multivariate Behavioral Research*, **40**, 207–233.

Hewitt, J. K., Eaves, L. J., Neale, M. C., & Meyer, J. M. (1988). Resolving causes of developmental continuity or "tracking." I. Longitudinal twin studies during growth. *Behavior Genetics*, **18**, 133–152.

Jablonka, E., & Lamb, M. J. (1995). *Epigenetic inheritance and evolution: The Lamarckian dimension*. Oxford: Oxford University Press.

Jablonka, E., & Lamb, M. J. (2005). *Evolution in four dimensions: Genetic, epigenetic, behavioral and symbolic variation in the history of life*. Cambridge, MA: MIT Press.

Jőreskog, K. G., & Sőrbom, D. (1993). *LISREL 8 user's reference guide*. Chicago: Scientific Software International.

Kan, K. J., Ploeger, A., Raijmakers, M. E. J., Dolan, C. V., & van der Maas, H. L. J. (2009). Nonlinear epigenetic variance: Review and simulations. *Developmental Science*, **13**(1), 11–27.

Kelderman, H., & Molenaar, P. C. M. (2007). The effect of individual differences in factor loadings on the standard factor model. *Multivariate Behavioral Research*, **42**, 435–456.

Krengel, U. (1985). *Ergodic theorems*. Berlin: Walter de Gruyter.

Lamiell, J. T. (2003). *Beyond individual and group differences: Human individuality, scientific psychology, and William Stern's critical personalism*. Thousand Oaks, CA: Sage.

Martin, N. G., & Eaves, L. J. (1977). The genetic analysis of covariance structures. *Heredity*, **38**, 79–93.

Mather, K., & Jinks, J. L. (1977). *Introduction to biometrical genetics*. London: Chapman & Hall.

McLachlan, J. C. (1999). Developmental morphologies not directly specified by the genome of the individual. In M. A. J. Chaplain, G. D. Singh, & J. C. McLachlan (Eds.), *On growth and form: Spatio-temporal pattern formation in biology* (pp. 157–172). Chichester: Wiley.

Meinhardt, H. (1982). *Models of biological pattern formation*. London: Academic Press.

Molenaar, P. C. M. (1985). A dynamic factor model for the analysis of multivariate time series. *Psychometrika*, **50**, 181–202.

Molenaar, P. C. M. (1997). Time series analysis and its relationship with longitudinal analysis. *International Journal of Sports Medicine*, **18**, 232–237.

Molenaar, P. C. M. (1999). *Longitudinal analysis*. In H. J. Ader & G. J. Mellenbergh (Eds.), *Research methodology in the social, behavioral and life sciences* (pp. 143–167). London: Sage.

Molenaar, P. C. M. (2004). A manifesto on psychology as idiographic science: Bringing the person back into scientific psychology, this time forever. *Measurement*, **2**, 201–218.

Molenaar, P. C. M. (2007). On the implications of the classical ergodic theorems: Analysis of developmental processes has to focus on intra-individual variation. *Developmental Psychobiology*, **50**, 60–69.

Molenaar, P. C. M. (2009). Note on optimization of individual psychotherapeutic processes. *Journal of Mathematical Psychology*, **54**(1), 208–213.

Molenaar, P. C. M., & Boomsma, D. I. (1987a). The genetic analysis of repeated measures II: The Karhunen-Loeve expansion. *Behavior Genetics*, **17**, 229–242.

Molenaar, P. C. M., & Boomsma, D. I. (1987b). Application of nonlinear factor analysis to genotype-environment interaction. *Behavior Genetics*, **17**, 71–80.

Molenaar, P. C. M., Boomsma, D. I., & Dolan, C. V. (1993). A third source of developmental differences. *Behavior Genetics*, **23**, 519–524.

Molenaar, P. C. M., & Boomsma, D. I., Neeleman, D., & Dolan, C. V. (1990). Using factor scores to detect G × E interactive origin of "pure" genetic or environmental factors obtained in genetic covariance structure analysis. *Genetic Epidemiology*, **7**, 93–100.

Molenaar, P. C. M., & Raijmakers, M. E. J. (1999). Additional aspects of third source variation for the genetic analysis of human development and behavior: A commentary on Eaves et al. *Twin Research*, **2**, 49–52.

Molenaar, P. C. M., Sinclair, K. O., Rovine, M. J., Ram, N., & Corneal, S. E. (2009). Analyzing developmental processes on an individual level using non-stationary time series modeling. *Developmental Psychology*, **45**, 260–271.

Neale, M. C., Walters, E. E., Eaves, L. J., Maes, H. H., & Kendler, K. S. (1994). Multivariate genetic analysis of twin data on fears: Mx models. *Behavior Genetics*, **24**, 119–139.

Nesselroade, J. R., Gerstorf, D., Hardy, S. A., & Ram, N. (2007). Idiographic filters for psychological constructs. *Measurement*, **5**, 217–235.

Plomin, R., & Daniels, D. (1987). Why are children in the same family so different from each other? *The Brain and Behavioral Sciences*, **10**, 1–16.

Robert, J. S. (2004). *Embryology, epigenesist, and evolution: Taking development seriously*. Cambridge: Cambridge University Press.

Rovine, M. J., & Molenaar, P. C. M. (2005). Relating factor models for longitudinal data to quasi-simplex and NARMA models. *Multivariate Behavioral Research*, **40**, 83–114.

Wohlwill, J. F. (1973). *The study of behavioral development*. New York: Academic Press.

Wright, S. (1920). The relative importance of heredity and environment in determining the piebald pattern of guinea pigs. *Proceedings of the National Academy of Sciences, USA*, **6**, 320–332.

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Songs My Mother Taught Me: Gene-Environment Interactions, Brain Development and the Auditory System: Thoughts on Non-Kin Rejection

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Gilbert Gottlieb's seminal works demonstrated that the auditory system, which is responsive to environmental influences before birth, provides an important pre-condition for subsequent "imprinting" of the newly hatched duckling to its mother. In this chapter, I explore the biological basis for environmentally responsive genes, with particular emphasis on the auditory system, and the role of prenatal auditory experience on subsequent identity, group specification, and rejection of individuals perceived to be foreign.

The discovery of a surprisingly small number of human genes, a large amount of non-coding DNA, and high degree of similarity in coding sequences between organisms as diverse as fruit fly and human, has undermined the "central dogma" of gene theory. The central dogma held that each gene produced one protein with one function. We now know that a "gene" includes both the DNA sequence encoding protein and non-coding "regulatory" sequences that play dynamic roles in modulating when the protein is produced (Hager, McNally & Misteli, 2009). Such regulation involves both the sequences that alter the length or internal composition of the resulting protein, and the DNA sites that regulate when and how much protein is produced. Recent genetic discoveries also reveal genes encoding RNA molecules, referred to as "ribozymes," that have enzymatic and regulatory functions just like proteins in cells (Fedor, 2009).

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Thus, discoveries enabled by the Human Genome Project since the release of the first full draft in 2001 (Lander et al., 2001; Venter et al., 2001) illuminate a fact long suspected by developmental and evolutionary biologists: that the chemically immutable genome is flexible in its expression. This flexibility is the biological basis of the varying outcomes observed in individuals with identical genotypes. Thus "epigenesis," the process by which factors other than genetic composition determine individual outcomes, is now widely acknowledged as a major contributor to both morphological and behavioral phenotypes at the cellular, organismal and even the psychosocial-behavioral levels. While these epigenetic chemical factors acting on genetic expression must be either directly encoded in the genome or synthesized by genome-encoded enzymes, their activity is responsive to the biological environment.

At the cellular level, the process of environmental responsiveness has been vigorously explored. The composition of the media bathing cells, their extracellular matrix, and the character of adjacent cells provoke cells to adopt varying responses. Such extracellular information is transmitted through events occurring across the cellular membrane called "signal transduction." Once the signal is transmitted, subsequent enzymatic events within the cell influence other activities in the cytoplasm and also travel to the nucleus where they turn on or off genes, or alter the length or composition of the protein produced. Highly evolved sophisticated signal transduction is the basis of neural circuitry, where one neuron secretes molecules that activate the next neuron across a focused contact point, the synapse. These are well-recognized biological facts now taught to students in such recently upadated texts as Alberts et al. (2007) *The Molecular Biology of the Cell*, Lodish et al. (2003) *Molecular Cell Biology* as well as many others. Throughout this chapter I will refer to generally accepted biological evidence, found in these textbooks, to behavioral attributes that we recognize in ourselves and others in everyday life.

Perhaps the most compelling question for research in human development is how the flexibility in genetic expression pertains to the controversy surrounding pre-determinism and free will. As Gilbert Gottlieb so elegantly empirically demonstrated, behavioral specification of even the most critical survival activity of a young chick to follow its mother is responsive to prenatal sensory input from the environment (Gottlieb, 1998), that is, it is mutable, flexible, and responsive. He coined the phrase "probabilistic epigenesis" to describe this idea (Gottlieb, 2007). In simpler language, the idea is that genes encode capabilities which can have variable outcomes based on external influences. His diagram, reproduced many times throughout later publications is shown in Figure 20.1 (Gottlieb, 1991).

Specifically, Gottlieb's research related to "instruction" of the pre-hatch auditory system by sounds experienced from within the eggshell. These sounds enabled the hatchling to identify its mother. The understanding of pre-hatch fetal instruction

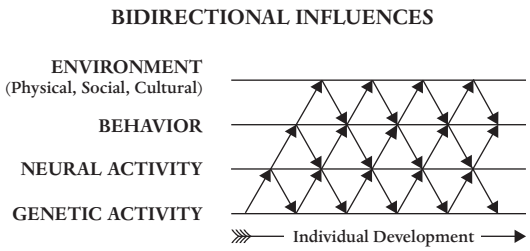

Figure 20.1. Bidirectional influences of genetic activity, neural activity, behavior and environment. A developmental-psychobiological systems framework. *Source*: Gottlieb (1992, p. 186).

came through careful observation of wood ducklings in the wild, and contradicted the more widely acknowledged work of Konrad Lorenz, whose Nobel Prize in Medicine and Physiology in 1973 recognized his re-awakening of the phenomenon "imprinting" (Lorenz, 1961). For Lorenz, imprinting was visual – incubator-hatched ducklings, who had little to no auditory stimuli from within the egg, attached to the first moving target they saw. Gottlieb's work added two dimensions – that auditory imprinting precedes birth, and thus that the visual experience only dominates when auditory is lacking.

My personal synthesis of these two processes, probabilistic epigenesis and imprinting, is that they are related phenomena, most likely part of a continuum of neural system instruction that I will refer to here as the PEI (*P*robabilistic *E*pigenesis and *I*mprinting). I propose that this process of instruction begins with the pluripotent stem cell, progresses to neural progenitors that may differentiate into either neural or glial cell type, depending on local substrate and cellular interactions. These events are then followed by a series of "fine-tuning" whereby each cell elaborates specific functions. For neurons, these include the arborizations of the dendritic tree; the pathway, length and branching of the axon; and the various biochemical and anatomical features of each synapse. The outcome of this complex choreography is a unique adult animal who, in the case of the human, may retain a subset of responsive elements that can also re-organize to "learn" or "forget" the lessons of experience and thereby modify the instructions acquired in development.

Here we review examples of biological "instruction" which likely underlie the PEI continuum drawn from biomedical wet-bench research results from my own laboratory, my collaborators and from others providing examples of several of the types of biochemical epigenetic mechanisms (Box 20.1). We will describe how these results demonstrate biochemical epigenesis at the cellular, organismal, and behavioral levels. We will then discuss computational approaches to simulation of epigenetic activity and their corollaries in behavioral modification. Finally we will explore implications of these human biological capabilities in the broader context of intellectual and social behaviors.

Box 20.1. Types of epigenetic regulation of cellular activity.

Transcriptional regulation
- Silencing (Permanently turned off and packaged into heterochromatin)
- Down-regulation (Capable of being activated under specific conditions)
- Up-regulation (Expression levels regulated by mRNA copy number produced)

Translational regulation
- Whether the mRNA is translated to protein or degraded
- Signal transduction-activated translation (mTOR, CPEB, etc)

Alternative splicing of RNA

Enzymatic activity-regulated
- Abundance, quantity of the enzyme
- Activity of each enzymatic molecule (post-translational modification)
- Presence of cofactors
- Substrate availability, substrate affinity
- Stability, longevity, rate of degradation
- Compartmentalization of the enzyme/location in the cell

DNA modification
- Insertions
- Nucleotide base-pair changes
- Deletions
- Amplifications (as in c-myc gene duplications)
- Expansions (as GAG repeats)
- Rearrangements (as in V(D)J recombination in human antibody specification)
- Transposable element hopping

Example 1: Yeast Mating Types are Regulated by Epigenetic Mechanisms

The single-celled yeast, *Saccharomyces cerevisiae*, has been useful since ancient times for baking and brewing. Perhaps the most extensively studied single-celled eukaryote, *S. cerevisiae*'s mating cycle has been a focus of high

attention (Klar, 2007). The yeast has two forms, diploid with a double complete set of chromosomes and two copies of every gene, and haploid, with a single complement of chromosomes and genes. Yeast cells primarily mate from the haploid form. In the haploid form, each yeast cell adopts either "α" or "a" mating type. The a-cell produces a factor to which the α-cell responds through a signal-transduction mechanism. The responding α-cell produces a "*schmoo*", which is a cellular extension projecting towards the source of the a-factor. The two cells "schmoo" towards each other and then mate by fusing. Cells of the same type do not normally mate, although recent evidence shows that they may do so in unusual circumstances (Soll, Pujol & Srikantha, 2009).

When diploid yeast are starved they undergo division into two haploid cells capable of mating. Each haploid daughter cell receives one complete set of chromosomes encoding either the MATa or MATα, different gene copies – alleles. MATa or MATα genes are different DNA sequences encoding a similar protein that regulates expression of cassettes of genes. MATa produces the a1 protein that activates expression of a-cell genes and represses α-cell genes, while MATα produces the α factor that activates α-protein expression and represses a-type gene expression (Weiss & Simpson, 1997). Thus, allelic variation produces epigenetic factors that differentiate these two cell types and restrict mating behavior through epigenetic mechanisms.

But yeast cells are even more clever than this. In the circumstance where there are not enough cells of any one mating type, haploid cells can switch mating types (Fasullo, Bennett & Dave, 1999). This switching is possible because yeast carry two copies of both MATa and MATα. One copy for each allele is silenced, and the other two copies are alternatively expressed. These silenced copies for each gene provide the template for replacement of that mating-type protein through DNA replication of the active allele in the haploid cell. This replacement is done through excision and digestion of the DNA sequence of the active allele and replication of a copy of the silenced DNA for the opposite mating type that is then ligated into the active site, switching the mating type (Haber, 1998; Peled et al., 2008). This process of DNA sequence alteration also occurs in the human immune system when antibodies are synthesized to recognize specific pathogenic microbes (Peled et al., 2008). For stable inheritance to occur, such gene-replacement must be tightly controlled so that the rest of the genome is not corrupted. Thus DNA sequence alteration is not thought to be a common event regulating non-inherited individual variation in adult organisms. In multicellular organisms, such DNA modifications occurring in somatic cells would not be inherited, as only germ cells produce new individuals. Hence these processes are uniquely designed to modify the genome only in specific cells within an individual and do not propagate to the individual's children.

Example 2: Hemoglobin Switching: Non-Coding DNA Sequences Specify Sites for Epigenetic Regulation

Prior to the human genome project, the large amount of DNA in the human chromosome that did not encode proteins was often referred to as "junk" DNA. However, once the draft of the human genome demonstrated that there were not enough genes or genetic differences within known coding regions to explain species variation between humans and chimpanzees, and even not enough to explain why fruitflies and humans were so different, new ideas for the role of these non-coding regions have emerged and are still being explored and proposed. One idea currently favored is that regulation of protein expression is the fundamental biological basis of species differentiation (Davidson & Erwin, 2006). Dynamic changes in protein expression from genes is far more frequently observed and common than DNA excision-repair-replacement described in Example 1.

Non-coding DNA plays a wide variety of roles in epigenetic regulation of the genetic material (see Box 20.2). The most common genetic disorders of humans are the hemoglobinopathies – mutations in the genes encoding the major red blood protein, hemoglobin (Weatherall, 2008). Hemoglobin carries oxygen in the blood and releases it to the tissue under defined chemical conditions. During human development, different types of hemoglobin are produced sequentially. Fetal hemoglobin has different oxygen binding characteristics than the hemoglobin produced after birth.

Box 20.2. Roles for non-coding DNA.

Mitotic spindle assembly and duplicated chromosome segregation

Meiotic separation of homologous chromosomes

Structural packaging of DNA

Binding sites for regulatory proteins:
- Transcription factors
- Silencing complexes
- Histone ordering

Signals for RNA processing

Two major subgroups of the hemoglobinopathies are sickle-cell anemia, where the adult hemoglobin gene has a single amino acid mutation, and thalassemia, a heterogeneous group of diseases in which not enough hemoglobin is produced, or the hemoglobin is of the fetal rather than the adult type. Because these mutations give some protection from the malaria parasite, they have been evolutionarily selected despite the illness they cause in people who inherit two copies of the mutated gene (Richer & Chudley, 2005).

Thalassemia is a disease of epigenetic mechanisms controlling hemoglobin protein production that protects against infections (Vento, Cainelli, & Cesario, 2006). In some forms of thalassemia, the DNA sequences that regulate hemoglobin expression contain mutations that alter hemoglobin protein expression levels, or prevent switching from fetal to adult hemoglobin genes.

Because most individuals with hemoglobinopathy also have other hemoglobin genes that are silenced during development, one avenue for potential therapy is to over-ride this epigenetic regulation and re-activate silenced genes.

Thalassemia thus exemplifies the importance of non-coding DNA regions that control expression. While thalassemia is a dramatic example of this type of epigenetic regulation, we believe that more subtle alterations in gene expression levels controlled by allelic variation in non-coding DNA underlie other susceptibility loci – that is, un-identified inherited DNA that in some environmental circumstances leads to a dysregulation causing disease states. Such single-nucleotide variations in human chromosomal alleles could explain many other differing characteristics between individuals as well.

Another example of epigenetic regulation leading to a disease state can be taken from data generated by my laboratory on a human deafness locus (Bearer et al. 2000). We were interested in the proteins that comprise the sensory apparatus of the auditory hair cell. Through unbiased comprehensive high-throughput screening aimed at identifying all proteins with the potential to create this apparatus, we identified several proteins present in the apparatus (Bearer, 1995; Bearer & Abraham, 1999). After expression cloning, we sequenced cDNA for one of these proteins and used the sequence to probe DNA from families with inherited deafness to find any with mutations in this gene (Bearer et al., 2000).

Surprisingly, two families were found with the location in the DNA that mapped to our gene, but with no mutations in the coding sequence (Bearer et al., 2000). We ultimately found that the mutation that caused the deafness was in the "regulatory" region of the gene – the part that specifies epigenetic control. Both of these families had hyper-sensitive hearing at early ages and became deaf in mid-life. One family included members that were professional violin-makers, using their heightened ability to create instruments of exceptional sound quality.

Thus, small alterations in regions of DNA responsible for regulation of gene expression may give rise to heightened capabilities as well as susceptibility to adult-onset disease.

Example 3: Specification of the Auditory System

During embryonic development the auditory system initiates through a series of cell-cell interactions occurring on the body surface at the position of the ear. Surface epithelium interacts with neural crest cells migrating out from the lateral

area around the neural tube to form the otic placode that ultimately differentiates into the inner ear, including the cochlea with its fluid-filled chambers that detect sound by translating pressure waves to oscillations of an inner membrane (Kil & Collazo, 2002).

The sensory epithelium that lines the cochlear membrane has rows of hair cells that project long finger-like processes, stereocilia, that reach across a fluid-filled space and touch the tectal membrane. Oscillations in the cochlear membranes displace the fingers resulting in an ion channel opening in the cell membrane. The anatomy of the sensory epithelium and the length and number of these stereocilia determines the pitch (frequency oscillations) to which each hair cell responds (Tilney & Saunders, 1983). The hair cells display many short stereocilia at one end of the sensory epithelium and fewer but longer stereocilia at the other end. The texture of sound (its complex frequency combinations) are probably specified by the set of hair cells that respond. Whether each hair cell responds to a pure sine wave or to more complex wave patterns remains a mystery.

Flux of ions into the hair cells produces an electrical current that induces each cell to release neurotransmitters onto sensory neurons contacting the base of the hair cell. The signal is propagated down the sensory process to the cochlear ganglion and thereby into the cochlear nerve to synapse in the brain on the neurons in the cochlear nucleus. Cochlear neurons transmit the electrical signal to the auditory cortex and other brain centers. Signals emanating from the auditory cortex also pass into the limbic system, where emotional events are detected and stored, into the hippocampus, where memory is initiated, and into cortical regions including those for speech and language.

Several stages of development in the auditory system respond to environmental input. First, the length of stereocilia may be influenced by sound perceived by the fetus. This length could be responsive to the environment and subsequently pre-determine the frequency or texture which the animal is capable of perceiving. While the number and length of the stereocilia develop appropriately in denervated cochlea cultured in the allantoic membranes of chick embryos (Corwin & Cotanche, 1989), the length of these processes may be influenced by sound waves prior to birth, as they continue to lengthen in the few days before hatching up to a week after hatching (Tilney & Saunders, 1983).

The dynamic responsiveness of the cochlear membranes and control of hair cell stimulation also appear to be modified in response to a sound (Song, McGee, & Walsh, 2008). Support cells that surround each hair cell may alter its local environment, tension, and structural features that influence the degree of response to membrane oscillations. In addition, the inner and outer hair cells may pull and push on the tectal membrane through dynamic oscillations of their own that also influence hair cell pitch sensitivity. These down-stream tunings of the tectal membrane-hair cell interaction could be regulated semi-permanently by acoustic experience. Speech perception as well as musical abilities and appreciation, which develop through experience, likely involve these dynamics.

Second, while the anatomy of the wiring for the auditory system is established at birth, in humans the wiring can be modified relatively easily until age 12, and less easily after that age (Milner, 1974). The process of sensory system tuning, as perhaps first established by Hubel and Weisel in the visual system, is likely also to hold true for the auditory system (Sanes & Bao, 2009). In childhood, practicing a musical instrument induces changes in fiber tracts within the brain detectable by MRI (Bengtsson et al., 2005).

Functional magnetic resonance imaging of the inferior colliculis has revealed that changes in the activity map in response to specific pitch stimulation occur in adults after consistent repetitive exposure to particular frequency noise. The frequency (Hz) of the sound influenced the location and size of the anatomical domain activated in mice by that sound (Yu et al., 2008). In humans multiple sites respond to the geometry of classical music (Janata et al., 2002). Results from functional magnetic resonance imaging are emerging and describe a complex pattern of inter-related cortical areas responding to, and modified by, musical perception and acquiring musical literacy and performance abilities.

Recently my lab has probed whether connectivity is greater or less in mental retardation such as found in Down syndrome, a trisomy of chromosome 21 (Bearer, Zhang & Jacobs, 2007). We discovered that connections within the memory circuit from hippocampus to forebrain were more robust in Down syndrome than in normals, a paradoxical finding. If we could extrapolate from these results, which may be dangerously speculative, we could infer that learning requires pruning of connectivity, and thus that the imprinting by pre-natal auditory stimuli as described by Gottlieb is a result of a limitation on otherwise more pluripotent capabilities in the fetus. In other words, that prenatal exposure to the mother's and sibling's noises limits the infant to recognize as "safe" only those objects that make the previously experienced noises. Those neonates with no prenatal auditory exposure are "blank slates" that post-natal visual cues imprint upon. Regardless of the process, imprinting of the mother is predestined, and ducklings that do not achieve it will die in the wild.

Example 4: Gamma Band Activation by Auditory Input

How then might "recognition" of a mother or other family member be influenced by auditory input experienced before birth? One notion is that the sound of the mother's voice would be linked in the nervous system to the sensation of pleasure. Indeed, data exist demonstrating the music activates what are known as "gamma waves," electrical oscillations in the brain with a frequency of 40 Hz. Gamma waves are thought to integrate input from various types of stimuli between neural nuclei within the brain (Bhattacharya & Petsche, 2001; Bhattacharya, Petsche,

& Pereda, 2001; Melloni et al., 2007; Sauve, 1999; Thaut, Peterson & McIntosh, 2005). Whether such gamma waves occur prenatally to induce pleasurable sensation through auditory input has not been explored.

Example 5: Consonant Learning Involves Visual as well as Auditory Input

As human babies learn to talk, they listen and also watch the faces of those who talk to them. Striking examples of altered perception of the consonant "b" versus "m" with and without visual cues are reported and easily replicated, otherwise known as the "McGurk effect" (McGurk & MacDonald, 1976). Hence, just as Gilbert Gottlieb described for the wood duckling, human language may be primed prenatally through auditory sensation and consolidated after birth by visual cues (Walker, Bruce, & O'Malley, 1995).

Another example that ties together all of the issues raised above is the role of the serotonin transporter genetic variation in behavior, environmental susceptibility to stress and cognition. Post-traumatic stress disorder may also involve a combination of auditory and visual experience that programs the mind to respond with fear to any resemblance of these sights and sounds out of the context of danger. Susceptibility for this disorder may involve the gene loci encoding proteins involved in the serotonin neurotransmitter pathway, including the serotonin transporter (SERT) (Champoux et al., 2002). Genetic alterations in the serotonin transporter correlate with differential response to stress in the mouse (Adamec, Burton, Blundell, Murphy, & Holmes, 2006). We recently reported profound anatomical changes in the limbic system circuitry of SERT knock-out mice as detected by live MR imaging of a track tracer being transported into the reward circuitry after injection into the frontal cortex (Bearer, Zhang, Janvelyan, Boulat, & Jacobs, 2009). This tracer methodology allows us to collect 3D images at different time points before and after experience and visualize cerebral pathways and connections. Others have now shown that alterations in the anatomy of the limbic system circuitry in rhesus monkeys correlate with different genetic alleles of the SERT gene with effects on cognition (Jedema et al., 2009).

How might the sensation of the mother's voice, heard in utero, pre-condition the neonate to recognize mother, her immediate family and other relatives? During infancy in humans, the sensitivity to non-native sounds diminishes (Pons, Lewkowicz, Soto-Faraco & Sebastian-Galles, 2009). How might auditory recognition serve to inform the child of its ethnic group? Does the unfamiliar speech patterns of non-group members prompt a negative response? If so, how is a negative response programmed – is it merely the absence of recognition, or is there a specific cue that activates rejection? These questions remain on the drawing board.

Conclusions

This book is being published to honor the life and work of Gilbert Gottlieb. Here I have proposed new theories based on his seminal discoveries of the role of auditory perception in the fetus and its role in specifying post-natal recognition of the mother. In a series of examples, I have described how flexible the implementation of a fixed genome can be in response to environmental/experiential cues. Focusing on the auditory system, I showed that both its primary sensory elements that detect and response to acoustic simulation, and its networking within deeper brain circuits are modified in response to experience.

Finally, I discussed how auditory stimuli might propel behavior through links developed in the prenatal period between the auditory system and pleasure circuits. Thus, the mother's vocal cadence, her lullaby heard prenatally, may indelibly prime the infant to respond to the sound of her voice by establishing the basic structure of the inner ear, and coupling the auditory pathway to the pleasure circuits. Much work remains to be done!

References

Adamec, R., Burton, P., Blundell, J., Murphy, D. L., & Holmes, A. (2006). Vulnerability to mild predator stress in serotonin transporter knockout mice. *Behavioural Brain Resesarch*, **170**(1), 126–140.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2007). *Molecular biology of the cell*. New York: Macmillan.

Bearer, E. L. (1995). Cytoskeletal domains in the activated platelet. *Cell Motility and the Cytoskeleton*, **30**(1), 50–66.

Bearer, E. L., & Abraham, M. T. (1999). 2E4 (kaptin): A novel actin-associated protein from human blood platelets found in lamellipodia and the tips of the stereocilia of the inner ear. *European Journal of Cell Biology*, **78**(2), 117–126.

Bearer, E. L., Chen, A. F., Chen, A. H., Li, Z., Mark, H. F., Smith, R. J. H. et al. (2000). 2E4/Kaptin (KPTN) – a candidate gene for the hearing loss locus, DFNA4. *Annals of Human Genetics*, **64**(3), 189–219.

Bearer, E. L., Zhang, X., & Jacobs, R. E. (2007). Live imaging of neuronal connections by magnetic resonance: Robust transport in the hippocampal-septal memory circuit in a mouse model of Down syndrome. *Neuroimage*, **37**(1), 230–242.

Bearer, E. L., Zhang, X., Janvelyan, D., Boulat, B., & Jacobs, R. E. (2009). Reward circuitry is perturbed in the absence of the serotonin transporter. *Neuroimage*, **46**(4), 1091–1104.

Bengtsson, S. L., Nagy, Z., Skare, S., Forsman, L., Forssberg, H., & Ullen, F. (2005) Extensive piano practicing has regionally specific effects on white matter development. *Nature Neuroscience*, **8**(9), 1148–1150.

Bhattacharya, J., & Petsche, H. (2001). Musicians and the gamma band: A secret affair? *NeuroReport*, **12**(2), 371–374.

Bhattacharya, J., Petsche, H., & Pereda, E. (2001). Long-range synchrony in the gamma band: Role in music perception. *Journal of Neuroscience*, **21**(16), 6329–6337.

Champoux, M., Bennett, A., Shannon, C., Higley, J. D., Lesch, K.P., & Suomi, S. J. (2002). Serotonin transporter gene polymorphism, differential early rearing, and behavior in rhesus monkey neonates. *Molecular Psychiatry*, **7**(10), 1058–1063.

Corwin, J. T., & Cotanche, D. A. (1989). Development of location-specific hair cell stereocilia in denervated embryonic ears. *The Journal of Comparative Neurology*, **288**(4), 529–537.

Davidson, E. H., & Erwin, D. H. (2006). Gene regulatory networks and the evolution of animal body plans. *Science*, **311**(5762), 796–800.

Fasullo, M., Bennett, T., & Dave, P. (1999). Expression of Saccharomyces cerevisiae MATa and MAT alpha enhances the HO endonuclease-stimulation of chromosomal rearrangements directed by his3 recombinational substrates. *Mutation Research*, **433**(1), 33–44.

Fedor, M. J. (2009). Comparative enzymology and structural biology of RNA self-cleavage. *Annual Review of Biophysics*, **38**, 271–99.

Gottlieb, G. (1991). *Individual development and evolution: The genesis of novel behavior*. New York: Oxford University Press.

Gottlieb, G. (1998) Normally occurring environmental and behavioral influences on gene activity: from central dogma to probabilistic epigenesis. *Psychological Review*, **105**(4), 792–802.

Gottlieb, G. (2007) Probabilistic epigenesis. *Developmental Science*, **10**(1), 1–11.

Haber, J. E. (1998) Mating-type gene switching in Saccharomyces cerevisiae. *Annual Review of Genetics*, **32**, 561–99.

Hager, G. L., McNally, J. G., & Misteli, T. (2009) Transcription dynamics. *Molecular Cell*, **35** (6), 741–753.

Janata, P., Birk, J. L., Van Horn, J. D., Leman, M., Tillmann, B., & Bharucha, J. J. (2002) The cortical topography of tonal structures underlying Western music. *Science*, **298**(5601), 2167–2170.

Jedema, H. P., Gianaros, P. J., Greer, P. J., Kerr, D. D., Liu, S., Higley, J. D., et al. (2009) Cognitive impact of genetic variation of the serotonin transporter in primates is associated with differences in brain morphology rather than serotonin neurotransmission. *Molecular Psychiatry*.

Kil, S. H., & Collazo, A. (2002). A review of inner ear fate maps and cell lineage studies. *Journal of Neurobiology*, **53**(2), 129–142.

Klar, A. J. (2007). Lessons learned from studies of fission yeast mating – type switching and silencing. *Annual Review of Genetics*, **41**, 213–236.

Lander, E. S., Linton, L. M., Birren, B., Nusbaum, C., Zody, M. C., Baldwin, J., et al. (2001) Initial sequencing and analysis of the human genome. *Nature*, **409**(6822), 860–921.

Lodish, H. B. A., Kreiger, M., Kaiser, C., Scott, M. P., Bretscher, A., & Ploegh, H. (2003). *Molecular cell biology*. New York: W. H Freeman.

Lorenz, K. (1961). *King Solomon's Ring*. London: Methuen.

McGurk, H., & MacDonald, J. (1976) Hearing lips and seeing voices. *Nature*, **264**(5588), 746–748.

Melloni, L., Molina, C., Pena, M., Torres, D., Singer, W., & Rodriguez, E. (2007). Synchronization of neural activity across cortical areas correlates with conscious perception. *Journal of Neuroscience*, **27**(11), 2858–2865.

Milner, B. (1974). Functional recovery after lesions of the nervous system. 3. Developmental processes in neural plasticity. Sparing of language functions after early unilateral brain damage. *Neuroscience Research Program Bulletic*, **12**(2), 213–217.

Peled, J. U., Kuang, F. L., Iglesias-Ussel, M. D., Roa, S., Kalis, S. L., Goodman, M. F., et al. (2008). The biochemistry of somatic hypermutation. *Annual Review of Immunology*, **26**, 481–511.

Pons, F., Lewkowicz, D. J., Soto-Faraco, S., & Sebastian-Galles, N. (2009) Narrowing of intersensory speech perception in infancy. *Proceedings of the National Academy of Sciences, USA*, **106**(26), 10598–10602.

Richer, J., & Chudley, A. E. (2005) The hemoglobinopathies and malaria. *Clinical Genetics*, **68**(4), 332–336.

Sanes, D. H., & Bao, S. (2009). Tuning up the developing auditory CNS. *Current Opinions in Neurobiology*, **9**(2), 188–199.

Sauve, K. (1999). Gamma-band synchronous oscillations: Recent evidence regarding their functional significance. *Consciousness and Cognition*, **8**(2), 213–224.

Soll, D. R., Pujol, C., & Srikantha, T. (2009). Sex: Deviant mating in yeast. *Current Biology*, **19** (13), R509–511.

Song, L., McGee, J., & Walsh, E. J. (2008) Development of cochlear amplification, frequency tuning, and two-tone suppression in the mouse. *Journal of Neurophysiology*, **99**(1), 344–355.

Thaut, M., Peterson, D., & McIntosh, G. (2005). Temporal entrainment of cognitive functions: Musical mnemonics induce brain plasticity and oscillatory synchrony in neural networks underlying memory. *Annals of the New York Academy of Sciences*, **2005** (1060), 243–254.

Tilney, L. G., & Saunders, J. C. (1983). Actin filaments, stereocilia, and hair cells of the bird cochlea. I. Length, number, width, and distribution of stereocilia of each hair cell are related to the position of the hair cell on the cochlea. *Journal of Cellular Biology*, **96**(3), 807–821.

Venter, J. C., Adams, M. D., Myers, E. W., Li, P. W., Mural, R. J., Sutton, G. G., et al. (2001). The sequence of the human genome. *Science*, **291**(5507), 1304–1351.

Vento, S., Cainelli, F., & Cesario, F. (2006) Infections and thalassaemia. *The Lancet Infectious Diseases*, **6**(4), 226–233.

Walker, S., Bruce, V., & O'Malley, C. (1995). Facial identity and facial speech processing: Familiar faces and voices in the McGurk effect. *Perception and Psychophysics*, **57**(8), 1124–1133.

Weatherall, D. J. (2008). Hemoglobinopathies worldwide: Present and future. *Current Molecular Medicine*, **8**(7), 592–559.

Weiss, K., & Simpson, R. T. (1997). Cell type-specific chromatin organization of the region that governs directionality of yeast mating type switching. *The EMBO Journal*, **16**(14), 4352–4360.

Yu, X., Zou, J., Babb, J. S., Johnson, G., Sanes, D. H., & Turnbull, D. H. (2008). Statistical mapping of sound-evoked activity in the mouse auditory midbrain using Mn-enhanced MRI. *NeuroImage*, **39**(1), 223–230.

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Applications of Developmental Systems Theory to Benefit Human Development

On the contributions of Gilbert Gottlieb to individuals, families, and communities

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Gilbert Gottlieb's scholarship (e.g., Gottlieb, 1970, 1976, 1983, 1991a, 1991b, 1992, 1997, 1998, 2004; Gottlieb, Wahlsten, & Lickliter, 1998, 2006) provided theory and data framing a developmental systems approach to developmental science, an approach that, while focusing on non-human organisms (e.g., ducks) in his own laboratory, legitimated an extension to the study of human development across the life span. Gottlieb's theory and research promoted the idea that development involved fully coactional relations across the multiple biological to contextual levels of the developmental system and, as such, rationalized the idea that plasticity (the potential for systematic change in structure and function across life) was a ubiquitous component of the developmental system. If coactional relations across the developmental system gave rise to the potential for plasticity, then such relations in their specific instantiation provided a base for a specific trajectory of human development. In turn, changes in plastic coactions could result in variation in these trajectories.

Such potential change in the course of human life affords an optimistic view about the potential of developmental science to optimize the course of human life. Accordingly, if developmental science is applied in ways that seek to

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promote positive outcomes in human development, it is within the power of researchers to engage the developmental system in ways that improve the human condition.

The purpose of this chapter is to describe Gottlieb's developmental systems model, and to explain how it provides a frame for the application of developmental systems theory. We discuss the contemporary features of human developmental science to illustrate Gottlieb's penetrating conceptual influence, and then discuss how, encouraged by Gottlieb's systematic approach to developmental systems theory, future research with humans can promote programs of research that address issues having the potential to improve both individual development and social justice.

The Contemporary Features of Human Developmental Science

Human development involves organized, systematic, and successive changes within a person across the life span (Lerner, 2002). Therefore, the study of development involves the description, explanation, and optimization of such intraindividual changes and, as well, the identification of interindividual differences in intraindividual changes across the life span (Baltes, Reese, & Nesselroade, 1977). While the goals of description and explanation are shared by all instances of science (for instance, from physics to genetics through astronomy), the optimization goal of developmental science stands in contrast to many other instances of science, where explanations are tested in the context of the researcher's ability to control or predict the full range of phenomena pertinent to a given scientific domain (e.g., physical particle movement, molecular change, neuromuscular connections, economic expansion in developing nations, or the orbits of comets).

Although developmental scientists are interested in prediction and, through laboratory-based experiments or community-based interventions, in "control" as well, the full range of variation of a phenomenon is not ethically available in the study of humans. For instance, learning ability or moral functioning may be normally distributed from low to high; however, the developmental scientist cannot ethically try to test the validity of his or her theory-based explanations by acting to decrease people's capacity to learn or to lower moral functioning or diminish character. The scientist is obligated to act to improve behavior, to make it more optimal, and not act to deteriorate it. In short, in developmental science the only ethical option available to the researcher seeking to test his or her explanations of why the changes he or she has described appear as they do is to attempt to move human functioning in a more positive or healthier direction, to attempt to move human behavior towards more optimal functioning.

At its core, then, the nature of developmental science involves the integration of basic issues of description and explanation with issues about how developmental science may be applied (e.g., through enacting community-based intervention programs or evaluating or testing the effects of social policies) to improve the human condition (and, as well, in the context of these applications, to test basic ideas about the individual and contextual bases of human development; Lerner, 2006). In short, contemporary developmental science involves a fundamental integration between basic and applied scientific work.

The conceptual foundation for this integration is predicated within modern developmental science by *developmental systems theories*. Today, these models of the mutually influential relations between individuals and their contexts (represented within the literature as individual ↔ context relations; Lerner, 2006) are at the cutting-edge of theory and research in contemporary developmental science (Damon & Lerner, 2006). As described in Box 21.1, these theories focus on *relations* among variables within and across the integrated levels of organization that comprise the ecology of human development (Bronfenbrenner & Morris, 2006; Overton, 2006).

Box 21.1. Defining features of developmental systems theories.

A Relational Metamodel

Predicated on a post-modern philosophical perspective that transcends Cartesian dualism, developmental systems theories are framed by a relational metamodel for human development. There is, then, a rejection of all splits between components of the ecology of human development, for example, between nature- and nurture-based variables, between continuity and discontinuity, or between stability and instability. Systemic syntheses or integrations replace dichotomizations or other reductionist partitions of the developmental system.

The Integration of Levels of Organization

Relational thinking and the rejection of Cartesian splits is associated with the idea that all levels of organization within the ecology of human development are integrated, or fused. These levels range from the biological and physiological through the cultural and historical.

Developmental Regulation across Ontogeny Involves Mutually Influential Individual ↔ Context Relations

As a consequence of the integration of levels, the regulation of development occurs through mutually influential connections among all levels of the developmental system, ranging from genes and cell physiology through individual mental and behavioral functioning to society, culture, the designed and natural ecology and, ultimately, history. These mutually influential relations may be represented generically as Level 1 ↔ Level 2 (e.g., Family ↔ Community) and, in the case of ontogeny may be represented as individual ↔ context.

Integrated Actions, or Individual ↔ Context Relations, are the Basic Unit of Analysis Within Human Development

The character of developmental regulation means that the integration of actions – of the individual on the context and of the multiple levels of the context on the individual (individual ↔ context) – constitute the fundamental unit of analysis in the study of the basic process of human development.

Temporality and Plasticity in Human Development

As a consequence of the fusion of the historical level of analysis – and therefore temporality – within all the levels of organization comprising the ecology of human development, the developmental system is characterized by the potential for systematic change, by plasticity. Observed trajectories of intraindividual change may vary across time and place as a consequence of such plasticity.

Plasticity is Relative

Developmental regulation may both facilitate and constrain opportunities for change. Thus, change in individual ↔ context relations is not limitless, and the magnitude of plasticity (the probability of change in a developmental trajectory occurring in relation to variation in contextual conditions) may vary across the life span and history. Nevertheless, the potential for plasticity at both individual and contextual levels constitutes a fundamental strength of all human development.

Intraindividual Change, Interindividual Differences in Intraindividual Change, and the Fundamental Substantive Significance of Diversity

The combinations of variables across the integrated levels of organization within the developmental system that provide the basis of the developmental process will vary at least in part across individuals and groups. This diversity is systematic and lawfully produced by idiographic, group differential, and generic (nomothetic) phenomena. The range of interindividual differences in intraindividual change observed at any point in time is evidence of the plasticity of the developmental system, and makes the study of diversity of fundamental substantive significance for the description, explanation, and optimization of human development.

Optimism about the Application of Developmental Science and the Promotion of Positive Human Development

The potential for and instantiations of plasticity legitimate an optimistic and proactive search for characteristics of individuals and of their ecologies that, together, can be arrayed to promote positive human development across life. Through the application of developmental science in planned attempts (i.e., interventions) to enhance (e.g., through social policies or community-based programs) the character of humans' developmental trajectories, the promotion of positive human development may be achieved by aligning the strengths (operationalized as the potential for positive change) of individuals and contexts.

Multidisciplinarity and the Need for Change-Sensitive Methodologies

The integrated levels of organization comprising the developmental system require collaborative analyses by scholars from multiple disciplines. Multidisciplinary knowledge and, ideally, interdisciplinary knowledge is sought. The temporal embeddedness and resulting plasticity of the developmental system requires that research designs, methods of observation and measurement, and procedures for data analysis be change-sensitive and able to integrate trajectories of change at multiple levels of analysis. Representative instances of change-sensitive methodologies may involve (a) innovations in sampling (e.g., theoretically predicated selection of participants and of x-axis divisions, or inverting the x- and the y-axis, that is, making time the dependent

variable; (b) using measures designed to be sensitive to change, and to possess equivalence across temporal levels (age, generation, history), different groups (sex, race, religion), and different contexts (family, community, urban-rural, culture); to provide relational indices (e.g., of person-environment fit); and to provide triangulation across different observational systems (convergent and divergent validation); (c) employing designs that are change-sensitive, such as longitudinal and sequential strategies, and person-centered, as compared to variable-centered, analyses ("P" versus "R" approaches); and (d) data analyses that afford multivariate analyses of change, for instance, procedures such as SEM, HLM, trajectory analysis, or time series analysis.

Gilbert Gottlieb's scholarship has been a visible and influential force in the field of developmental psychology, and of human development more specifically, for some time (e.g., Gottlieb, 1970, 1997, 1998; Gottlieb et al., 2006). Moreover, his perspective converges with that found in the scholarship of other long-term leaders of developmental comparative science (e.g., Kuo, 1967, 1976; Lehrman, 1953; Schneirla, 1956; Tobach, 1971).

The developmental systems framework of Gottlieb (1991a, 1991b, 1992, 1997) indicates that all organismic characteristics (e.g., genes, cells, tissues, organs), as well as the whole organism itself, function in a bidirectional, reciprocal, or "dynamic interactional" (Lerner, 1978) relation with the contexts within which the organism is embedded. With the dimension of time, it is this multilevel, integrated functioning which constitutes the course of individual development. Gottlieb's (1991a, 1991b, 1992) examples of dynamic interactions involve integrated, multilevel exchanges of material (e.g., nutritional, hormonal) variables, energy (e.g., light) variables, or informational (i.e., psychological and behavioral) variables. Within the human development literature, examples of dynamic interactions have most often involved integrated, multilevel exchanges involving the latter type of variables (Ford & Lerner, 1992; Lerner, 1991).

Although these types of examples refer to exchanges having contents which are qualitatively different, their structure and function can be integrated within a common, developmental systems perspective, such as the one forwarded by Gottlieb (1991a, 1991b, 1992; see too Ford & Lerner, 1992). Indeed, whether illustrated by data from the field of comparative psychology or from the field of human development, the developmental systems model underscores the idea that the basic process of development is a relational one (Overton, 1998). That is, the basic process of development is changing relations between the organism and the multilevel context comprising the ecology of the organism's development.

Moreover, the reciprocity between organism and context, and the temporality that derives from the embeddedness of all levels of this system in history, provides a change component to the organism, to the context, and to the relation between the two. In addition, the singularity of the array of variables from the multiple, integrated levels that characterizes an organism across its life span, assures that lawful individual difference (i.e., individuality that is neither mere error variance nor substantively trivial) characterizes the course of ontogeny. Thus, as Gottlieb (1992, p. 95) points out: "Ontogeny in each generation is a consequence of the coaction of hereditary or genetic factors and many different local environmental circumstances that determine the expression of the phenotype during the course of development."

Accordingly, the key features of Gottlieb's developmental systems perspective provide an intellectually important and societally timely frame for the study of human development. These features include:

- changing organism-context coactions;
- focus on the actual physical and social ecology within which the organism develops;
- individual differences (or, as they are now more often labeled within the human development field, "diversity"); and
- a sensitivity to the entire life span as a legitimate frame within which to study interactions and individuality.

In short, within the context of Gottlieb's (1997) developmental systems view, epigenesis is a probabilistic process of individual development and "The most important feature of the developmental systems view is the explicit recognition that the genes are an integral part of the system and their activity (i.e., genetic expression) is affected by events at other levels of the system, including the environment of the organism" (p. 82). Indeed, Gottlieb emphasizes that "The principal ideas concern the epigenetic characterization of individual development as an emergent, coactional, hierarchical system" (Gottlieb, 1997, p. 89).

Accordingly, Gottlieb (1997) draws a distinction between the probabilistic view of epigenesis and the predetermined version of epigenesis. The latter

> viewpoint holds that behavioral epigenesis is predetermined by invariant organic factors of growth and differentiation (particularly neural maturation), and the... [former]... viewpoint holds that the sequence and outcome of prenatal behavior is probabilistically determined by the critical operation of various endogenous and exogenous stimulative events. (Gottlieb, 1970, p. 111)

These intra-individual and extra-individual (contextual) events are parts of the organism's experience.

Within the relational perspective forwarded by Gottlieb, experience represents, then, a concept that is central in attempts to distinguish between an integrative,

developmental systems perspective and a split conception. Akin to the views advanced by Schneirla (1957), Gottlieb stated,

> experience should be broadly defined to include activity produced within the organism itself (endogenous motor as well as sensory-system activity).... It is only by denying (or not acknowledging) the role of spontaneous endogenous activity within the nervous system as playing a formative role in neural and behavioral development that the outmoded nature versus nurture conception can be kept alive. (Gottlieb, 1997, p. 55)

Indeed, by explaining the various roles of experience in development, Gottlieb "forces us to think in a new way about the role of experience in the development of behavior that is thought of as instinctive" (1997, p. 76). Accordingly, it is useful to discuss Gottlieb's conception of the roles of experience within developmental systems.

In contrast to nativist notions, such as those forwarded by Waddington (1957; 1971), and to further illustrate the character of experiential influences within the developmental system, Gottlieb offers several examples of the nonobvious role of experience in individual development. For instance, he explains how experiential factors (such as social interactions, the introduction of particular gasses into the proximal atmosphere of the developing individual, or changing day length) may influence physiological functioning (for instance, hormone secretions) which, in turn, may result in the turning on of genes, that is, in the activation of DNA transcription in the cell nucleus. Outcomes of such experiences may involve effects as dramatic as the development of teeth-like structures in chickens, sex reversals in coral reef fish, and a second set of wings in otherwise normal fruit flies (Gottlieb, 1997). In fact, there is now so much evidence suggesting that experience (e.g., sensory stimulation) can activate DNA in the individual that the phenomenon has a name: Immediate early gene expression (Gottlieb, 1997).

These examples and the others provided by Gottlieb (1997) about the role of experience in individual development, derived from his and others' research, underscore that coactions, among the integrated levels of organization of the developmental system, provide the basis of ontogenetic change (Gottlieb et al., 1998). As such, Gottlieb notes that "when certain scientists refer to behavior or any other aspect of organismic structure or function as being 'genetically determined,' they are not mindful of the fact that genes synthesize protein (not behavior) and that they do so in the context of a developmental system of higher influences" (1997, p. 93). Indeed, such genetic determinist (nature-nurture split) conceptions (e.g., Plomin, 1986; Rowe, 1994; Rushton, 1997) "have provided impediments to thinking clearly about the need for conceptual and empirical analysis at all levels of the developmental systems hierarchy" (Gottlieb, 1991a, p. 7).

Overton (1998) explains that the casting of "our fundamental understanding of development into an inclusive relational frame has profound implications for the concepts and theories, as well as the methodology and methods, of developmental

inquiry" (p. 114). We would in addition stress a point regarding the notion of plasticity, when considered within a developmental systems view. That is, that a developmental systems perspective supports optimism about the potential efficacy of developmentally-appropriate public policies and of preventive and optimizing developmental interventions; the enactment and evaluation of such policies and programs serve as a way of testing or demonstrating this developmental systems perspective (cf. Brim & Kagan, 1980; Gottlieb, 1997, p. 138; Lerner, 1995).

Gilbert Gottlieb's View of Epigenesis

Gottlieb (1992) presents a developmental systems perspective within which changing gene (or organism)-context relations are the key foci of both developmental and evolutionary analysis. As such, he builds on the work of Garstang (1922), de Beer (1930, 1958), and Goldschmidt (1933), and notes that "Phylogeny is thus not the cause but the product of a succession of different ontogenies" (Gottlieb, 1992, p. 90). In other words, variation in development – for instance, behavioral novelty arising through the plasticity of dynamic organism-context relations (e.g., Lerner, 1984) – produces evolution; evolution does not produce development. "Ontogeny in each generation is a consequence of the coaction of hereditary or genetic factors and many different local environmental circumstances that determine the expression of the phenotype during the course of development" (Gottlieb, 1992, p. 95). In essence, then, Gottlieb agrees with Goldschmidt (1933, p. 543) that "The nature and working of the developmental process of the individual then should, if known, permit us to form certain notions regarding the possibilities of evolutionary changes." Therefore, what is the character of the developmental process as envisioned by Gottlieb?

Gottlieb's conception of the developmental process "is one of a totally interrelated, fully coactional system in which the activity of genes themselves can be affected through the cytoplasm of the cell by events originating at any other level in the system, including the external environment" (Gottlieb, 1992, pp. 144–145). Accordingly, based on the work of Schneirla (e.g., 1957), Kuo (1976), Lehrman (1970), and others (e.g., Tobach, 1981, and of course Gottlieb himself, 1970, 1976, 1983, 1991a, 1991b), Gottlieb (1992) provides a new definition of epigenesis:

> Individual development is characterized by an increase of complexity of organization – that is, the emergence of new structural and functional properties and competencies – at all levels of analysis (molecular, subcellular, cellular, organismic) as a consequence of horizontal and vertical coactions among its parts, including organism-environment coactions (pp. 159–160).

Moreover, Gottlieb explains that within the developmental system of coactions that he described there exists both horizontal and vertical coactions. Horizontal

coactions "are those that occur at the same level (gene-gene, cell-cell, tissue-tissue, organism-organism), whereas vertical coactions occur at different levels (gene-cytoplasm, cell-tissue, behavioral activity-nervous system) and are reciprocal, meaning that they can influence each other in either direction, from lower to higher, or from higher to lower, levels of the developing system" (Gottlieb, 1992, pp. 160–161).

Accordingly, in presenting his views of a developmental systems conception of development, Gottlieb notes that when one speaks of coaction between genes and the other levels of the system as being at the "heart of developmental analysis or causality what we mean is that we need to specify some relationship between at least two components of the developmental system" (Gottlieb, 1992, pp. 161–163). Indeed, Gottlieb (1992) contends that this systems view of individual development is the only "way to envisage the manner in which development must occur if a harmoniously functioning, fully integrated organism is to be its product" (pp. 165–166). "[G]enes are part of the developmental system in the same sense as other components (cell, tissue, organism), so genes must be susceptible to influence from other levels during the process of individual development" (Gottlieb, 1992, p. 167).

The theory and data Gottlieb (1997; Gottlieb et al., 1998) marshals in support of this developmental systems view are compelling. Many of these examples involve integrated, multilevel exchanges of material (e.g., nutritional, hormonal) or energy (e.g., light) variables. Gottlieb explains that such evidence underscores that the action of genes (gene expression) is "affected by events at other levels of the [developmental] system" (Gottlieb, 1991a, p. 5), that "all levels of the system may be considered potentially equal" (Gottlieb, 1991a, p. 6), and therefore that "genetic activity does not by itself produce finished traits such as blue eyes, arms, legs, or neurons. The problem of anatomical and physiological differentiation remains unsolved, but it is unanimously recognized as requiring influences above the strictly cellular level" (Gottlieb, 1991a, p. 5). Thus, intra-organism variables making up the proximal context of the gene, as well as extra-organism contextual variables, are shown in Gottlieb (1991a, 1991b), as well as in the literature he cites (e.g., Edelman, 1987, 1988; Grouse, Schrier, Letendre, & Nelson, 1980; Kollar & Fisher, 1980; Uphouse & Bonner, 1975; see also Lerner, 1984), to exist in a reciprocally influential relation with genes.

Given this evidence, one conclusion is inescapable: The idea that genes are impenetrable and fixed entities that direct a person's development in a manner independent of the supragenetic, organismic, and environmental (contextual) levels of organization within which the genes are embedded, is absurd (Ho, 1984; Strohman, 1993). No feature of biology is so encapsulated, so automated, and so invulnerable to moderation by the context that it can stand as an example of such an impenetrable entity. Simply, then, just as genes may influence supragenetic levels, both within and outside of the organism, these levels of organization influence genes. It is these multilevel co-actions that produce development, and that are embodied in Gottlieb's (1992) new definition of epigenesis noted earlier.

From Phylogeny to Ontogeny

Whether studying infancy, childhood, adolescence, or the adult and aging portions of the life span, the cutting-edge of contemporary scholarship in human development involves attempting to integrate information from the several levels of organization comprising the ecology of human development (Bronfenbrenner & Morris, 2006). Such work aims to explain how mutually influential (i.e., bidirectional, reciprocal, synergistic, or fused; e.g., Thelen & Smith, 2006; Tobach & Greenberg, 1984) relations between individuals and their contexts provide the basis for behavior and development. As such, to describe, explain, and optimize developmental changes, the developmental scientist focuses on systematic and successive alterations in the course of the relations an individual has with the multiple levels of the ecology of human development, ranging from the inner-biological level through the sociocultural and historical levels (Bronfenbrenner & Morris, 2006; Lerner, 2002, 2006). In short, through conducting research that is focused on or, at the least, informed by, individual ↔ context relations, developmental scientists can describe, explain, and optimize trajectories of developmental changes across the life span (Baltes et al., 1977).

Not all developmental scientists pursue all three goals at one time (or even within one career). Nevertheless, all goals are needed to have a complete and vibrant developmental science. Indeed, the view that the application of developmental science is regarded as a foundational component of the contemporary study of human development is evidenced both by the broad interest in the conceptual facets of developmental systems models (e.g., see Volume 1, "Theoretical Models of Human Development," in the 6th edition of the *Handbook of Child Psychology*; Damon & Lerner, 2006) and by the active research associated with issues of application in developmental science (e.g., see the four volumes of the *Handbook of Applied Developmental Science: Promoting Positive Child, Adolescent, and Family Development through Research, Policies, and Programs*; Lerner, Jacobs, & Wertlieb, 2003; the two-volume *Applied Developmental Science: An Encyclopedia of Research, Policies, and Programs*; Fisher & Lerner, 2005; or the articles published in the quarterly journal, *Applied Developmental Science*, which at this writing is now in its 13th volume year). Given the centrality of the application of developmental science within the field of human development, it is useful to review briefly the history and defining features of this domain of scholarship.

Applied Developmental Science: An Overview

Applied developmental science (ADS) seeks to advance the integration of developmental research with actions that promote positive development and/or

enhance the life chances of vulnerable children, adolescents, younger and older adults, and their families (e.g., see discussions by Eccles, 1996; Fisher & Lerner, 1994; Lerner, 2006; Lerner, Fisher, & Weinberg, 2000; Sherrod, 1999; Takanishi, 1993). Given its roots in developmental systems theory, ADS challenges the usefulness of decontextualized knowledge and, as a consequence, the legitimacy of isolating scholarship from the pressing human problems of our world.

Accordingly, when focused on the first two decades of the life span, scientists applying developmental science use biological, behavioral, and social science theory and data to describe, explain, and optimize the course of child and adolescent development, and to enhance the key settings within which young people develop. These settings are families, schools, after-school programs, community social service settings, or health settings.

The Scope of ADS Activities

In the late 1980s, scholars from several disciplines (ones associated with the American Psychological Association, the Society for Research in Child Development, the Society for Research on Adolescence, the International Society for Infant Studies, the Gerontological Society of America, the National Black Child Development Institute, and the National Council on Family Relations) came to the realization that issues of child, youth, and adult development, of family structure and function, of economic competitiveness, of environmental quality, and of health and health care were interdependent. To understand these phenomena requires creative and integrative research. To improve the status of these issues involves the design, deployment, and evaluation of innovative public policies and intervention programs. Moreover, as a consequence of the presence of the interrelated problems confronting global society, there has been over the last two decades increasing societal pressure for universities, and for the scholars within them, to design and deliver knowledge applications addressing the problems of individuals and communities across the life span (Boyer, 1990; Chibucos & Lerner, 1999; Ralston et al., 1999).

These applications involve the ability to understand and assist the development of individuals who vary with respect to cultural and ethnic background; economic and social opportunity; physical and cognitive abilities; and conditions of living (e.g., in regard to their family, neighborhood, community, and physical settings). Moreover, infants at biological or social risk (e.g., due to being born into conditions of poverty); gifted children or those with developmental disabilities; adolescents considering health-compromising behaviors; single- and dual-worker parents; the frail elderly; ethnic minorities; and impoverished families are just some of the populations requiring applications of knowledge based on the work of scholars – in fields such as psychology, sociology, nursing, human ecology/human development, social work, criminology, political science, medicine, biology, anthropology, and economics – who adopt a developmental perspective to their science.

The multiplicity of disciplines called on to apply their scientific expertise in the service of enhancing the development of individuals, families, and communities resulted in a collaboration among the above-noted learned societies. These groups organized a "National Task Force on Applied Developmental Science" in order to synthesize research and applications aimed at describing, explaining, and promoting optimal developmental outcomes across the life cycle of individuals, families, and communities.

To accomplish these objectives, the National Task Force defined the nature and scope of applied developmental science (ADS). The Task Force forwarded these definitions in the context of convening a national conference (at Fordham University, in October, 1991), on "Graduate Education in the Applications of Developmental Science Across the Life Span." The conference inaugurated ADS as a formal program of graduate study and specified the key components involved in graduate education in ADS (Fisher et al., 1993).

The National Task Force indicated that the activities of ADS span a continuum of knowledge generation to knowledge application which includes, but is not limited to:

1. research on the applicability of scientific theory to growth and development in "natural" (i.e., ecologically valid) contexts;
2. the study of developmental correlates of phenomena of social import;
3. the construction and utilization of developmentally and contextually sensitive assessment instruments;
4. the design and evaluation of developmental interventions and enhancement programs; and
5. the dissemination of developmental knowledge to individuals, families, communities, practitioners, and policymakers through developmental education, written materials, the mass media, expert testimony, and community collaborations.

This articulation of ADS activities by the several scholarly societies involved in the National Task Force has, in a sense, involved the rearticulation of the philosophy and scholarly and outreach agenda of the land-grant university (Bonnen, 1998; Kellogg Commission on the Future of State and Land-Grant Universities, 1999; Lerner & Miller, 1993; Ralston et al., 1999). In addition, ADS has involved an embracing of an approach to scholarship that merges basic and applied research within an integrated developmental system (Lerner, 2002, 2006).

Accordingly, consistent with a developmental systems perspective, applied developmental scientists seek to synthesize research and outreach in order to describe, explain, and enhance development in individuals and families across the life span (Fisher & Lerner, 1994). Fisher et al. (1993) characterized the "principles," or core substantive features, of applied developmental science (ADS) in terms of the following five conceptual components:

1. The first component is the *temporality of change*; there is a temporal dimension – a chronosystem (Bronfenbrenner & Morris, 2006) – that pertains to individuals, families, institutions, and community experiences. In other words, the "arrow of time" (history) runs through the structure and function of the human development system. Simply, things change!

   Some temporal features (change parameters) of individual and contextual development or historical variation reflect continuity; other features may reflect discontinuity. Continuous or discontinuous changes may occur at different rates across different levels of organization of the human development system (requiring, therefore, that x-axis divisions of time be different for, for instance, gauging the pattern of change for infant neuromuscular development, adolescent identity development, the family life cycle, or the course of school reforms subsequent to the introduction of new educational policies; Lerner, Schwartz, & Phelps, 2008).

   Accordingly, the temporality of change has important implications for research design, service provision, and program evaluation. Because of temporality, generalizations across historical periods or birth cohorts may not be warranted (e.g., Elder, 1974; Elder & Shanahan, 2006).
2. The second component is *sensitivity to individual differences and within-person change*. This component means that interventions must take into account individual differences, which means the diversity of racial, ethnic, social class, and gender groups and, as well, other important variations that may moderate how an intervention may influence development (e.g., such variation may involve family socialization practices, youth motivation, or youth intellectual functioning). This component emphasizes that developmental science must be attentive to both intraindividual change and to interindividual differences in intraindividual change (Baltes et al., 1977). It also means that diversity is a core, substantive component of developmental science theory and research. In other words, diversity – within individuals across time and between individuals within time – *is* the essence of human development; it comprises the "fabric" of human development.
3. The third component of ADS involves *the centrality of context in terms of individual and family development*. Predicated on the relational focus of developmental systems models, the basic unit of analysis in developmental science is the relation between features of the individual and features of his or her context, the multiple, integrated levels of organization comprising the ecology of human development (Bronfenbrenner & Morris, 2006). In other words, context pertains to all levels within the developmental system, that is, the biological/physiological, individual (psychological/behavioral), social relational (e.g., dyadic or peer group), family, community, cultural (e.g., educational, religious, political, and economic), and physical/ecological levels of organization. Accordingly, the focus on the individual $\leftrightarrow$ context relation requires scholarship that involves systemic, multilevel approaches to research and program design and implementation.

4. The fourth component is *an emphasis on (descriptively) normative developmental processes and on primary prevention and optimization – on the promotion of positive development – rather than on remediation*. This component is linked, first, to the focus on diversity within ADS (and within a developmental systems approach to developmental science more generally; Damon & Lerner, 2006; Lerner, 2006).

   Across its history, the study of human development has all-too-often been equated with the study of White, middle-class American samples (e.g., Graham, 1992; Lerner, 2006). As such, considerably less is known about the normative development of other racial, ethnic, and national/cultural groups. For instance, at this writing, normative information about the life-span development of Native Americans, Kenyans, Venezuelans, or Singaporeans does not exist, and normative descriptions of the family life cycle of African Americans, Cubans, Norwegians, Indians, or Chinese do not exist. Nevertheless, despite the absence of such normative information about the diversity of humanity, the normative study of White, middle class American samples was often mistakenly construed as research identifying the characteristics of what was normative for all of humanity and, as well, as what was reflective of optimal or ideal development.

   Such egregious – and potentially socially pernicious – overgeneralization is rejected within ADS. The need to identify what is both normal and optimal among diverse individuals, families, communities, and cultural groups is regarded as a fundamental goal of the field.

   The multiplicity of normative or optimal developmental paths raises the second aspect of the fourth component of ADS, and involves the emphases on temporality and plasticity within developmental systems theories. The optimism about promoting positive human development that derives from the concepts of plasticity and temporality means that individual $\leftrightarrow$ context relations can be found or created to enhance the likelihood of positive, healthy development (see Box 21.1). As a consequence, ADS emphasizes that programs and policies may be aimed at promoting positive development and not only on reducing or remediating problems. Accordingly, deficit perspectives are eschewed within ADS (e.g., Lerner, 2005; Lerner & Steinberg, 2009). Although problems of, and challenges to healthy human development do exist, such issues are not regarded as inevitable or as necessarily characteristic of particular age, racial, ethnic, or cultural groups. While work continues to need to be done to prevent or to reduce problems of development that do arise, especially because features of both positive and problematic development can develop at the same time (e.g., Phelps et al., 2007), researchers and practitioners using an ADS perspective to frame their work emphasize the possibility of promoting positive human development across the life span (e.g., Baltes, Lindenberger, & Staudinger, 2006). Indeed, a goal for the field is to describe, explain, and optimize the life course of the diversity of humanity.

5. The fifth component of ADS is *respect for the bidirectional relationship between knowledge generation and knowledge application*. Fisher and Lerner (1994, p. 7) have discussed this component by noting that:

> there is an interactive relationship between science and application. Accordingly, the work of those who generate empirically based knowledge about development and those who provide professional services or construct policies affecting individuals and families is seen as reciprocal in that research and theory guide intervention strategies and the evaluation of interventions and policies provides the bases for reformulating theory and future research.... As a result, applied developmental [scientists] not only disseminate information about development to parents, professionals, and policy makers working to enhance the development of others, they also integrate the perspectives and experiences of these members of the community into the reformulation of theory and the design of research and interventions.

In sum, the components of ADS together foster a dynamic agenda of collaborations between researchers and practitioners that involves developmental, contextually-sensitive research and applications directed to enhancing the individual ↔ context relations characterizing the course of development of diverse individuals and groups. ADS scholarship inherently takes a life-span approach to human development in that individuals always interact in a social context composed of people of diverse age levels. In other words, part of any person's development involves relations with individuals who may be at any one of many other stages of life. These social relations are a basis of the course of intraindividual changes a given person will experience in life. Moreover, the life-span purview of ADS means also that research and application may be focused on understanding and promoting the positive development of individuals at any portion of the life course.

Conclusions

Applied developmental scientists seek ways to apply their scientific expertise in manners that promote the life chances of the individuals, social groups, and communities participating in developmental scholarship. The key challenge in such efforts is to generate scientifically rigorous evaluations of the usefulness of the policies and the programs associated with such ADS and, as well, to use such information in the day-to-day operation of programs (e.g., Fetterman, Kaftarian, & Wandersman, 1996; Jacobs, 1988; Lerner, 2002). How may this challenge be addressed?

The expertise of the research and practitioner communities can provide much of the human resources needed to meet this challenge if means can be created to foster collaborations with the goal of creating empowered communities. At this writing, efforts in the US, such as the America's Promise Alliance (APA), are aimed at

precisely this sort of collaboration (America's Promise Alliance, 2007). APA is the country's largest multi-sector collaboration dedicated to the well-being of all young people in America (more information about APA may be found at http://americaspromise.org). Through partnering with researchers to conduct basic developmental research and evaluations of its work, the Alliance uses data to guide its strategy. Importantly, the Alliance disseminates the best practices derived from research to national partners, policy makers, and community leaders throughout the country. Based on the efforts such as those being pursued by APA, policies promoting such researcher-practitioner-community coalitions could become an integral component of a national youth and family development policy aimed at creating caring communities that possess the capacity to further the healthy development of youth and families (e.g., Kennedy, 1999; Sherrod, 1999; Spanier, 1999; Thompson, 1999; Zaff & Smerdon, 2009).

These partnerships, if facilitated and rewarded by an engaged university (Kellogg Commission, 1999; Spanier, 1999), will enable scholars and their community collaborators to enhance social justice and contribute to civil society. As such, these collaborations will model how universities, and the applied developmental scientists working within them, may be part of a multi-institutional system changing American society by moving it in the direction of greater equity and access to democratizing resources for all its diverse citizens.

Given the enormous, indeed historically unprecedented, challenges facing the families of America and the world, perhaps especially as they strive to raise healthy and successful children capable of leading civil society productively, responsibly, and morally across the 21st century (Lerner, 2004, 2007), there is no time to lose in the development of such collaborations. Indeed, by enhancing its motivation for and capacity to engage in outreach research, the field of human development can improve upon the often-cited idea of Kurt Lewin (1943), that there is nothing as practical as a good theory. We can, through the application of developmental science, serve our world's citizens and demonstrate that there is nothing of greater value to civil society than a science devoted to using its scholarship to improve the life chances of all people.

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References

America's Promise Alliance (2007). *From a meeting to a movement: Building a blueprint for action. An interim report on the Leadership Forum*. Washington, DC: Author.

Baltes, P. B., Lindenberger, U., & Staudinger, U. (2006). Life span theory in developmental psychology. In: W. Damon & R. M. Lerner (Eds. in Chief) & R. M.Lerner (Vol. Ed.), *Handbook of child psychology, Vol. 1. Theoretical models of human development* (6th ed., pp. 569–664). Hoboken, NJ: Wiley.

Baltes, P. B., Reese, H. W., & Nesselroade, J. R. (1977). *Life-span developmental psychology: Introduction to research methods*. Monterey, CA: Brooks/Cole.

Bonnen, J. T. (1998). The land-grant idea and the evolving outreach university. In: R. M. Lerner & L. A. K. Simon (Eds.), *University-community collaborations for the twenty-first century: Outreach scholarship for youth and families* (pp. 25–71). New York: Garland.

Boyer, E. L. (1990). *Scholarship reconsidered: Priorities of the professoriate*. Princeton, NJ: The Carnegie Foundation for the Advancement of Teaching.

Brim, O. G., & Kagan J.K. (Eds.). (1980). *Constancy and change in human development*. Cambridge, MA: Harvard University Press.

Bronfenbrenner, U. & Morris, P. A. (2006). The bioecological model of human development. In W. Damon & R. M. Lerner (Eds. in Chief) & R. M. Lerner (Vol. Ed.), *Handbook of child psychology, Vol. 1. Theoretical models of human development* (6th ed., pp. 793–828). Hoboken, NJ: Wiley.

Chibucos, T., & Lerner R.M. (Eds.). (1999). *Serving children and families through community-university partnerships: Success stories*. Norwell, MA: Kluwer.

Damon, W., & Lerner R.M. (Eds.). (2006). *Handbook of Child Psychology* (6th ed.). Hoboken, NJ: Wiley.

de Beer, G. R. (1930). *Embryology and evolution*. Oxford: Clarendon Press.

de Beer, G. R. (1958). *Embryos and ancestors* (3rd ed.). Oxford: The Clarendon Press.

Eccles, J. S. (1996). The power and difficulty of university-community collaboration. *Journal of Research on Adolescence*, **6**, 81–86.

Edelman, G. M. (1987). *Neural Darwinism: The theory of neuronal group selection*. New York: Basic Books.

Edelman, G. M. (1988). *Topbiology: An introduction to molecular biology*. New York: Basic Books.

Elder, G. H. (1974). *Children of the Great Depression*. Chicago: University of Chicago Press.

Elder, G. H., & Shanahan, M. J. (2006). The life course and human development. In W. Damon & R. M. Lerner (Eds. in Chief) & R. M. Lerner (Vol. Ed.), *Handbook of child psychology, Vol. 1. Theoretical models of human development* (6th ed., pp. 665–715). Hoboken, NJ: Wiley.

Fetterman, D. M., Kaftarian S.J., & Wandersman, A. (Eds.). (1996). *Empowerment evaluation: Knowledge and tools for self-assessment and accountability*. Thousand Oaks, CA: Sage.

Fisher, C. B., & Lerner, R. M. (1994). Foundations of applied developmental psychology. In: C. B. Fisher & R. M. Lerner (Eds.), *Applied developmental psychology* (pp. 3–20). New York: McGraw-Hill.

Fisher, C. B., & Lerner R.M. (Eds.). (2005). *Encyclopedia of applied developmental science*. Thousand Oaks, CA: Sage.

Fisher, C. B., Murray, J. P., Dill, J. R., Hagen, J. W., Hogan, M. J., Lerner, R. M., et al. (1993). The national conference on graduate education in the applications of developmental science across the life-span. *Journal of Applied Developmental Psychology*, **14**, 1–10.

Ford, D. H., & Lerner, R. M. (1992). *Developmental systems theory: An integrative approach*. Thousand Oaks, CA: Sage.

Garstang, W. (1922). The theory of recapitulation: A critical re-statement of the biogenetic law. *Journal of the Linnean Society of London Zoology*, **35**, 81–101.

Goldschmidt, R. (1933). Some aspects of evolution. *Science*, **78**, 539–547.

Gottlieb, G. (1970). Conceptions of prenatal behavior. In: L. R. Aronson, E. Tobach, D. S. Lehrman, & J. S. Rosenblatt (Eds.), *Development and evolution of behavior: Essays in memory of T. C. Schneirla* (pp. 111–137). San Francisco: Freeman.

Gottlieb, G. (1976). Conceptions of prenatal development: Behavioral embryology. *Psychological Review*, **83**, 215–234.

Gottlieb, G. (1983). The psychobiological approach to developmental issues. In: M. M. Haith & J. Campos (Eds.), *Handbook of child psychology: Infancy and biological bases*. (Vol. 2, pp. 1–26). New York: Wiley.

Gottlieb, G. (1991a). Experiential canalization of behavioral development: Theory. *Developmental Psychology*, **27**, 4–13.

Gottlieb, G. (1991b). Experiential canalization of behavioral development: Results. *Developmental Psychology*, **27**, 39–42.

Gottlieb, G. (1992). *Individual development and evolution: The genesis of novel behavior*. New York: Oxford University Press.

Gottlieb, G. (1997). *Synthesizing nature-nurture: Prenatal roots of instinctive behavior*. Mahwah, NJ: Erlbaum.

Gottlieb, G. (1998). Normally occurring environmental and behavioral influences on gene activity: From central dogma to probabilistic epigenesis. *Psychological Review*, **105**, 792–802.

Gottlieb, G. (2004). Normally occurring environmental and behavioral influences on gene activity: From central dogma to probabilistic epigenesis. In C. Garcia Coll, E. Bearer, & R. M. Lerner (Eds.), *Nature and nurture: The complex interplay of genetic and environmental influences on human behavior and development* (pp. 85–106). Mahwah, NJ: Erlbaum.

Gottlieb, G., Wahlsten, D., & Lickliter, R. (1998). The significance of biology for human development: A developmental psychobiological systems view. In W. Damon (Ed. in Chief) & R. M. Lerner (Vol. Ed.), *Handbook of child psychology, Vol. 1. Theoretical models of human development* (5th ed., pp. 233–273). New York: Wiley.

Gottlieb, G., Wahlsten, D., & Lickliter, R. (2006). *The significance of biology for human development: A developmental psychobiological systems view*. Hoboken, NJ: Wiley.

Graham, S. (1992). "Most of the subjects were white and middle class": Trends in published research on African Americans in selected APA journals, 1970–1989. *American Psychologist*, **47**, 629–639.

Grouse, L. D., Schrier, B. K., Letendre, C. H., & Nelson, P. G. (1980). RNA sequence complexity in central nervous system development and plasticity. *Current Topics in Developmental Biology*, **16**, 381–397.

Ho, M. W. (1984). Environment and heredity in development and evolution. In M. W. Ho & P. T. Saunders (Eds.), *Beyond neo-Darwinism: An introduction to the new evolutionary paradigm* (pp. 267–289). London: Academic Press.

Jacobs, F. (1988). The five-tiered approach to evaluation: Context and implementation. In H. B. Weiss & F. Jacobs (Eds.), *Evaluating family programs* (pp. 37–68). Medford, MA: Eliot-Pearson Department of Child Development, Tufts University.

Kellogg Commission on the Future of State and Land-Grant Universities (1999). *Returning to our roots: The engaged institution*. Washington, DC: National Association of State Universities and Land-Grant Colleges.

Kennedy, E. M. (1999). University-community partnerships: A mutually beneficial effort to aid community development and improve academic learning opportunities. *Applied Developmental Science*, **3**(4), 197–198.

Kollar, E. J., & Fisher, C. (1980). Tooth induction in chick epithelium: Expression of quiescent genes for enamel synthesis. *Science*, **207**, 993–995.

Kuo, Z. Y. (1967). *The dynamics of behavior development*. New York: Random House.

Kuo, Z. Y. (1976). *The dynamics of behavior development: An epigenetic view*. New York: Plenum Publishing.

Lehrman, D. S. (1953). A critique of Konrad Lorenz's theory of instinctive behavior. *Quarterly Review of Biology*, **28**, 337–363.

Lehrman, D. S. (1970). Semantic and conceptual issues in the nature-nurture problem. In L. R. Aronson, D. S. Lehrman, E. Tobach, & J. S. Rosenblatt, (Eds.), *Development and evolution of behavior: Essays in memory of T. C. Schneirla* (pp. 17–52). San Francisco, CA: W. H. Freeman.

Lerner, R. M. (1978). Nature, nurture, and dynamic interactionism. *Human Development*, **21**, 1–20.

Lerner, R. M. (1984). *On the nature of human plasticity*. New York: Cambridge University Press.

Lerner, R. M. (1991). Changing organism-context relations as the basic process of development: A developmental contextual perspective. *Developmental Psychology*, **27**, 27–32.

Lerner, R. M. (1995). The place of learning within the human development system: A developmental contextual perspective. *Human Development*, **38**, 361–366.

Lerner, R. M. (2002). *Concepts and theories of human development* (3rd ed.) Mahwah, NJ: Erlbaum.

Lerner, R. M. (2004). *Liberty: Thriving and civic engagement among America's youth*. Thousand Oaks, CA: Sage.

Lerner, R. M. (2005, September). *Promoting positive youth development: Theoretical and empirical bases*. White paper prepared for the Workshop on the Science of Adolescent Health and Development, National Research Council/Institute of Medicine. Washington, DC: National Academies of Science.

Lerner, R. M. (2006). Developmental science, developmental systems, and contemporary theories of human development. In W. Damon & R. M. Lerner (Eds. in Chief) & R. M. Lerner (Vol. Ed.), *Handbook of child psychology, Vol. 1. Theoretical models of human development* (6th ed., pp. 1–17). Hoboken, NJ: Wiley.

Lerner, R. M. (2007). *The good teen: Rescuing adolescents from the myths of the storm and stress years*. New York: The Crown Publishing Group.

Lerner, R. M., Fisher, C. B., & Weinberg, R. A. (2000). Toward a science for and of the people: Promoting civil society through the application of developmental science. *Child Development*, **71**, 11–20.

Lerner, R. M., Jacobs F., & Wertlieb, D. (Eds.) (2003). *Handbook of applied developmental science: Promoting positive child, adolescent, and family development through research, policies, and programs*. Thousand Oaks, CA: Sage.

Lerner, R. M., & Miller, J. R. (1993). Integrating human development research and intervention for America's children: The Michigan State University model. *Journal of Applied Developmental Psychology*, **14**, 347–364.

Lerner, R. M., Schwartz, S. J., & Phelps, E. (2008). *Studying the processes of individual development: Theoretical problematics and methodological possibilities*. Medford, MA: Tufts University, Institute for Applied Research in Youth Development.

Lerner, R. M., & Steinberg L. (Eds.). (2009). *Handbook of adolescent psychology* (3rd Ed.). Hoboken, NJ: Wiley.

Lewin, K. (1943). Psychology and the process of group living. *Journal of Social Psychology*, **17**, 113–131.

Overton, W. F. (1998). Developmental psychology: Philosophy, concepts, and methodology. In W. Damon (Ed. In Chief) & R. M. Lerner (Vol. Ed.), *Handbook of child psychology, Vol. 1. Theoretical models of human development* (5th ed., pp. 107–187). New York: Wiley.

Overton, W. F. (2006). Developmental psychology: Philosophy, concepts, methodology. In W. Damon & R. M. Lerner (Eds. in Chief) & R. M. Lerner (Vol. Ed.), *Handbook of child psychology, Vol. 1. Theoretical models of human development* (6th ed., pp. 18–88). Hoboken, NJ: Wiley.

Phelps, E., Balsano, A., Fay, K., Peltz, J., Zimmerman, S., & Lerner, R. M. et al. (2007) Nuances in early adolescent development trajectories of positive and of problematic/risk behaviors: Findings from the 4-H Study of Positive Youth Development. *Child and Adolescent Clinics of North America,* **16**(2), 473–496.

Plomin, R. (1986). *Development, genetics, and psychology*. Hillsdale, NJ: Erlbaum.

Ralston, P., Lerner R.M., Mullis, A., Simerly C., & Murray, J. (Eds.). (1999). *Social change, public policy and community collaboration: Training human development professionals for the twenty-first century*. Norwell, MA: Kluwer Academic Publishers.

Rowe, D. C. (1994). *The limits of family influence: Genes, experience, and behavior*. New York: Guilford Press.

Rushton, J. P. (1997). Cranial size and IQ in Asian Americans from birth to age seven. *Intelligence*, **25**, 7–20.

Schneirla, T. C. (1956). Interrelationships of the innate and the acquired in instinctive behavior. In P. P. Grassé (Ed.), *L'instinct dans le comportement des animaux et de l'homme*. Paris: Mason et Cie.

Schneirla, T. C. (1957). The concept of development in comparative psychology. In D. B. Harris (Ed.), *The concept of development: An issue in the study of human behavior* (pp. 78–108). Minneapolis, MN: University of Minnesota Press.

Sherrod, L. R. (1999). "Giving child development knowledge away": Using university-community partnerships to disseminate research on children, youth, and families. *Applied Developmental Science*, **3**, 228–234.

Spanier, G. B. (1999). Enhancing the quality of life: A model for the 21st century land-grant university. *Applied Developmental Science*, **3**(4), 199–205.

Strohman, R. C. (1993). Book reviews: *Final solutions* (Richard M. Lerner, Penn State Press, 1992) and *Individual development and evolution: The genesis of novel behavior* (Gilbert Gottlieb, Oxford University Press, 1992). *Integrative Physiological and Behavioral Sciences*, **28**, 99–104.

Takanishi, R. (1993). An agenda for the integration of research and policy during early adolescence. In R. M. Lerner (Ed.), *Early adolescence: Perspectives on research, policy, and intervention* (pp. 457–470). Hillsdale, NJ: Erlbaum.

Thelen, E., & Smith, L. B. (2006). Dynamic systems theories. In W. Damon & R. M. Lerner (Eds. in Chief) & R. M. Lerner (Vol. Ed.), *Handbook of child psychology, Vol. 1. Theoretical models of human development* (6th ed., pp. 258–312). Hoboken, NJ: Wiley.

Thompson, L. (1999). Creating partnerships with government, communities, and universities to achieve results for children. *Applied Developmental Science*, **3**, (4), 213–216.

Tobach, E. (1971). Some evolutionary aspects of human gender. *Journal of Orthopsychiatry*, **41**, 710–715.

Tobach, E. (1981). Evolutionary aspects of the activity of the organism and its development. In R. M. Lerner & N. A. Busch-Rossnagel (Eds.), *Individuals as producers of their development: A lifespan perspective* (pp. 37–68). New York: Academic Press.

Tobach, E., & Greenberg, G., (1984). The significance of T. C. Schneirla's contribution to the concept of levels of integration. In G. Greenberg & E. Tobach (Eds.), *Behavioral evolution and integrative levels* (pp. 1–7). Hillsdale, NJ: Erlbaum.

Uphouse, L. L., & Bonner, J. (1975). Preliminary evidence for the effects of environmental complexity on hybridization of rat brain RNA to rat unique DNA. *Developmental Psychobiology*, **8**, 171–178.

Waddington, C. H. (1957). *The strategy of genes*. London: George Allen and Unwin.

Waddington, C. H. (1971). *Towards a theoretical biology Vol. 3: Drafts*. Edinburgh: Edinburgh University Press.

Zaff, J., & Smerdon, B. (2009). Putting children front and center: Building coordinated social policy for America's children. *Applied Developmental Science*, **13**(3), 105–118.

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