

Phenotypic Plasticity: Molecular Mechanisms and Adaptive Significance

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ABSTRACT

Phenotypic plasticity can be broadly defined as the ability of one genotype to produce more than one phenotype when exposed to different environments, as the modification of developmental events by the environment, or as the ability of an individual organism to alter its phenotype in response to changes in environmental conditions. Not surprisingly, the study of phenotypic plasticity is innately interdisciplinary and encompasses aspects of behavior, development, ecology, evolution, genetics, genomics, and multiple physiological systems at various levels of biological organization. From an ecological and evolutionary perspective, phenotypic plasticity may be a powerful means of adaptation and dramatic examples of phenotypic plasticity include predator avoidance, insect wing polymorphisms, the timing of metamorphosis in amphibians, osmoregulation in fishes, and alternative reproductive tactics in male vertebrates. From a human health perspective, documented examples of plasticity most commonly include the results of exercise, training, and/or dieting on human morphology and physiology. Regardless of the discipline, phenotypic plasticity has increasingly become the target of a plethora of investigations with the methodological approaches utilized ranging from the molecular to whole organismal. In this article, we provide a brief historical outlook on phenotypic plasticity; examine its potential adaptive significance; emphasize recent molecular approaches that provide novel insight into underlying mechanisms, and highlight examples in fishes and insects. Finally, we highlight examples of phenotypic plasticity from a human health perspective and underscore the use of mouse models as a powerful tool in understanding the genetic architecture of phenotypic plasticity. © 2012 American Physiological Society. *Compr Physiol* 2:1417–1439, 2012.

Introduction

Phenotypic plasticity can be broadly defined as the ability of a genotype to produce different phenotypes in response to different environmental conditions (see Fig. 1 and 64, 122, 124, 126, 143, 178, 181). Defined broadly then, phenotypic plasticity will include cases of fixed, irreversible, and distinct developmental trajectories that cannot be expressed in a single individual, cases of rapid, reversible, flexible physiological responses during the course of single individual's lifetime, and just about every conceivable case in between (Table 1) (see also Table 1 in reference 120). Genotypes may differ phenotypically within one environment, differ phenotypically in yet another environment, but all show the same basic developmental or physiological response to this environmental variation (Fig. 2B). In such a case, these genotypes are all phenotypically plastic—that is, they exhibit “reaction norms” of nonzero slope—for the trait of interest, but the reaction norms are parallel. The environmentally induced phenotypic differences within each genotype are often referred to as “nongenetic” or “environmental” difference. However, because we assume that at least some of the phenotypic differences between environments, even within a single genotype, are due to environmentally sensitive differences in gene expression, such plasticity is still, of course, “genetic”

(38). However, in some cases, even the magnitudes of the developmental/physiological responses of the different genotypes differ; that is, the slopes of these reaction norms are not equal (Fig. 2C). Cases of nonparallel reaction norms indicate genotype-by-environment interactions; that is, underlying the variation in organismal responses to a fluctuating environment is a regulatory genetic architecture capable of responding to selection (see Fig. 2 in reference 56). In the examples later, we discuss cases of both types, and attempt to explain, when possible, which scenario applies.

As described in the introductory text of DeWitt and Scheiner (38), depending on how the word “phenotype” is defined (e.g., developmental event, physiological adjustment, behavioral shift, environment-dependent gene expression, etc.) all biological process are in some fashion influenced by the environment, and consequently any resulting

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Figure 1 Two genetically identical water fleas, *Daphnia lumholtzi*. The helmet and extended tail spine of the individual on the left were induced as a result of chemical cues from a predaceous fish and serve as protection (67). This figure is recreated, with permission, from (3) Agrawal, A. “Phenotypic plasticity in the interactions and evolution of species”, *Science*, October 12, 2001, 294:321-326, Figure 1, with permission of D. Laforisch.

modification could be categorized as plasticity (see also reference 120). We, like DeWitt and Scheiner (38), do not see a broad definition of plasticity as problematic. Thus, we present the contents of this article using a liberal designation while providing a range of examples that examine plasticity from both a proximate (i.e., environmentally induced changes that occur within individual organisms during their lifetimes or physiological adaptation) and ultimate (i.e., selection acting directly on phenotypic plasticity or evolutionary adaptation) context (e.g., see references 50,51,74) across a wide range of organismal and physiological systems.

The use of the term phenotypic plasticity to describe environmentally induced changes is common place for ecological and evolutionary biologists. Mykles et al. (108) propose phenotypic plasticity as a “unifying and guiding framework” for the disciplines of comparative physiology and evolutionary biology. Conversely, subfields within the biomedical sciences frequently partition phenotypic plasticity and apply additional descriptive terms such as “muscle hypertrophy,” “weight disregulation,” or simply “training adaptations.” Furthermore, “learning,” a commonplace behavioral trait, is a general form of phenotypic or behavioral plasticity that can be applied in an ecological (discussed in reference 3) or biomedical context (e.g., 135, see Table 1 for additional specific terminology). Moreover, the underlying mechanisms of learning may also be phenotypically plastic (e.g., changes neuronal plasticity) (see reference 107). Regardless of the subfield, phenotypic

Table 1 Selected Definitions of “Phenotypic Plasticity,” Associated Terms, and More Specific Terminology

Definitions of “phenotypic plasticity”	Source (page number)
“All types of environmentally induced phenotypic variation”	Stearns (166) (p. 436)
“Alteration of organismal form by changes in the environment”	Pigliucci and Schlichting (127) (p. 21)
“The property of a given genotype to produce different phenotypes in response to distinct environmental conditions”	Pigliucci (122) (p. 1)
“Is any change in an organism’s characteristics in response to an environmental signal”	Schlichting and Smith (146) (p. 190)
“The ability of an organism to react to an internal or external environmental input with a change in form, state, movement, or rate of activity”	West-Eberhard (181) (p. 34)
“Environment-dependent phenotype expression”	DeWitt and Scheiner (38) (p. 1)
“Ability of a single individual to develop into more than one phenotype”	Gilbert and Epel (61) (p. 6)
Associated terms	
Canalization: “The stability of a particular developmental trajectory in the face of random, but not persistent and predictable, environmental changes”	Pigliucci and Schlichting (127) (p. 83)
Developmental instability: “The within-environment phenotypic variance for a given genotype”	DeWitt et al. (39) (p. 79)
Developmental noise: “Random fluctuations that arise during development that alter the phenotypic product of development”	DeWitt and Scheiner (38) (p. 5)
Homeostasis: “Physiological canalization stemming from plasticity in other physiological, morphological, and behavioral traits.”	Woods (190) (p. 656)
Specific terminology for phenotypic plasticity	
Phenotypic flexibility: “Reversible changes in individual phenotypes comprising flexible responses to changing tasks”	Piersma and Lindstrom (119) (p. 135)
Acclimation and acclimatization: “The adjustments of physiological traits to ambient environmental conditions in the laboratory and the field, respectively”	Piersma andDrent (118) (p. 228)
Developmental plasticity: “Environmental factors can influence development by acting at any time after formation of the zygote, or in some cases even before (e.g., maternal effects acting on the unfertilized egg).” . . . “Whenever they act, the consequences of environmental effects are often termed developmental or phenotypic plasticity.”	Garland and Kelly (56) (p. 2345)
Polyphenism: “The ability of one genotype to produce two or more discrete phenotypes in response to an environmental signal”	Stearns (166) (p. 438)

Note: In a few cases, words have been rearranged for purposes of clarity and consistency, but the meaning has not been altered.

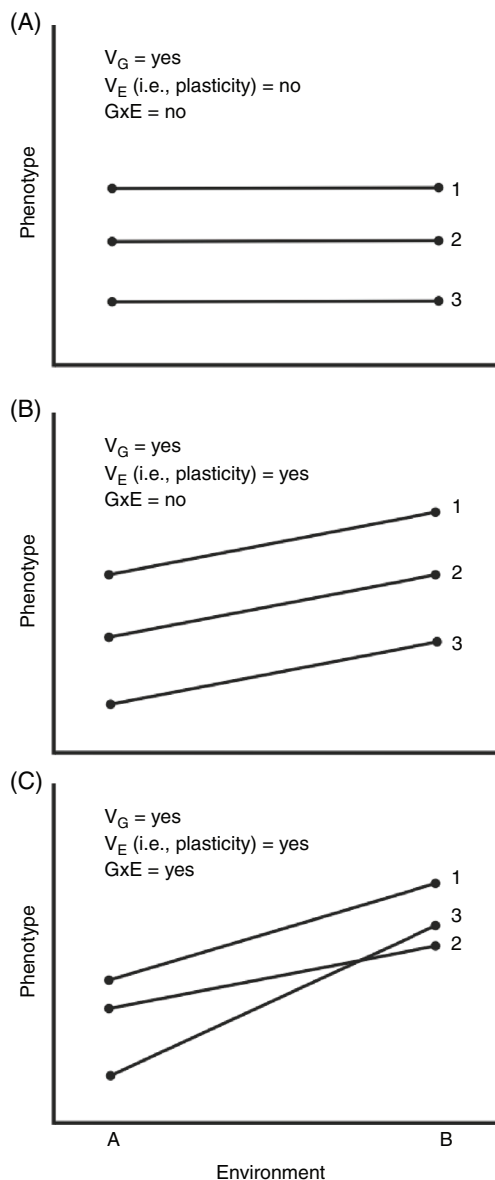


Figure 2 Possible relationships among plasticity and genetic variation. In each panel, dots connected by lines represent the phenotypes expressed by each of three genotypes (or families), numbered 1, 2, and 3, in each of two alternative environments (A, B). These lines are the reaction norms. (A) The three genotypes differ in their phenotypes within each environment, indicating genetic variation ($V_G = \text{yes}$). However, a given genotype expresses the same phenotype, regardless of environment; that is, the reaction norms are flat. Hence, there is no environmental effect on the phenotype (V_E , or plasticity, is absent). Because the reaction norms are parallel, there is no genotype-by-environment interaction ($G \times E = \text{no}$). (B) As in panel “A,” the three genotypes differ in phenotype within a given environment, but in addition, each genotype expresses a different phenotype in environment “B,” relative to that expressed in environment “A.” That is, the reaction norms are not flat; each genotype is plastic for the trait of interest. However, because the slopes of all reaction norms are parallel, there is no genotype-by-environment interaction. The genotypes, although plastic, are all similarly plastic (for the trait of interest). (C) Genotypes differ within environments, show plasticity, and differ in plasticity. That is, the reaction norms are not parallel, indicating genotype-by-environment interactions. In this case, reaction norms 2 and 3 cross, but this may not always be the case. This figure is conceptually similar to, and derived, with permission, from Figure 1.4 from Pigliucci (121; p. 15) and Figure 1.1 from reference 38; p. 4)

plasticity is an important concept in modern ecological, evolutionary, and biomedical literature that has been the primary focus of variety of investigations (108).

As an indication of the volume of work performed relating to phenotypic plasticity, we searched the PubMed (MEDLINE; <http://www.ncbi.nlm.nih.gov/pubmed/>) and Web of Science (ISI; <http://apps.isiknowledge.com/>) databases during December of 2010. Using the keywords “phenotypic plasticity,” the PubMed search results revealed 1748 articles of which 352 were classified as review articles and out of the total approximately 39% were published in the last 3 years (2008–2010). Alternatively, the Web of Science query revealed 6741 articles of which 630 were classified as review articles. Of these 6741 articles, approximately 31% were published between 2008 and 2010. These very general searches of a limited number of digital resources reveal two points of note with regard to phenotypic plasticity. First, as indicated by the fact that over a third of the articles in our searches were published in the last 3 years, phenotypic plasticity is increasingly the primary target of investigations as opposed to being considered secondarily or treated as a nuisance (for a historical evolutionary perspective see reference 124). Second, as discussed previously, phenotypic plasticity is often referred to by a variety of additional descriptive terms, which partially explains the discrepancy between the quantities of resulting articles from each of the two search engines. For instance, if instead of “phenotypic plasticity,” we utilize “cardiac remodeling (a form of phenotypic plasticity) (e.g., see reference 173)” in our PubMed (a service of the U.S. National Library of Medicine at the National Institutes of Health) query the resulting number of articles increases from 1748 articles to 12,001, a 587% increase.

Regardless of the nomenclature, physiological adjustments in response to environmental heterogeneity are generally considered to be adaptive or advantageous (e.g., Figs. 3 and 4). We discuss why this assumption may or may not be true later (see Section “Adaptive Significance of Phenotypic Plasticity”), but first it is important to define the term “adaptation” as it is used widely across a variety of biological disciplines (10, 55). As reviewed and defined in Garland and Kelly (56), herein “physiological adaptation” refers to changes that occur within individual organisms during their lifetimes driven by environmental perturbations (e.g., see reference 92). “Evolutionary adaptation” refers to cross-generational changes in the genetic composition of a population in response to natural selection; however, with recent advances in epigenetics there is mounting evidence pointing toward necessary flexibility in this definition (see references 29, 70, 125). Accordingly, although phenotypic plasticity can be thought of in terms of numerous physiological adaptations, plasticity of any particular trait may also be an evolutionary adaptation, although the latter is difficult to demonstrate definitively (see Figs. 2 and 3 and reference 42). The answer to the difficult question of whether phenotypic plasticity is adaptive has been partially eased by the advancement of modern technological advancements, which have shed light on

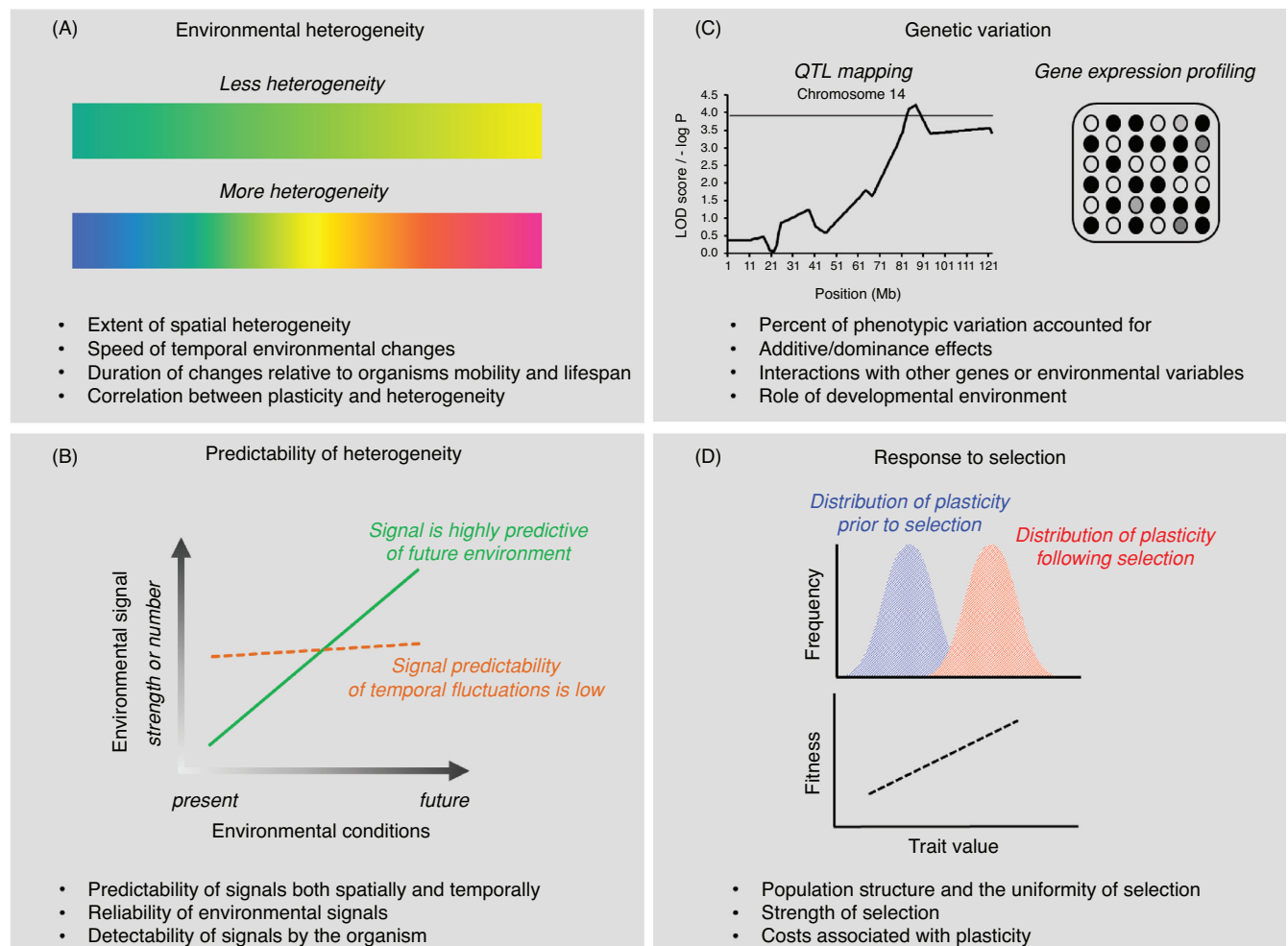


Figure 3 The panels above (A-D) are representations of the general requirements needed for recognizing phenotypic plasticity as an adaptation. Although the representation of each of these criteria is pictorial simplistic, we acknowledge that their conclusive demonstration is quite complex. Accordingly, under each of the four general criteria we have listed additional considerations that should be taken into account. For extensive discussion of these criteria see text (also see reference 42). (A) Environmental heterogeneity must exist, and the degree of heterogeneity may determine the evolutionary (see panel D) consequences as opposed to an alteration in population mean. Heterogeneity may be biotic (e.g., predator presence) or abiotic (e.g., temperature), and special care should be taken to consider the extent of the spatial heterogeneity, the speed of fluctuations, and how these relate to the behavior and life history of the investigated organism. (B) Organisms must be able to reliably predict heterogeneity using environmental signals and these signals must be highly correlated with future environmental conditions. These signals must be spatial and temporally reliable and the organism must have the ability to sense and respond (even if the response is imperfect, see reference 87). (C) There must be an underlying genetic architecture regulating the plastic response. Here, we have presented methodologies for the evaluation of genetic variation. Using quantitative trait locus (QTL) mapping researchers may identify plasticity regions that directly affect the reaction norm (see text and Fig. 3 for examples), or evaluate differential gene expression in different environments with microarray technology. (D) There is a measurable response to selection and the response confers a fitness benefit.

the underlying molecular mechanisms of plasticity (e.g., see Table 2 and Fig. 3). Specifically, technological advancements have now provided the ability to identify genomic regions, or specific genes, underlying the plastic response in higher level traits. Consequently, affording the opportunity to examine cross-generational fluctuations in allele frequencies of genes directly affecting phenotypic plasticity. With the caveat that one would need to then need to see if cross-generational fluctuations are caused by random genetic drift or natural selection, with only the later suggesting an evolutionary adaptation.

In this review, we first provide a very brief primer detailing the historical context of phenotypic plasticity. We then put into context the study of phenotypic plasticity as an adaptive trait, and the molecular tools currently being utilized to better understand how plasticity might respond to selection. We follow this discussion with examples from ecological and evolutionary literature that examines these aspects of plasticity in natural populations of fishes and invertebrates. Finally, we describe the use of laboratory selection in providing additional insight into the genetic basis of plasticity and how this knowledge may inform the physiology of human health conditions.

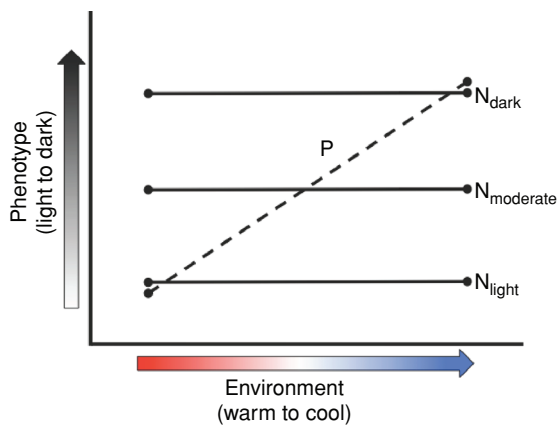


Figure 4 Conceptual representation of how one would assess whether plasticity is adaptive. Imagine environmentally induced variation in coloration, from light to dark, which is associated with seasonal, latitudinal, or elevation variation in temperature. One plastic (P) and three alternative nonplastic (N) genotypes expressing phenotypes in warm and cool environments are shown, with the three nonplastic genotypes expressing dark coloration, light coloration, and one of intermediate (i.e., moderate) coloration (N_{dark} , N_{light} , and N_{moderate} , respectively). The plastic genotype expresses light coloration when it is warm (e.g., summer phenotype), but dark coloration when it is cool (e.g., spring or autumn phenotypes). Suppose that when it is warm, a light-colored phenotype avoids overheating. Thus, in the warm environment, the fitness of the plastic genotype (P) is similar to that of the nonplastic light-colored genotype (N_{light}), both of which are more fit than either of the other two nonplastic genotypes. Suppose also that in the cool environment, the plastic genotype (which now expresses a dark phenotype) has similar fitness to that of the nonplastic dark-colored phenotype, because both can convert the absorbed solar radiation into higher body temperatures necessary for some components of fitness. Provided all of the previously mentioned conditions are true (and making certain assumptions about the probability of encountering both environments), we would conclude that the plasticity is adaptive, because there is no single nonplastic genotype that has, on average, higher fitness. However, if there is no cost to being dark under warm conditions, such that the fitness of the N_{dark} genotype is as high as that of the plastic genotype under the warm conditions, then there is no fitness advantage to being plastic. Therefore, we could not conclude that the plasticity is adaptive (and indeed, if there are costs to being plastic, *per se*, we might expect the plastic genotype to have lower fitness than the N_{dark} genotype, despite that both have the same beneficial, dark-coloration phenotype under the cool conditions.)

Phenotypic Plasticity: A Brief History

The beginning of the scientific study of phenotypic plasticity is often credited to Woltereck's studies on the head height of *Daphnia* in response to varying nutrient levels (188), wherein he coined the term "reaction norm" to describe the relationship between environmental variation and phenotypic variation. This assessment is probably reasonable, provided that one keeps in mind biologists before Woltereck were certainly aware of the effects of the environment on phenotypes. For example, two decades before Woltereck's (now) famous paper, biologists Weismann and Poulton described the effects of environmental manipulations on the colors of butterflies and moths (129, 180). In any event, as a field of serious sci-

entific inquiry, phenotypic plasticity was slow to blossom. This is thought to be due, at least in part, to the development of population genetic theory during the 1930s and 1940s, which placed a great emphasis on the relationship between the genotype and phenotype (122, 141). Although some important papers addressing phenotypic plasticity (though not necessarily using the term) were published in the field of evolutionary biology in the few decades following the "evolutionary synthesis" (e.g., see reference 147), credit for the beginning of the modern scientific study of phenotypic plasticity is often attributed to a 1965 paper by Bradshaw (18). In this review of phenotypic plasticity of plants, Bradshaw suggested that plasticity, *per se*, is a trait that can have a genetic basis and that therefore can evolve (18, also see reference 50). Despite Bradshaw's important paper, research in plasticity did not immediately take off, as it has more recently. This may be because Bradshaw's publication occurred at a time of rapid progress in molecular biology; with these new tools evolutionary biologists had even more sophisticated ways to quantify the relationship between genotype and phenotype. Traits that were plastic—that threatened to make less clear the genotype/phenotype relationship—would be less likely to endear themselves to biologists seeking to connect genetic variation to phenotypic variation.

The development of evolutionary quantitative genetics and optimization theory led to an explosion of work into phenotypic plasticity, mostly from an evolutionary perspective (141). This period was characterized by active controversies addressing a variety of problems in the field, but much of the debate concerned whether plasticity itself was a target of natural selection (and as such, whether it made sense to speak of "plasticity genes," for example) (141).

The debates that started in the 1980s are not over, but new ideas, new techniques, and the revisiting of older ideas have added new debates to the field of phenotypic plasticity research. The rise of evolutionary developmental biology, that is, "evo-devo," has increased interest in the processes that lead to the generation of phenotypic variation (as opposed to a focus largely on the selective consequences of that variation). As such, research into the underlying developmental molecular mechanisms of phenotypic plasticity is an active area of inquiry (34, 146). Also of interest is the relationship between phenotypic integration and phenotypic plasticity—because traits do not develop in isolation, plasticity of some traits, even forms of adaptive phenotypic plasticity, should affect, and be affected by, other traits (e.g., see reference 123). The early years of the rise of interest in phenotypic plasticity placed a major emphasis on understanding how plasticity might evolve. More recent approaches have flipped that question on its head, to ask how phenotypic plasticity itself affects evolution. In other words, is the extent of the adaptive plastic response a primary factor in driving future evolution? And, specifically, does adaptive plasticity influence the evolution of phenotypic diversity (e.g., see references 106, 181)?

Table 2 Examples of Common Molecular Approaches Used to Study the Molecular Basis of the Phenotypic Plasticity of Physiological Traits

Molecular approaches	Brief methodology	Organism	Example	Reference
Candidate gene	Expression patterns of a priori candidate genes are compared between individuals from different environmental conditions (e.g., normoxia vs. hypoxia or high vs. low salinity).	Black Porgy (<i>Acanthopargus schlegelii</i>)	Expression of osmoregulatory candidate genes (e.g., hormone receptors, Na/K ATPase) in response to acute salinity stress	Tomy et al. (175)
Transcriptomes	Global gene expression patterns are compared between individuals experiencing different environmental conditions or phenotypes. This analysis may utilize microarrays, next-generation sequencing, or more traditional sequencing approaches.	Killifish (<i>Fundulus heteroclitus</i>)	Global transcriptome of gill tissue in response to osmotic shock.	Whitehead et al. (184)
Heterologous hybridization to DNA microarrays	A cDNA microarray from one species is used as the template to study the expression patterns in a different species. Performed across different environmental conditions. Useful for the study of nonmodel organisms.	Bluefin Tuna (<i>Thunnus orientalis</i>)	Thermal acclimation (temperature stress) in selected tissues (e.g., skeletal muscle fibers).	Castilho et al. (23)
Transgenics (Gene knockouts)	Transgenics involve the introduction of DNA into an organism's genome. The DNA is typically an interspecific gene of interest or in the case of a knockout system it may be a replacement gene that is used to knock out the function of a specific locus. This technique has been useful for understanding functional genes involved in physiological traits and has been combined with microarray analyses to shed light on genetic foundations of phenotypic plasticity.	Mice (<i>Mus musculus</i>)	Expression profiles of soleus muscle from HIF1 alpha heterodeficient mice [HIF-1(alpha)-/+] compared to wild-type mice subjected to 24 h of hypoxia or normoxia.	References reviewed in reference (51)
Regulatory mechanisms of gene expression ^B	Processes such as hormonal influence on gene expression and epigenetic ^C mechanisms such as DNA methylation and chromatin remodeling. With respect to phenotypic plasticity, these processes may be influenced by specific internal or external environmental cues. Global analyses of DNA methylation is now being used to study the epigenome of an organism and these epigenomes may be compared between phenotypes or developmental stages (4). Epigenetics is now argued by some to be at the "core of several types of phenotypic plasticity" (16). Multiple molecular techniques can be used for epigenetic investigations.	Humans	Comparison of genome wide methylation patterns between obese and lean control cases.	Wang et al. (179) Also reviewed in Franks and Ling, (53)

^aTopics and descriptions presented here are adapted from recent discussions and reviews in references (4 and 8). This is not meant to be a complete list of examples but is intended only as an illustration of the molecular approach. We also specifically highlight examples based on physiological traits (as opposed to behavioral or morphological). These examples do not include studies on developmental plasticity and genomics; we refer you instead to reference (8).

^bEpigenetics refers to heritable changes in the expression of genes that are not the result of changes in the DNA sequence [reference (16) and references therein].

^cSee Bossdorf et al. (16) for examples of common molecular techniques in the study of epigenetics.

Adaptive Significance of Phenotypic Plasticity

The concept that plasticity may be an important part of evolutionary change has been controversial (60). It was traditionally proposed that phenotypic plasticity did not play a

significant role in adaptive evolution or even had the effect of hindering adaptive evolutionary rates (e.g., see references 47,60,65,91,155,185,192). However, recent arguments make the case that plasticity may make evolutionary change possible and that evolution may precede by genetic assimilation (e.g., see references 126,181). As detailed later, it has

been recognized that plasticity may be adaptive (maintained by natural selection) or nonadaptive and this aspect partly determines plasticity's contribution to genetic differentiation in a new or changing environment (reviewed in references 5, 52, 60).

There are examples and reviews of numerous taxa that underscore the ubiquitous nature of plasticity (see reference 60) and which suggest morphological, behavioral, and physiological traits all have the possibility of being plastic and all may participate in adaptive evolution (99, 181). Physiological (and behavioral) traits are argued to be "inherently" plastic and likely to evolve relatively quickly upon colonization of new environments (reference 99 and references therein). Furthermore, physiological traits may be "particularly germane to the question of plasticity's role in adaptation to environmental heterogeneity" (99). McCairns and Barnatchez (99) mention several reasons for this potential phenomenon; (i) physiological traits are typically "labile and reversible" which can be useful during establishment in a new environment, (ii) physiological traits tend to be less complex than other traits and many physiological processes occur at the biochemical level of organization directly influenced by proteins, (iii) this direct connection to transcriptional products means that physiological processes may be "more immediately susceptible to changes in the composition and/or confirmation of proteins resulting from mutation in the coding DNA sequence," and mutations at regulatory regions will alter the transcription rate and potentially create phenotypic variation susceptible to selection, and (iv) the plasticity of physiological traits can occur during individual development or as a rapid and reversible response during the adult lifetime of the individual.

The general assumption that phenotypic plasticity is adaptive from the perspective of improving organismal function and/or overall fitness (e.g., see reference 113) seems to be especially true when examining physiological adjustments during acclimation and acclimatization (reviewed in reference 187). Physiologists have often assumed every difference among species is adaptive in the evolutionary sense (48), and every physiological response is adaptive in a proximate sense (see reference 55). However, "evolutionary physiology," as a concentrated discipline, has raised issues with regard to the overuse of adaptive explanations among comparative physiological investigations, (see references 48, 55). Regardless, these assumptions, historically accepted by comparative physiologists, empirically may or may not be true (see Figs. 3 and 4) and thus has been termed the beneficial acclimation hypothesis (71, 72, 90, 187). Additionally, it is important to note that several investigations (examining acclimation to thermal environments) have concluded that phenotypic plasticity does not always lead to increased fitness in an altered environment, and in fact, some alterations may be maladaptive. For example, Leroi et al. (90) acclimated genetically identical lines of bacteria (*Escherichia coli*) to 32°C and 41.5°C for 24 h (~ 6.7 cell generations per day at 37°C), and then assessed the comparative overall fitness of each group at their own temperature and the alternative temperature. Consistent with the

assumptions of the beneficial acclimation hypothesis, Leroi et al. (90) found that prior acclimation to 32°C enhanced fitness at 32°C (relative to acclimation at 41.5°C). However, contrary to the predictions of the beneficial acclimation hypothesis, *E. coli* adapted to 41.5°C had lower relative fitness (when compared to *E. coli* adapted to 32°C) at 41.5°C. As a demonstration of the complexity of phenotypically plastic responses, Leroi et al. (90) also found that although prior acclimation to 41.5°C comparatively reduced fitness at 41.5°C, it increased fitness at 50°C (a lethal temperature). Leroi et al. (90) hypothesized that both outcomes may be a consequence of the same physiological process (the induction of stress proteins). In a follow-up study, Bennett and Lenski (12) acclimated groups of *E. coli* to a range of temperatures (22, 27, 32, 37, and 40°C) and tested comparative fitness between the groups in a similar fashion to that of Leroi et al. (90). In 7 of 12 cases, Bennett and Lenski (12) observed a fitness benefit as predicted by the beneficial acclimation hypothesis. Additionally, in *Drosophila melanogaster*, Gibert et al. (59) tested effects of population (Congo and France), developmental temperature (18, 25, and 29°C), adult temperature (18, 25, and 29°C), and age (2, 7, and 13 days) on walking speed, in the context of the beneficial acclimation hypothesis. Gibert et al. (59) did not find support for the beneficial acclimation hypothesis, but instead found that flies reared at intermediate temperatures ran comparatively faster across all temperatures, not just their own, lending support to the optimal developmental hypothesis (31, 71, 72). For an additional and more extensive review of the three studies presented previously, we encourage the reader to see Wilson and Franklin (187), who argue that the detailed investigations are "elegant analyses of the adaptive significance of developmental plasticity" as opposed to "direct nor complete tests of the functional benefit of thermal acclimation, as defined from traditional physiological studies of acclimation."

Molecular Mechanisms of Phenotypic Plasticity

As highlighted previously, beginning with investigations by Woltereck (188), the study of phenotypic plasticity has become incredibly diverse with the capacity to address questions across virtually every biological discipline. Accordingly, numerous research methodologies have been developed and utilized to investigate plasticity's adaptive significance, ecological consequences, importance for evolutionary change, molecular basis, costs and limits, and significance for human health and disease. Perspectives on these methodologies, their past use and future implications, have been previously chronicled generally (38), and more specifically from an ecological and evolutionary perspective (123, 124, 181) and pertaining to the physiological adaptations to high altitude in vertebrates (167). This list, of course, is not meant to be exhaustive and we encourage the reader to seek out the ever-increasing

volume of literature regarding the study of phenotypic plasticity (see Section “References”).

In Table 2, we present examples of common molecular approaches that are being utilized to better understand the molecular basis of phenotypic plasticity of physiological traits. Much work focuses on the changes in gene expression patterns involved under different environmental conditions and thus highlight the potential for what has been termed genomic reaction norms and the plasticity of gene expression (4, 8). We also refer you to detailed reviews and discussions of these topics in references 4 and 8. With the advent of new molecular technologies and techniques, both biomedical and ecological and evolutionary fields of study are moving beyond the traditional candidate gene approach to a whole genome wide analysis (8). Even work on nonmodel organisms has been made more feasible due to techniques such as heterologous hybridization to DNA microarrays, advances in next-generation sequencing, and the increase in the number of reference genomes (Table 2; see also reference 4). An additional phenomenon of molecular study is highlighted in Table 2—regulatory mechanisms of gene regulation. This covers both hormonal regulation of gene expression and epigenetic mechanisms such as DNA methylation. Bossdorf et al. (16) make the case of incorporating this area of research into ecological studies of phenotypic plasticity and other researchers have highlighted the study of epigenomics when comparing between phenotypes or developmental stages (reviewed in reference 4). Although these techniques have and continue to dramatically advance our general understanding of the phenotypic plasticity, perhaps the most compelling contribution thus far is their ability to shed light on the genetic basis of plasticity and how plasticity may respond to and potentially drive evolution.

Examples in Natural Populations

In this section we highlight the role that ecological and evolutionary studies play in our understanding of phenotypic plasticity of physiological traits. The goal of many of these studies is to understand how the environment and organisms within that environment interact and how these interactions may result in phenotypic trait shifts (genetic or “nongenetic,” *sensu* Fig. 2B) across populations or species. Historically, studies have addressed the role of the environment with respect to natural selection of phenotypes adapted to specific habitats. That is, how heritable traits are modified over time as environmentally induced selection results in changes in gene frequency. More recently, researchers are studying the role of phenotypic plasticity (typically characterized as nongenetic effects) in generating intra- and interspecific phenotypic variation and the idea that plasticity can be evolutionarily adaptive and facilitate evolutionary change, especially in rapidly changing environments or colonization to novel habitats (discussed in references 52, 60, 132, and references therein).

Here, we will showcase recent ecological and evolutionary examples of studies that address the phenotypic plasticity of physiological traits, adaptive evolution, and the use of molecular techniques to better understand the genetic basis of plasticity (see also Table 2). We chose to highlight examples from fish and insects although there has been considerable work from other taxa that could be included here as well.

Hypoxia tolerance in fish

Aquatic environments can vary greatly in their pH, salinity, temperature, and oxygen levels and some environments can experience seasonal or daily fluctuations in these variables. This habitat heterogeneity provides a source for divergent phenotypes among fishes and has provided an excellent model system for studying environmental adaptations, phenotypic plasticity, and the role of plasticity in evolutionary change (43, 102, 183). Here, we will briefly discuss several fish examples that highlight plasticity, evolutionary adaptations, and gene expression activity to varying environmental oxygen and salinity levels. These examples are not meant to be an exhaustive review and so, we refer the reader to more comprehensive publications throughout the section.

Aquatic respiring fish must acquire adequate oxygen from the water to maintain sufficient metabolic rates. When the oxygen levels decrease (become hypoxic), behavioral, morphological, and physiological changes that improve oxygen acquisition or reduce the consequences of low oxygen at the tissues will allow for greater hypoxia tolerance (94, 136). These modifications may be the outcome of selection (fixed genetic changes), developmental plasticity, or acute changes as the result of acclimatization, and may vary between species (or populations). Species that are from low-oxygen environments are commonly more tolerant to hypoxia than species from well-oxygenated waters (e.g., see references 27, 28, 98) and display differences in several physiological mechanisms, such as hemoglobin-oxygen binding affinity [see examples in reference 94 and Richards’ interspecific comparisons of Sculpin fishes (136)]. Other morpho-physiological modifications induced by hypoxia include changes in gill surface area and perfusion, hematocrit and hemoglobin concentrations, tissue oxygen characteristics, metabolic rate, and biochemical factors (as summarized in references 94, 136, 174).

Here, we introduce part of a series of work published by Chapman and colleagues on haplochromine cichlids (*Pseudocrenilabrus multicolor victoriae* Seegers). This work highlights the morpho-physiological phenotypic plastic response of these fish to hypoxic environments (24, 26, 35, 98). And by comparing different populations of *P. multicolor*, this work illustrates the interplay between local adaptation (genetic differences between populations), phenotypic plasticity (nongenetic environmentally induced differences between populations), and the potential for adaptive plasticity (see also Table 1).

Populations of *P. multicolor* are found in rivers, lakes, and swamps in East Africa. These water systems vary in their

level of oxygen concentrations. For example, many of the lakes and parts of the rivers maintain constant high concentrations of oxygen, while the swamps stay at much lower oxygen levels (24, 26). Some river areas have seasonally fluctuating oxygen concentrations due to flooding or droughts (24, 26). Several different experiments with *P. multicolor* populations have been performed over recent years using a common garden F1 design (see details in references 24, 26, 35, 98). Briefly, individuals from fish populations that are caught from rivers, lakes, or swamps with different oxygen concentrations are brought into the lab. Typically, offspring (F1) from several families per population are raised in either normoxic or hypoxic conditions (exception to this design in references 26 and 98). The response to hypoxic stress in morpho-physiological traits (e.g., gill size, brain size, body size, hematocrit, and lactate dehydrogenase specific activities) is examined to look for the degree of phenotypic plasticity to hypoxic stress both within and between populations. Studying different *P. multicolor* populations has allowed the researches to address the hypothesis that both environmentally induced selection and phenotypic plasticity are responsible for population phenotypic variances seen in this species (24, 26, 35, 98).

One of several morpho-physiological traits that is examined in this series of work (24, 26, 35) is gill size and the relationship between hypoxic stress and phenotypic plasticity of gill size. Gills are the important cite of gas exchange in these aquatic respiring fish and as gill size increases the surface area for oxygen uptake increases (35, 73), most likely affecting the physiology underlying aquatic respiration efficiency and critical oxygen tension (P_{crit}) (136). As highlighted by Martinez et al. (98), research on this system established that field populations of fish differ in their gill size phenotype and this correlates with oxygen levels in the water (25, 26). Fish from hypoxic habitats have larger gill size than fish populations from normoxic environments (25, 26). Martinez et al. (98) investigated the extent to which the population phenotypic trait variation was attributable to genetic or environmental influences. It was shown that among all studied *P. multicolor* populations, regardless of the water system naturally inhabited, there is a high degree of plasticity in gill size (24, 26, 35). F1 fish raised in low-oxygen conditions (hypoxic) have significantly larger gill size than fish raised in relatively high-oxygen tanks. This plastic response varied little between populations (24, 26, 35). The authors suggest that although there is genetic variation among populations in gill size, the phenotypic variations are driven mainly by the environment (plasticity) (35). Whether or not this plastic gill response is adaptive is not directly tested in these studies; however, it is argued that because a larger gill increases the surface area for gas exchange, that it would be beneficial under hypoxic stress (see details in reference 35). Furthermore, when oxygen levels are normal, the tradeoff between a large gill size and osmoregulation and head structures would favor a smaller gill size (24, 26, 35). These studies also addressed the effect of hypoxia and population on both brain and body size traits. Brain mass was found to have a plastic response to

hypoxia, a genetic effect, and the plasticity is suggested to be adaptive (details in reference 35).

This work on *P. multicolor* also includes research from Martinez et al. (98), who revealed phenotypic plastic responses in two physiological traits, hematocrit and lactate dehydrogenase activities, to hypoxic stress. Using a F1 design, *P. multicolor* fish populations from either a well-oxygenated lake or low-oxygen swamp were raised in normoxia and then acclimatized for several weeks in hypoxic or normoxic tanks. Changes in hematocrit and lactate dehydrogenase specific activities from several tissues were assessed after the acclimatization. Results were population dependent—fish from the hypoxic swamp populations showed the predicted increase in hematocrit and lactate dehydrogenase activity relative to the normoxic controls. However, F1 fish from the well-oxygenated lake populations did not show a plastic response (or showed a decreased response) after hypoxic acclimation. There was also support for population divergence in hematocrit levels. Even after a generation of being raised in normoxia (F1 generation), fish from the swamp populations had higher hematocrit levels, regardless of acclimation treatment, compared to lake population fish. This result, like the brain mass result in Crispo and Chapman (35), suggests both a genetic (as demonstrated by population-dependent differences) and plastic influence on the hypoxia response in *P. multicolor* (98).

Salinity tolerance in fish

Among fish, euryhaline species show remarkable plasticity (acclimatization) to changing salinity conditions. For fish to acclimate to salinity changes compensatory osmoregulation responses in several physiological body systems is required (184). As summarized by Whitehead et al. (184) although potentially species specific, in general, the kidneys alter the concentration of excreted urine, the digestive tract adjusts the absorption and secretion patterns of water and ions across the intestine, endocrine glands regulate secretion of hormones that control the body systems, and the gills change the direction of ion absorption and secretion across the epithelial surface (for a detailed review of marine and freshwater osmoregulation mechanisms in the gills see reference 44).

The Killifish species, *Fundulus heteroclitus*, serve as an excellent model for physiological adaptations and plasticity (acclimation) to salinity and other environmental factors (reviewed in references 21, 185). Some populations of killifish are distributed along the eastern coast of North America and are found in salt marshes and brackish estuaries (153). These fish populations experience diverse ecosystems that vary in temperature, salinity, oxygen levels, and pollution (21 185). *F. heteroclitus* have been characterized by their extreme acclimation (a specific type of plasticity) ability to these varying environmental factors. Phenotypic variation among populations has also been the result of local evolutionary adaptation. For example, as described in the introduction of Scott et al. (153), populations along a temperature gradient have

specific thermal adaptations and differ in “glycolytic enzyme expression and activity (117, 130), endocrinology (36, 116), metabolism (128), morphology, and behavior (131).” Some populations of killifish are found in highly toxic environments and show extreme tolerance to pollutants such as dioxin compounds and polycyclic aromatic hydrocarbons (109, 177, 184). This tolerance has been shown to be the result of heritable fixed population differences and not subject to plasticity (184). Given these population differences, *F. heteroclitus* are sometimes distinguished as being from a northern group or a southern group (153).

Osmotic tolerance has also been extensively investigated in *F. heteroclitus* (e.g., see references 80, 96, 189, reviewed in references 21, 44) and has been shown to be both highly plastic and the result of local adaptation. Killifish habitats experience routine fluctuations in salinity and fish can acclimate to freshwater and up to four times the salinity of seawater (68, 152, 153, 184). One necessary acclimation response to these environmental changes is a response in ion movement across the gills (152). As reviewed in the introduction of Scott et al. (152), gill epithelium mitochondria-rich cells alternate ion absorption and secretion processes with salinity changes. The ion transporters responsible for ion movement across the gills have been characterized (summarized in references 21, 44, 151). Depending on the species, these include ion transporters specific for absorption, (basolateral Na^+ , K^+ -ATPase, an apical Na^+ channel coupled V-type H^+ -ATPase, and a Na^+ - H^+ or a Cl^- - HCO_3^- exchanger) or secretion [basolateral Na^+ , K^+ -ATPase, Na^+ , K^+ , 2Cl^- cotransporter (NKCC)], and an apical cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel). The discovery of these ion transporters is recently being utilized to examine expression profiles of candidate genes that code for proteins during acclimation to salinity fluctuations (e.g., see reference 99, 149–153, 184) and are an important contribution to our understanding of the genetic and molecular processes of physiological plasticity and local adaptation (reviewed in references 21, 185; see detailed examples later).

Two companion papers, Scott et al. (153) and Scott and Schulte (149) have investigated *Fundulus heteroclitus* intraspecific variation in the physiological responses to salinity fluctuation. Here, we will discuss this work in detail to emphasize (i) the osmoregulatory responses that occur in Killifish gills during changes in salinity, (ii) the intraspecific variation present between northern and southern fish populations which suggest a level of local evolutionary adaptation in osmotic tolerance, and (iii) the use of candidate gene expression analysis in discovering the genomic and molecular basis of phenotypic plasticity (also see reference 99 for a recent example in *Sticklebacks* and review in reference 21; and Table 2).

In Scott et al. (153) the osmotic tolerance (acclimation/plasticity) differences in two populations of killifish were examined to look for local adaptations that might favor the invasion of freshwater from saltwater habitats. Adult fish from either a northern population or a southern population were abruptly transferred from a high salinity tank (mimics the

preferred native salinity environment) to a freshwater tank. In freshwater, the northern population fish had higher survival rates compared to the southern population, which suggests greater osmotic tolerance in the northern population (153). As pointed out by the authors (153), other studies have shown that northern inhabitants also show greater survival among larval stages and fertilization success than southern populations in low salinities (1) and that northern genotypes are proportionally more represented in freshwater habitats (131). Scott et al. (153) revealed that part of the population difference in osmotic tolerance maybe explained by physiological differences in ion regulation. They found that once transferred to freshwater, northern fish were better able to regulate the level of plasma Na^+ and Cl^- compared to the southern fish—southern fish had lower plasma levels of both ions for a longer period of time and Cl^- concentrations remained low for the entire experimental period (compared to northern fish that stopped Cl^- loss). The authors suggest that the difference in Na^+ regulation may be partly explained by the observation that the mRNA expression of the gill Na^+ / K^+ -ATPase was greater in the northern population relative to the southern population when analyzed 1 to 14 days post freshwater transfer. Cl^- maintenance in freshwater was managed better in the northern population and is explained by the authors as the possible retention of a seawater gill physiology and morphology in the southern population (153). This difference in Cl^- regulation is suggested to be the main physiological factor contributing to the intraspecific variation in freshwater acclimation (153). This work revealed the potential for evolved differences in plasticity among the killifish populations (153). As pointed out by Whitehead (183), these population differences in osmotic tolerance also reflect variation in the physiological plasticity and evolutionary lability to salinity changes that have been observed between species of killifish.

A question that arose from Scott et al. (153) was whether or not fish from the northern population pay cost in ion regulation in seawater for their higher freshwater tolerance (149). The answer to this question was addressed by Scott and Schulte (149). The authors compared the results for both northern (152) and southern (149) fish populations when transferred from brackish water to seawater. Both populations were able to efficiently balance their plasma levels of Na^+ and Cl^- ions (unlike in freshwater transfers), suggesting that the northern population’s freshwater adaptation has not come at an ionoregulatory cost in seawater (149). Although both populations show a similar acclimation response to seawater transfer and no cost is apparent, Scott and Schulte (149) found that the northern and southern populations have diverged in their molecular response to seawater transfer. The mRNA expression patterns of gill ion transporters varied between the two populations. Seawater transfer resulted in a longer duration increase of gill Na^+ / K^+ -ATPase and NKCC expression for the southern population compared to the northern. While an increase in CFTR expression was more transient for southern fish relative to northern. The cause of the population variation in the molecular response to seawater transfer

was not examined by the authors, but they speculate that potential differences in the levels of the hormone cortisol may play a role (149). Cortisol is an important stress hormone and as pointed out by the authors and others it may regulate cortisol-responsive gene expression during salinity changes in killifish (95,97,149,151,154,161). Scott and Schulte (149) highlight that killifish research has revealed population variation in both plasma cortisol levels and expression of genes that respond to cortisol during stress (see reference 149). This work sought to address an important question regarding the potential trade-offs or costs that may occur with an acclimation response. Such trade-offs have been suggested for other plastic and adaptive responses in various systems (e.g., see references 142,174).

A recent genomic study (161) sheds light on the potential mechanisms regulating gill ion transporter gene transcription (specifically for the *CFTR* gene) and, therefore, may help explain the differences that have been observed by Scott et al. (152,153) and Scott and Schulte (149). Using a comparative genomics approach with both freshwater and seawater killifish (*Fundulus*) species, the authors identify and reveal differences in the putative glucocorticoid (GRE) and osmotic responsive elements (OREs) in the promotor of the killifish *CFTR* gene. These regions may be important in the upregulation of *CFTR* during seawater transfer (161). The species variation in GRE is in a predicted direction with a greater number of GREs in the saltwater species (*F. heteroclitus* and *Fundulus grandis*) relative to the freshwater taxa (see details in reference 161). The ORE differed by a single base pair between the saltwater (and one brackish-water species) and freshwater species. The possible functional differences of the killifish *CFTR* promoter (and putative GRE and ORE) and the influence of cortisol and high osmolarity were further characterized using *in vivo* and *in vitro* techniques (see details in reference 161). The results suggest that the promoter responds with an increase in transcription of *CFTR* under high osmolarity (luciferase *in vitro* cell-culture results) (161). The authors caution that although it is tempting to conclude an adaptive functional promoter in saltwater species that the close phylogenetic relationship (the two saltwater species form a single clade) may explain the identical putative ORE sequence (identical by common descent), rather than be an adaptation to a high-salinity habitat (161). Using an *in vitro* approach (see the transgenic fish and cortisol or the RU486 cortisol antagonist injection experiments in reference 161), the authors examined a potential functional role for the GRE differences. The results were mixed, but some data were supportive of a functional role for GRE within the killifish *CFTR* promoter (161). In summary, this study shows how a comparative genomics approach may be useful in identifying mechanisms involved in the killifish osmoregulatory response (see also Table 2 for examples), but additional work on this system will benefit from controlling for phylogeny (161). Also of interest will be a population level analysis of potential variation in these genetic regulatory mechanisms of ion transporter candidate genes.

Whitehead et al. (184) extends the power of genomics in understanding both plasticity and local adaptation in killifish, but instead of focusing on the expression differences of specific candidate genes (e.g., gill ion transporter genes, like *CFTR* or $\text{Na}^+/\text{K}^+-\text{ATPase}$) the authors take a global approach and identify a transcriptomic response in the acclimation to osmotic shock (see also Table 2). Whitehead et al. (184) reestablished the known physiological plasticity of adult *F. heteroclitus* fish from a northern population that have been transferred from high-salinity water to freshwater and then determine the transcriptome regulation following the water salinity transfer and acclimation. Gill RNA was hybridized to a microarray chip using *F. heteroclitus* expressed sequence tags (ESTs). Replicate fish samples following a time course after freshwater transfer and a seawater control were examined using the microarray design (see reference 184 for details). A principal component analysis was analyzed to determine the overall transcriptome response across time and gene annotation tools identified potential genes involved in osmotic tolerance. As outlined by Whitehead et al. (184), when fish experience hypo-osmotic shock the gill epithelia cells swell, which must be quickly controlled before tissue damage occurs. Within a short time, nonregulatory mechanisms kick in to facilitate a decrease in the cell volume via the transport of ions out of the cell and water movement by osmosis. If osmotic stress continues, a compensatory response via gene regulation will occur. The authors characterize the gene expression patterns in response to osmotic shock with two phases. Phase one is said to be a quick, yet transient, “sensing and early effector signaling” response (184). This phase involves the upregulation of genes characterized by gene ontology terms, such as “nucleosome, intracellular signaling cascade, signal transduction, protein kinase activity, and negative regulation of cell differentiation” (184). Specific genes involved in phosphorylation cascades, thyroid hormone, and calcium signaling were identified and suggested to play an important role in osmotic acclimation (specific genes and more detail can be found in reference 184). Phase two of the compensatory response to osmotic shock is said to be “associated with regulation of cellular effectors, functioning to return the cell to a state of osmotic homeostasis” and includes genes responsible for maintaining the cell integrity, tissue remodeling, and the previously identified ion transporters that regulate ion balance (184). For example, gill ion regulators (*CFTR* and *NKCC*) seen in the acclimation to seawater are permanently downregulated early on in the time course (6 and 24 h, respectively), while freshwater gill transporters ($\text{Na}^+/\text{K}^+-\text{ATPase}$ and V-type H^+-ATPase) are upregulated later on in the time series (72 and 168 h posttransfer, respectively) (184). Figure 5 (replicated from reference 184) highlights the changes in gene expression through time during the hypo-osmotic challenge based on functional category. Figure 5 is a useful illustration of how researchers demonstrate gene expression changes over time as a result of an altered environment. In this case, if we consider gene expression to be the phenotype of interest and time in a hypo-osmotic challenge to be the altered

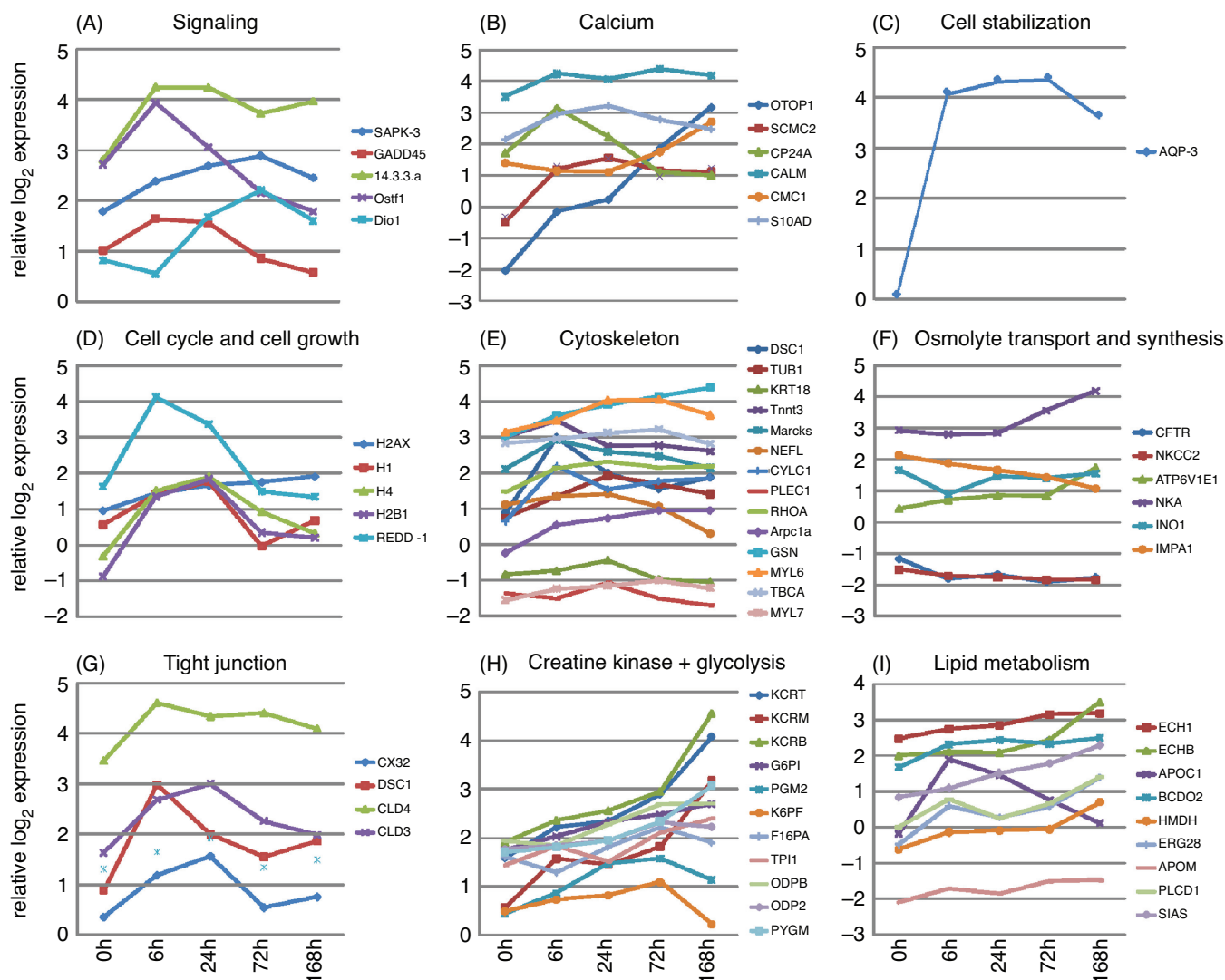


Figure 5 Depiction of the use of molecular techniques to better understand the genetic basis of phenotypic plasticity. Gene expression data were collected using a global transcriptome analysis of *Fundulus heteroclitus* gill tissue during hypo-osmotic challenge. \log_2 gene expression values are plotted as a function of time of exposure and partitioned by functional category. This figure is recreated, with permission, from (184) Whitehead et al. "Functional genomics of physiological plasticity and local adaptation in killifish", *Journal of Heredity*, June 25, 2010, Figure 3, with permission of Oxford University Press on behalf of the American Genetic Association. Gene names are as follows: "14.3.3.a, 14-3-3.a protein; APOC1, apolipoprotein C-1; APOM, apolipoprotein M; AQP-3, aquaporin-3; Arpc1a, actin-related protein 2/3 complex subunit 1A; ATP6V1E1, V-type proton ATPase subunit E 1; BCDO2, beta-carotene dioxygenase 2; CALM, calmodulin; CFTR, cystic fibrosis transmembrane conductance regulator; CLD3, claudin-3; CLD4, claudin-4; CMC1, calcium-binding mitochondrial carrier protein Aralar1; CP24A, 1,25-dihydroxyvitamin D(3) 24-hydroxylase; CX32, gap junction connexin-32.2 protein; CYLC1, cylicin-1; Dio1; DSC1, desmocollin-1; ECH1, delta(3,5)-delta(2,4)-dienoyl-CoA isomerase; ECHB, acetyl-CoA acyltransferase; ERG28, probable ergosterol biosynthetic protein 28; F16PA, fructose-1,6-bisphosphatase class 1; G6PI, glucose-6-phosphate isomerase; GADD45, growth arrest and DNA damage-inducible protein GADD45 beta; GSN, gelsolin; H1, histone H1; H2AX, histone H2A.x; H2B1, histone H2B.1; H4, histone H4; HMDH, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; IMPA1, inositol monophosphatase; INO1, inositol-3-phosphate synthase; K6PF, 6-phosphofructokinase; KCRB, creatine kinase B-type; KCRM, creatine kinase M-type; KCRT, creatine kinase, testis isozyme; KRT18, keratin, type I cytoskeletal 18; Marcks, myristoylated alanine-rich C-kinase substrate; MYL6, myosin light polypeptide 6; MYL7, myosin regulatory light chain 2, atrial isoform; NEFL, neurofilament light polypeptide; NKA, sodium/potassium-transporting ATPase subunit alpha-1; NKCC2, sodium/calcium exchanger 2; ODP2, pyruvate dehydrogenase complex E2 subunit; ODPB, pyruvate dehydrogenase E1 component subunit beta; Ostf1, *Oreochromis mossambicus* osmotic stress transcription factor 1; OTOP1, otopenin-1; PGM2, phosphoglucomutase-2; PLCD1, phospholipase C-delta-1; PLEC1, plectin-1; PYGM, glycogen phosphorylase; REDD-1, DNA damage-inducible transcript 4 protein; RHOA, transforming protein RhoA; S10AD, S100 calcium-binding protein A13; SAPK-3, MAP kinase p38 γ ; SCMC2, small calcium-binding mitochondrial carrier protein 2; SIAS, sialic acid synthase; TBCA, tubulin-specific chaperone A; Tnni3, troponin T, fast skeletal muscle; TPI1, triosephosphate isomerase; TUB1, tubulin alpha chain" (184).

environment, Figure 5 illustrates what we would classically term a reaction norm. This result reveals the time it may take for mitochondria-rich cells and ion transporters to proliferate in the gill epithelium postfreshwater transfer (184). This paper is arguably an important contribution to our understanding of how the transcriptome responds during osmotic shock and acclimation in Killifish and highlights how modern genomic tools (e.g., microarray analysis) are being used to better understand phenotypic plasticity of physiological traits (184). It should be mentioned that this paper also reveals the complex nature of gene expression patterns and genome sequence variation associated with local evolutionary adaptation of killifish in pollution tolerance—contributing more to our understanding of the interplay between phenotypic plasticity and evolutionary change in creating phenotypic trait variation (184).

The previous work on both *P. multicolor* and *F. heteroclitus* represent just a few of the fish ecological and evolutionary studies that focus on the potential interplay between phenotypic plasticity and adaptive evolution when investigating physiological (and other traits) trait variation within and between species. The use of advanced genetic techniques in these studies and others holds promise for a more complete picture of the molecular and genomic underpinnings of physiological plasticity (as emphasized in reference 21; see also Table 2).

Insect immune defense

In this section, we discuss plasticity in insect immune defenses. We chose this example for a number of reasons, including but not limited to the fact that has practical implications for physiology and health, generally, but also for understanding insect physiology, ecology, behavior and evolution, and control (e.g., pest control). Focusing on this trait or, more precisely, a suite of traits with similar physiological functions—that is, the elimination or control of disease—allows us to investigate the kinds of environmental factors or cues that affect immunity and identify what factors shape patterns of plasticity.

Although a lengthy description of the mechanisms of insect immune defenses is beyond the scope of this article, a brief description of the basics may help orient the reader. Unlike the vertebrate immune response involving so-called “adaptive” immunity mediated by antigen-specific antibodies, the insect immune system lacks this particular form of specificity. However, invertebrates can still successfully fend off disease through several forms of innate immunity. Among these mechanisms is the “encapsulation” response, typically a response to “macro” parasites such as parasitoids, and various mechanisms resulting in the production of peptides that, through different mechanisms, eliminate or control infections by bacteria, viruses, and other “micro” parasites. The encapsulation response is a multiple-step process by which the host recognizes an invader as foreign and then subsequently encloses (or “encapsulates”) that invader in hemocytes, which

are specialized cells of the invertebrate hemolymph. Typically, this capsule is then melanized. This melanization process proceeds through the action of series of steps involving various enzymes, most notably phenoloxidase. This process of encapsulation followed by melanization can often effectively kill the invading parasite or pathogen. The immune defenses that do not involve encapsulation include things such as the production of antimicrobial peptides and highly reactive cytotoxic substances. Readers interested in the details may consult any number of good reviews (148, 162).

Research in the field that has come to be called “ecological immunology” makes it very clear that all forms of invertebrate immune defenses are phenotypically plastic. The adaptive benefits of many forms of phenotypic plasticity do not seem to present major intellectual challenges—for example, it is not hard to understand why some mammals develop white pelage in winter, but nonwhite pelage during other (i.e., nonsnowy) times of year. The puzzle that immune defense presents, and why it is interesting in the context of phenotypic plasticity, can be summed up fairly simply as this: why would immune defense be plastic? Or, put another way: why would an organism ever express anything other than a maximal immune defense? A number of hypotheses have been proposed, and some of these are briefly reviewed here. The central unifying theme in the study of phenotypic plasticity of immune defense is the idea of costs—despite the obvious benefits of a maximal immune defense for fighting disease, such responses are hypothesized to come at a cost, and as such, the balance of benefits and costs may sometimes, or often, result in the optimal immune response being something less than what an organism is capable of (139, 148). Therefore, much of the work in this field is focused on trying to identify what those costs might be and what environmental factors (broadly defined) affect these costs (and hence, the cost-benefit balance).

The potential costs of immune defense are many and varied (192). Costs can be considered at different “levels”; that is, one might consider the evolutionary costs of immunity, by which we mean, for example, the negative genetic correlations between components of immune defense and other components of fitness. But for our purposes, that is, from the perspective of phenotypic plasticity, perhaps of primary interest are those costs of mounting an immune response. One such cost might include the risk of immunopathology; that is, damage to self from a maximal immune response. However, much of the recent research on plasticity and costs in immune defense has addressed costs in the context of a resource-allocation problem. That is, immune responses, like other traits, are hypothesized to require nontrivial resources and because of this, immune responses will compete with other traits for those resources. Later, we discuss a few examples that illustrate examples of research into plastic immune defenses that take this perspective.

Perhaps the most direct way to test the hypothesis that plasticity in immune response results from resource-based trade-offs is simply to restrict resources (i.e., nutrients) in an

organism and measure the subsequent immune responses. If resource restriction reduces the magnitude of the response, we have compelling evidence that immune defense requires nontrivial resources. For example, Siva-Jothy and Thompson (163) found that short-term restriction of nutrients resulted in reduced (but reversible) decreases in immune defenses in mealworm beetles. More recent research has suggested nutrient quality, such as the ratios of important nutrients, may be more important than nutrient quantity (33, 89), but such findings do not refute the idea of costs of immunity; rather, they simply emphasize that such costs are complex.

A number of studies have examined plasticity and immunity by examining trade-offs more directly. That is, such studies address how immune defense varies when organisms invest resources in other traits, such as reproduction. For example, studies have found that increased sexual activity can result in decreased immune response in damselflies (164), fruit flies (100), and mealworm beetles (138). In addition to the environmental factors responsible for plasticity in immunity outlined previously, we would expect, if immune defense were costly, that other ecological factors might result in variation in defenses. For example, when insects occur at low densities, risks of disease might be relatively low and so the benefits of strong immune defenses might likewise be low. Conversely, then, we would predict that high densities would increase investment in immunity. This so-called “density-dependent prophylaxis” has, indeed, been found (186). Other, more complicated life-history factors also seem to play a role in immune defense plasticity. For example, damselfly larvae grow in aquatic environments where they are at risk from predatory fish as well as from conspecific cannibals (104). Predation increases the risks of foraging, which in turn influence optimal growth rates and timing of developmental transitions. It appears that immune defense also responds in complex and sex-specific ways: phenoloxidase activity is lowered in males in response to increased predation risk, but in females phenoloxidase is insensitive to these environmental differences (104).

Finally, it is worth noting that although all of these various mechanisms of immune defense have a similar function, by definition, in fighting infection, it is not the case that in a given organism all of these different components of immune defense will show the same patterns of phenotypic plasticity. Factors that reduce the magnitude of one type of immune response may have little or no (or even the opposite) effect on a different immune response. For example, mating reduced the magnitude of the response of three components of immunity in ground crickets in both males and females, but in a fourth component of defense there was no effect of mating in males and an increase in the magnitude of the effect in females (49). Why this is so is not clear, but it seems reasonable to hypothesize that it must have something to do with the relative costs and benefits of different mechanisms of immunity in a given context, and this, in turn, is likely to depend to a considerable degree on the underlying physiological processes responsible for each component of immunity. Indeed, understanding the complexity of phenotypic plasticity of immunocompetence is

widely recognized as one of the primary goals of the field of “ecological immunology” (162).

Insect locust polyphenism

A particularly striking form of phenotypic plasticity, occurring in several insect taxa, is that of “polyphenism.” Polyphenism is typically defined as environmentally induced, discrete phenotypes (but see reference 22, for a history of and criticism of current usage of the term). That is, the term not only implies phenotypic plasticity (and thus distinguishes those cases from cases of genetic polymorphisms), but also contrasts with cases of continuous phenotypic variation due to environmental cues, making polyphenism a special case of phenotypic plasticity. However, as some have pointed out, the discontinuity of phenotypes may not reflect any underlying biology but instead simply be a product of discontinuity in the environmental determinants of phenotypic variation (for example, seasons) (112). In any event, what typically characterizes cases of so-called polyphenisms in insects is that the different phenotypes are typically characterized by differences in a number of traits. The polyphenisms of the locusts are particularly illustrative here.

Locusts are members of the insect order orthoptera (crickets, grasshoppers, and relatives) that (by definition) often form very dense aggregations but that also exhibit nongrouping phenotypes (156). That is, they exhibit a polyphenism consisting of both a gregarious and “solitarious” form. Typically, individuals of the solitarious form avoid other locusts but if ecological conditions bring many individuals together, so that densities are sufficiently high, the locusts change behavior and become gregarious. It appears that a number of proximate cues, due to close proximity to other locusts, trigger the cascade of phenotypic changes, among them visual, olfactory, and tactile cues. For example, experimentally stimulating the hind legs (with a paint brush) of a solitarious form locust is sufficient to induce gregariousness (159). The behavioral changes, in turn, reinforce gregariousness in what has been called a case of positive feedback. The behavioral changes are then followed by a number of morphological and physiological changes.

The details of this form of plasticity are beyond the scope of this review, and have been covered in detail in reviews by Pener and Yerishalmi (115), Simpson et al. (158), Simpson and Sword (157) and, most thoroughly and recently, in an almost-300-page review of the current understanding of locust polyphenism by Pener and Simpson (114). What we wish to do here is briefly introduce the reader to some of the various changes that occur in these locusts to illustrate just how all-encompassing this plasticity is. In addition to difference in behavior between the forms, the most outwardly obvious difference is in coloration. There are different kinds of color variation that occur in different species of plastic locusts, and the adaptive significance (if any) of the color differences is not always clear, but in one species, the desert locust (*Schistocerca gregaria*), it seems as if the conspicuous

black and yellow coloration of the gregarious form is a form of aposematic coloration. These locusts are toxic to vertebrates because they (the locusts) are able to sequester toxins from their host plants (172). In this same species, the solitary form is much less conspicuous, being green or beige in color (e.g., see Fig. 2 from reference 114).

Locust phase polyphenism is far from simply a phenomenon of color differences. For example, gregarious form locusts are more resistant to fungal infection than are the solitary forms (186). This effect is interpreted as being a form of adaptive “density-dependent prophylaxis” mentioned previously. That is, given that the gregarious form lives in dense populations, disease risk (from exposure) is expected to be higher and as such, one would predict this form to be more disease resistant. The dramatic differences in population density experienced by the two forms should also affect other important aspects of their ecology, such as competition over food. In addition, the migratory nature of the gregarious form likely also exposes it to greater dietary heterogeneity than the solitary form. Finally, the energy demands of the gregarious (and migratory) form are higher. These factors together lead to the hypothesis that the nutritional physiology of the two forms should differ, and indeed it does (160). When provided with defined diets differing in nutrient levels (and nutrient balance), the forms differed in consumption, in the ability to convert protein to growth, in lipid deposition, and in development time (160). Color, immunity, and nutritional physiology are just some of the traits that differ between gregarious and solitary-phase locusts, and such “phenotype-wide” forms of plasticity are not limited to the locusts. For example, temperature-induced differences in butterfly wing color patterns (19) are also associated with dramatic differences in courtship behaviors (133). These examples highlight what is certainly more likely the norm than the exception: because of the integration of phenotypes, it is probably rare that environmental variation affects only single traits (121, 124).

Because locusts are “nonmodel” organisms, the ability to investigate the molecular mechanisms of locust phase polyphenism has lagged behind that of other insect groups, particular the Diptera (flies), Hymenoptera (bees and wasps), and Lepidoptera (moths and butterflies) (37). However, this is changing with the advancements in genomic technologies. For example, the analysis of large numbers of ESTs in one polyphenic locust, *Locusta migratoria*, has identified over 500 genes that show expression differences between the solitary and gregarious forms (78). What is particularly promising about the Kang et al. (78) study is that many of the genes showed not only phase but also tissue specificity; for example, some of the genes showing head-tissue specificity were those associated with hormone-binding proteins (in other insect orders) whereas some associated with leg tissues were muscle-related genes. These genomic approaches, including the compilation of resource databases (93), hold great promise for rapidly speeding up the process of identifying some of the genes responsible for the regulation of plasticity in the locusts, which can then be examined in greater detail. For example,

Guo et al. (69) used comparative gene expression profiling, at multiple time points during phase shifting in locusts, to investigate expression patterns in the heads of locusts. From these results they identified two gene families of particular interest (CSP, or chemosensory protein genes and *takeout* genes), the function of which they then investigated via RNAi and behavioral assays. Their results indicate that these genes play a role in olfaction, which in turn plays a role in the early behavioral phase changes observed when the locusts are in close proximity.

As noted previously, behavior is just one way the locust phases differ, and work continues to try to uncover the molecular mechanisms associated with the many phenotypic differences between the phases (De Loof et al., 2006). One of the most promising applications of locust phase polyphenism research will likely be in eventually shedding light on the evolution of the molecular mechanisms of this form of adaptive plasticity. Locusts are, by definition, grasshoppers that exhibit phase polyphenism, but they are not a monophyletic taxon; instead, this form of plasticity has evolved multiple times within the group that includes the locusts (165). However, not all forms of phase polyphenism are alike, with some species exhibiting the full suite of morphological and physiological changes and others exhibiting just some of the differences (and indeed, some members of the group express density-dependent color changes that are not associated with the “swarming” behavior that defines the locusts) (165). This phylogenetic distribution of phase polyphenism provides not only the independence, but also the variation, to ask very important questions about how and why this form of plasticity evolves. But even more, if molecular techniques can eventually be applied to at least some of these species in a phylogenetic framework, we might eventually be able to determine whether the evolution of (this form of) adaptive plasticity evolves independently through unique molecular mechanisms or if some or all cases involve modifications of similar molecular pathways.

Experimental evolution and phenotypic plasticity

Selection experiments have proven a powerful tool in the study of evolutionary biology and comparative and evolutionary physiology (11, 54, 57, 169). Moreover, selection experiments have undoubtedly established that phenotypic plasticity is a heritable trait capable of responding to selection, with an underlying complex genetic architecture (reviewed in reference 144). Additionally, in the subsequent examples, it is important to note that artificial selection, with evolutionary physiology as a central focus, has in many cases furthered the study of phenotypic plasticity and provided insight into areas previously unstudied (additionally see reference 55). Later, examples are provided of studies that have identified the genetic basis of plasticity in body weight and adiposity in response to exercise in mice (81, 88). For similar cases in the human literature see references in Bray et al. (20).

In an extensive review of the study of phenotypic plasticity utilizing selection experiments, Scheiner (144) distinguishes between two types of selection experiments "... (i) artificial selection in which the experimenter selects on a focal trait or trait index and (ii) quasinatural selection (or laboratory natural selection) in which the experimenter establishes a set of environmental conditions and then allows the population to evolve." Utilizing artificial selection, numerous investigations have successfully demonstrated the evolution of phenotypic plasticity through selection directly on the reaction norm (e.g., see reference 145), and selection on a single trait in one environment and selection on a single trait across multiple environments. In the latter two instances, the evolution of plasticity is most commonly examined as a correlated response to selection (e.g., see references 45, 46 and the examples presented later). In addition to artificial selection, investigators have also used quasinatural selection to investigate the evolution of plasticity (e.g., see reference 134). In contrast to artificial selection experiments, quasinatural selection allows for the simultaneous examination of spatial and temporal environmental variation and their role in the evolution of phenotypic plasticity. For a general review of quasinatural selection experiments see Irschick and Reznick (75). Later, we summarize the methods and results of a handful of examples of selection experiments that have investigated the potential for the evolution of phenotypic plasticity. The examples presented later are not intended to be a representation of the entirety of the literature on regarding plasticity and selection experiments; consequently we encourage the reader to explore alternative and certainly more comprehensive examinations into selection experiments, their methods, and their applications (9, 54, 57, 144).

Selecting directly on the reaction norm, Scheiner and Lyman (145) provide one of the most thorough examples of the heritability of plasticity using *D. melanogaster*. Scheiner and Lyman (145) caught wild *D. melanogaster* and subsequently maintained them in the laboratory at 21°C for multiple generations. Next, Scheiner and Lyman (145) directly selected for increased and decreased thorax length at 19 and 25°C, and directly selected for increased and decreased plasticity. Scheiner and Lyman (145) defined plasticity as the difference in average thorax length for sets of full-sibs raised at 19 and 25°C. Scheiner and Lyman (145) observed several important findings. First, selection on plasticity of thorax length revealed a realized heritability of 0.088 ± 0.027 , indicating that plasticity did indeed respond to selection. Second, Scheiner and Lyman (145) did not observe a correlation between the extent of plasticity of thorax length and the amount of genetic variation for thorax length. And, finally, Scheiner and Lyman (145) demonstrated that the genetic architecture of plasticity in thorax length was a result of genetic interactions among multiple loci and not reflective of the previously predicted overdominance model (amount of plasticity is negatively correlated with the number of heterozygous loci—resulting in elevated fitness of heterozygotes). More specifically, with regard to the genetic basis of plasticity, Schiener and Lyman (145) tested

models of overdominance, pleiotrophy, and epistasis and conclude that: "The results mostly support the epistasis model, that the plasticity of a character is determined by separate loci from those determining the mean of the character." However, Scheiner and Lyman (145) later offer that "... some paradoxical results suggest that reality may be even more complex than originally envisaged."

In another example, investigators have utilized a long-term murine artificial selection experiment for high voluntary wheel-running behavior on days 5 and 6 of a 6-day wheel exposure (168) to examine the evolution of phenotypic plasticity as a correlated response to selection. This mouse model and the investigations into the evolution of plasticity are extensively reviewed elsewhere (56). Therefore, here, we will only provide relevant background and briefly focus on two examples. By the 16th generation of selection, the high-running lines (HR, four replicates) displayed a 2.5- to 3.0-fold increase in daily revolutions as compared to the nonselected control lines (four replicates), mainly by running faster (especially in females), a differential maintained through at least 40 subsequent generations. To test for differences in phenotypic plasticity of physiological relevant traits, Garland and colleagues have performed multiple experiments in which HR and control mice are housed with and without access to running wheels for days, weeks, or months (56). In response to these housing conditions (access to voluntary exercise or not), Garland and colleagues have observed a variety of responses. The patterns observed in exercise training responses are often trait dependent and variable, and have included the following: little to no response in either HR or control lines, similar changes in HR and control lines, greater change in HR lines but in the same direction as control lines, and opposing directional change in the HR as compared to the control lines. For the cases where HR mice exhibit a greater training response, Garland and colleagues have concluded that the greater plasticity exhibited by HR mice can in some cases be statistically explained by the increased running of HR mice (i.e., "more pain, more gain"). Alternatively, as explained by Garland and Kelly (56) the greater training response may not be a simple linear function of the amount of wheel running; rather there is the potential for HR lines to have evolved greater plasticity as a correlated response to selection. In the subsequent instance, we highlight an example of physiological plasticity at the molecular level, and the potential to have evolved as a correlated response to selection. It is worth noting, that in either of the two scenarios described previously (more pain, more gain or the evolution of greater plasticity) we have described circumstances in which a behavior (voluntary running) stimulates plastic changes in morphological or physiological traits that in turn may improve the ability to perform such a behavior. This phenomenon has been previously termed "self-induced adaptive plasticity," (170, 171).

In addition to examining the plasticity of wheel-running behavior (not discussed here), Garland and Kelly (56) highlight several examples of increased plasticity of physiological traits in the HR lines that appear to represent adaptations for

high wheel running. We want to emphasize the phrase “appear to represent” and encourage the reader to review the text presented by Garland and Kelly (56) discussing at length why identifying evolutionary adaptations for high wheel running is “nontrivial” (see also references 10, 42, 48, 55). Although Garland and Kelly (56) detail numerous examples, we will only highlight the experiment performed by Gomes et al. (63) as it appears to be the most dramatic example of the difference in plasticity between HR and control lines over the shortest period of exercise exposure. Gomes et al. (63) measured the abundance of a glucose transporter, GLUT-4, in the gastrocnemius muscle of female HR and control mice in the absence of running wheels and following 5 days of running activity. Gomes et al. (63) report that in the absence of running wheels, HR and control mice did not significantly differ in the amount of GLUT-4 in the gastrocnemius muscle. After 5 days of wheel access, Gomes et al. (63) observed a 271% increase in GLUT-4 abundance in HR lines compared to only a 79% increase in control lines, a statistically significant difference. However, Gomes et al. (63) also observed a statistically significant elevation in the amount of running (distance, speed, and duration) across all 5 days in HR mice as compared to controls. As described in detail by Garland and Kelly (56), the postexercise elevation in GLUT-4 levels could be a function of the increased wheel running of the HR mice (i.e., more pain, more gain) or the differences could reflect greater plasticity among the HR mice (i.e., “for a given amount of stimulus, such as wheel running per day, individuals in the HR lines show a greater response as compared with individuals in the control lines”). In fact, Gomes et al. (63) observed that greater increases in GLUT-4 after 5 days of wheel access was not a linear function of the amount of running, rather the increase represented the evolution of adaptive phenotypic plasticity as a correlated response to selection. We should acknowledge that Gomes et al. (63) did not simply assume that the differences in plasticity were an adaptive response to the high levels of voluntary wheel running (i.e., the beneficial acclimation hypothesis), and discuss at length the importance of glucose transport in the context of sustained aerobic running.

Phenotypic plasticity: Mouse models and human health

Within biomedical disciplines the examination of physiological adaptations in response to multiple environments most ardently focuses on the role of plasticity as it relates to the prevention and treatment of health-related disease. Perhaps one of the most clinically relevant examples of physiological adaptations in response to an altered environment, with far-reaching implications for administration of public health (30), is variation in physical activity levels or exercise training programs. In this example, we consider the absence (inactivity) or presence of exercise to be the altered environmental variable (as opposed to variability in hypoxia or salinity, as discussed previously) (see Fig. 6), although we acknowledge (and discuss later) that within an altered exercise environment there

may be a continuum of activity durations, frequencies, and intensities. Although we will not discuss it here, Garland and colleagues (2011) provide a thorough review of the biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in both humans and rodents. The morphological and physiological adaptations in response to exercise are complex and involve both central and peripheral inputs at a variety of organismal levels within an array of physiological systems (e.g., see references 2, 13, 15, 32, 101). Although we could spend the entirety of this article focusing on the physiological adaptations to exercise, here we will only briefly examine how alterations in voluntary exercise regimes affect relevant measures of obesity, namely, body weight and adiposity (Fig. 6). In this specific case phenotypic plasticity is the change in body weight and adiposity in response to exercise. Furthermore, we will highlight examples, in animal models, where changes in body weight and adiposity resulting from exercise (the reaction norm) have been mapped to genetic regions (quantitative trait loci or QTL). Finally, we will conclude this section by discussing the mounting evidence suggesting that variable environmental conditions during development (e.g., nutritional level) and the resulting phenotypic responses (developmental plasticity or developmental canalization) are capable of having long-term influences on the adult phenotypes which in turn may underlie variation in disease susceptibility (6, 7, 62).

The beneficial effects of regular exercise have been chronicled for a number of health-related phenotypes such as reduction in triglyceride and low-density lipoprotein (LDL) levels, increased high-density lipoprotein (HDL), enhanced insulin sensitivity, weight loss, and reductions in adiposity (86, 111, 176). We do acknowledge that the extent of change in response to exercise (or any environmental perturbation for that matter) is potentially limited due to the initial level of physical capacity, a concept termed the principle of initial values. That is, “if capacity is low then the percentage gain in capacity in response to training will be high, and vice versa,” a concept directly tested in rat genetic models of exercise capacity (85). For general health benefits and the prevention of weight gain, with a potential for modest weight loss, the exercise recommendation is generally between 150 and 250 min/week (40, 76, 191). Furthermore, more than 250 min/week of exercise have been associated with clinically relevant reductions in overall weight (40). However, physiological adaptations in response to an altered exercise environment are often variable and individual dependent, especially with regard to weight loss and reduction of adiposity (79). Specifically, in a 12-week exercise intervention among overweight and obese sedentary men and women, the variability in weight loss changed ranged from -14.7 kg to $+1.7$ Kg (84). A portion of this variability has been attributed to the frequency, duration, and intensity of physical activity, although results appear to be inconsistent (66, 77, 137). Moreover, in a review of MEDLINE literature from 1996 to 2000, Ross and Janssen (140) concluded that for short-term studies (≤ 16 weeks) there was substantial evidence that

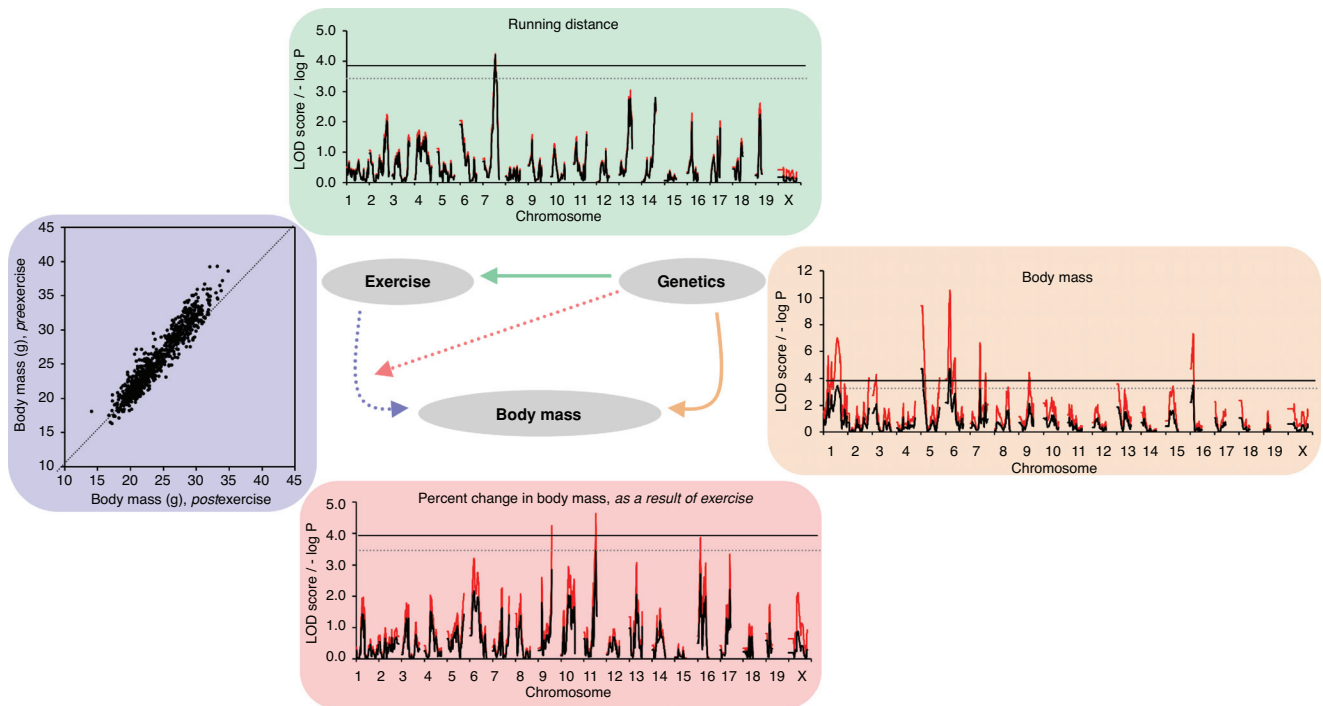


Figure 6 An integrative approach to the study of phenotypic plasticity utilizing a mouse model. This figure is partially recreated, with permission, from (82) Kelly et al. "Genetic architecture of voluntary exercise in an advanced intercross line of mice" *Physiological Genomics*, 2010, 42: 190-200, Figures 1 and 2; and (81) Kelly et al. "Exercise, weight loss, and changes in body composition in mice: phenotypic relationships and genetic architecture," *Physiological Genomics*, 2011, 43: 199-212, Figures 1 and 5. In this integrative example, we illustrate the complex control of a single phenotype, body mass, and the intricacies of its response to an altered environment, exercise. It has been well established that the variation in body mass is partially regulated by genetics (orange panel). Additionally, as demonstrated by the presence of an altered environment (exercise, as opposed to none) there is a plastic response in body weight (blue panel). Although this change is most commonly a reduction in weight, there is substantial variation among a given population (blue panel). Additionally, this variation in the change in body weight in response to exercise has a genetic basis as indicated by the presence of a quantitative trait locus (QTL) on chromosome 11 (red panel). Furthermore, the predisposition to engage in the altered environment (exercise or not) has an underlying genetic architecture (green panel). This type of comprehensive approach demonstrates the complexities of the study of phenotypic plasticity and the power of a molecular approach using a mouse model. For further discussion of examples from this mouse model see text and Garland and Kelly (56). Additionally, for a similar theoretical approach studying bone structure and performance see Middleton et al. (103). For the genome wide QTL plots, red traces are the simple mapping output, and black traces are corrected for family structure in this fourth generation population. The solid black and gray dotted lines represent the permuted 95% [logarithm of odds (LOD) ≥ 3.9 , $P \leq 0.05$] and 90% (LOD ≥ 3.5 , $P \leq 0.1$) LOD thresholds, respectively.

exercised-induced weight loss is positively related to reductions in total fat in a dose-response manner although for long-term studies (≥ 26 weeks) dose-response relationship was not present. An additional source of the variation underlying the physiological response to exercise, with regard to weight and adiposity, is the relationship between exercise and food consumption (58, 83, 182), although this relationship varies with exercise duration and intensity, with sex (41), and among individuals (14). Regardless of the shifting results depending on population, duration of exercise program, or food intake compensation, even when exercise doses and the resulting energy expenditure are tightly controlled, changes in weight remain variable (17).

Given the importance of weight maintenance to general health and the complex interactions between exercise and body composition, investigations into the genetic architecture underlying the change in weight and adiposity as a result of exercise are ever increasing (20, 81, 88, 105, 110). To illustrate, Leamy et al. (88) investigated the genetic basis of

weight change in response to physical activity using a murine model. Three hundred and seven F_2 mice, resulting from an intercross between inbred strains C57L/J and C3H/HeJ were provided running wheels at 9 weeks of age and given access for 21 days during which daily distance, duration, and speed were recorded, and later averaged across all 21 days of the voluntary activity. With 129 single nucleotide polymorphisms (SNPs), Leamy et al. (88) utilized an interval mapping approach to identify five (four suggestive and 1 significant) QTL affecting weight change as a result of exercise. Only two of these loci colocalized with previously identified physical activity QTL and only one of the two displayed a positive result for direct pleiotropy, indicating the possibility that this same QTL simultaneously affects distance, duration, speed, and the resulting weight change. Although only one significant QTL was observed, Leamy et al. (88) concluded that the epistatic interactions contributed significantly to the genetic variation underlying the relationship between weight change and physical activity traits. Kelly et al. (81) provide the only

other example of a direct test of the genetic architecture of weight change in response to physical activity, while providing additional results identifying QTL underlying the change in adiposity and lean mass as a result of exercise (see results in Fig. 6). Kelly et al. (81) utilized an advanced intercross line of mice originating from reciprocal crosses between a high-running line and the inbred strain C57BL/6J. Body composition measures (weight, %fat, and %lean) were measured at 4, 6, and 8 weeks of age, at which time the mice were granted running wheel access for 6 days. Following the 6-day voluntary exercise period, body composition measures were assessed again. Utilizing 530 evenly spaced SNPs across the genome, Kelly et al. (81) identified several QTL underlying the change in body composition as a result of exercise: one suggestive locus for percent change in body mass (Fig. 6), two loci (one significant and one suggestive) for percent change in percent fat, and one significant locus for percent change in percent lean mass. The QTL identified by Kelly et al. (81) did not colocalize with previously identified physical activity loci (see reference 82 for comparison) and did not directly overlap with those identified by Leamy et al. (88). In conclusion, Kelly and colleagues state,

"Taken together, our results are demonstrative of the complexity of weight regulation and the relationships between genetics, body composition, exercise, and food consumption. Body composition, exercise, and food consumption each have their own complex underlying genetic architectures, but they clearly interact in a complex way, making it, in our opinion, imperative to begin to unify isolated investigations of each of these traits (81)."

Figure 6 depicts one such attempt at integrating behavioral, morphological, and genomic results to understand the general patterns of phenotypic plasticity, the genetic mechanisms, and the potential human health implications.

Conclusion

As we emphasized in the introductory paragraphs to this review, under a broad definition of plasticity (which we prefer), plasticity is the norm, not the exception. That is, we should be more surprised to find traits that are insensitive to environmental variation than to find those that are sensitive to the environment. As the selected, and far from unique, examples in this review illustrate, not only is plasticity widespread taxonomically, it is also an attribute of virtually all kinds of traits, from behavior and morphology to physiology and gene expression. The near ubiquity of plasticity is a double-edged sword: that it is so widespread means that it almost certainly must be important to the biology of the organisms expressing it. However, its pervasiveness and complexity mean that it is challenging to study, and identifying when plasticity is adaptive and when it is neutral or even maladaptive is far from trivial.

However, these challenges to studying phenotypic plasticity should not be discouraging, but should instead be in-

spiring. The field is as active now as it has ever been, and shows no signs of slowing down. Without doubt, the most active area of research into phenotypic plasticity right now concerns the incorporation of genomic and molecular tools and concepts into the investigation of the mechanisms, and evolutionary significance, of plasticity. This is, and should, continue to be an area of rapid progress. We would encourage this approach, but emphasize that it will be most beneficial if incorporated into a highly integrative research program that utilizes the full range of approaches—field studies, selection experiments, and so on—at its disposal (e.g., Fig. 6). These methodological approaches are most effective when paired with a highly integrative perspective on plasticity, that is, one that considers the broad-based view of plasticity outlined in the previous paragraph. Modern biology is, and will continue to become, more integrative than ever before. Perhaps no field of inquiry is more suited to the integrative approach than that of the study of phenotypic plasticity.

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