EVOLUTIONARY CELL BIOLOGY

PHENOTYPIC PLASTICITY & EVOLUTION

CAUSES, CONSEQUENCES, CONTROVERSIES

EDITED BY

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FOREWORD BY

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CRC Press

Taylor & Francis Group

Phenotypic Plasticity & Evolution

Evolutionary Cell Biology

Science publisher

Charles R. Crumly – CRC Press/Taylor & Francis Group

PUBLISHED TITLES

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Causes, Consequences, Controversies

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CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

First edition published 2021
by CRC Press
6000 Broken Sound Parkway NW, Suite 300, Boca Raton, FL 33487-2742

and by CRC Press
2 Park Square, Milton Park, Abingdon, Oxon, OX14 4RN

ISBN: 9780367357047 (hbk)
ISBN: 9780367676551 (pbk)
ISBN: 9780429343001 (ebk)

DOI: 10.1201/9780429343001

Typeset in Times
by codeMantra

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In recent decades, evolutionary principles have been integrated into biological disciplines such as developmental biology, ecology and genetics. As a result, major new fields emerged, chief among which are Evolutionary Developmental Biology (or Evo-Devo) and Ecological Developmental Biology (or Eco-Devo). Inspired by the integration of knowledge of change over single life spans (ontogenetic history) and change over evolutionary time (phylogenetic history), evo-devo produced a unification of developmental and evolutionary biology that generated unanticipated synergies: Molecular biologists employ computational and conceptual tools generated by developmental biologists and by systematists, while evolutionary biologists use detailed analysis of molecules in their studies. These integrations have shifted paradigms and enabled us to answer questions once thought intractable.

Major highlights in the development of modern Evo-Devo are a comparison of the evolutionary behavior of cells, evidenced in Stephen J. Gould's 1979 proposal of changes in the timing of the activity of cells during development — heterochrony — as a major force in evolutionary change, and numerous studies demonstrating how conserved gene families across numerous cell types 'explain" development and evolution. Advances in technology and in instrumentation now allow cell biologists to make ever more detailed observations of the structure of cells and the processes by which cells arise, divide, differentiate and die. In recent years, cell biologists have increasingly asked questions whose answers require insights from evolutionary history. As just one example: How many cell types are there and how are they related? Given this conceptual basis, cell biology — a rich field in biology with history going back centuries — is poised to be reintegrated with evolution to provide a means of organizing and explaining diverse empirical observations and testing fundamental hypotheses about the cellular basis of life. Integrating evolutionary and cellular biology has the potential to generate new theories of cellular function and to create a new field, "*Evolutionary Cell Biology*."

Mechanistically, cells provide the link between the genotype and the phenotype, both during development and in evolution. Hence the proposal for a series of books under the general theme of "*Evolutionary Cell Biology: Translating Genotypes into Phenotypes*", to document, demonstrate and establish the central role played by cellular mechanisms in the evolution of all forms of life.

Brian K. Hall and Sally A. Moody

Foreword
A perspective on 'plasticity'

Mary Jane West-Eberhard
Smithsonian Tropical Research Institute

Anyone interested in the nature of living organisms and their adaptive evolution will be stimulated by reading this book. I am no exception. But I have an advantage, the opportunity to write a Foreword, with freedom to comment—or even emote, opine, and reminisce—about the topics at hand without even the corrective of peer review to put a brake on runaway ruminations. I apologize in advance for citing my own work in sections where I am trying to substantiate a thought without doing a proper review.

Start with the word 'plasticity.' It seems designed to befuddle the uninitiated because it does not bring to mind any obvious characteristic of living organisms, which are not plastic quite in the manner of elastic belts, rubber bands, bubble gum, or food packaging. I could be accused of having promoted the word plasticity by using it as one of four in a book title: *Developmental Plasticity and Evolution* (West-Eberhard 2003). But those four words cost more hours of agonizing indecision than any other set of four in a book of more than 300,000 words (yes, too long—so it was designed to be read in pieces). The problem with 'plasticity' was that it already had a definition, in terms of reaction norms of primarily quantitative traits. But that definition did not readily suggest all that needed attention, which included plasticity-facilitated developmental reorganization of qualitatively distinctive phenotypes. [Schlichting (2021) in this volume, citing Uller and colleagues (2020), notes some of the same reservations that I had about beginning with reaction norms.] I decided to adopt a broad version of the established definition: the ability to respond to an environmental input with a phenotypic change. For my purposes, plasticity might better have been called 'responsiveness' or 'condition sensitivity.' But plasticity already had a public. And the plasticity public was being enlarged at the time by important books and reviews. So I stuck with the word 'plasticity.'

This quandary over terms indicates why the word 'developmental' as a modifier of plasticity is important. Development implies attention to mechanisms and invites looking at responsiveness to both external and internal environments—responsiveness of the phenotype at all levels of organization from the molecular to the behavioral, including internal responsiveness to gene products.

Attention to developmental plasticity picks up Darwin's thoughts where they left off—with a struggle to understand the causes of selectable variation. Those struggles were summarized in Darwin's theory of pangenesis, which includes what we could now call a molecular theory of the gene, postulating tiny gemmules that, like genes, were seen as being found throughout the body and as mediating both the transmission and the expression of traits. The history of Darwin's ideas about development

and evolution, including the idea of phenotypic plasticity, is traced in Costa's (2021) masterful chapter in this volume, beginning with Darwin's ideas as a young man and intertwined in his thoughts about variation and selection until the end of his life, most remarkably in his two volumes on variation (Darwin 1868). Costa then traces the fate of those ideas through the history of evolutionary biology into the present, giving an unprecedented account of the history of developmental plasticity in evolutionary biology.

Costa's history is exciting because it shows the connections between Darwin's ideas and current ones that seem to us to be 'new' (see also West-Eberhard 2003, Chapter 8; 2008b). Rediscoveries are no less exciting when their origins can be traced to Darwin or before. But Darwin's achievement in this area is a lesson in humility for those who suffer from the amnesia that seems to generate new-discovery cycles with a periodicity of about 30 years for almost any idea in evolutionary biology. We now benefit from data that Darwin lacked, including concrete information on gene expression that allows us to see how the material basis for transmission and expression is the same—the dual nature fulfilled by the 'gemmules' he imagined. I think that Darwin would have been especially fascinated by the chapter in this book (Lister 2021) showing how fossils can now be used to substantiate the importance of developmental flexibility in the origin of morphological transitions. The same could be said regarding a chapter by Bonduriansky (2021) on non-genetic inheritance and epigenetic effects of the environment, a good entrée into an area that has enormously expanded in the last two decades.

This book is sprinkled with histories. Costa's (2021) history on Darwin and the causes of phenotypic variation is complemented by Sultan's (2021) reminder of the long history of studies of plastic responses; Scheiner and Levis (2021) on the history of ideas about genetic assimilation; Diamond and Martin (2021) on the history of the plasticity as buying time for genetic adjustments to environmental change; Levis and Pfennig (2021) on the history of ideas about plasticity-led evolution; and Futuyma (2021) and Pfennig (2021, Box 3.2), both of whom track plasticity concepts to the 20th-century synthesis with genetics.

Histories invite asking: what has changed in the past? And what might be changing now? The rest of this essay reflects thoughts about those questions. They are based on 60 years of personal observation that started in 1959 with a lab section in zoology at the University of Michigan, taught by a newly hired assistant professor, Richard Alexander, who later became a prominent evolutionary biologist. As an undergraduate and graduate student in zoology, I learned—and internalized—the synthesis that put genetics at the center of evolutionary biology. Mayr (1991), in his own history, characterized 'The Synthesis' as a consensus that developed between geneticists (who focused on genes) and naturalists (who focused on phenotypes). It meant that we were all thinking and talking in terms of genes, even those of us who, like me, were naturalists working primarily on phenotypes (e.g., morphology, behavior, or taxonomic traits).

A major change between that era (what I will call it the 'Synthesis Era') and the present age is an increased focus on the phenotype, including its development and plasticity. The 20th-century synthesis had not too much of genes but too little of phenotypes and their development. Here I briefly discuss some of the changes implied

by a shift in evolutionary biology toward increased attention to the phenotype, especially its developmental plasticity. I also respond to some opinions, expressed in the present volume, that indicate resistance to such change.

On the nature of selection. It is sometimes said that the role of plasticity for evolution is 'controversial' (see Futuyma 2021; Levis and Pfennig 2021; Pfennig 2021 and references therein). That seems to raise doubts about its importance. There may be unresolved questions about the role of developmental plasticity in a particular case or in a particular pattern of evolutionary genetic change, just as there may be unresolved questions about the role of mutation or of selection. But there can be no doubt that developmental plasticity needs to be recognized, alongside genes, as playing an important role in Darwinian (adaptive and social/sexual) evolution, one that needs to be taken into account by any general description of how Darwinian evolution works. Here is a quick summary of the rationale for that assertion:

- Darwinian evolution requires heritable change due to selection.
- Selection depends on the existence of phenotypic variation.
- All phenotypic variation comes from variation in development.
- All variation in development comes from its responsiveness to inputs from two major sources—the genome and the environment.
- Therefore, developmental plasticity—developmental responsiveness to environmental inputs—is important for Darwinian evolution.

An important point is that selection does not depend on the presence of *genetic* variation. It only requires *phenotypic* variation that affects fitness, regardless of the proximate cause of that variation. Of course, a genetic response to selection (genetic *evolution*) does depend on the presence of genetic variation. Note also that genetic change in response to selection—adaptive evolution—if it occurs, necessarily depends on, and therefore follows, selection. In this sense genes are virtually always followers in adaptive phenotypic evolution, a point (with one kind of exception) further discussed below.

Failure to appreciate the fact that selection acts on variation in phenotypes—not genotypes—has led to some interesting mistaken ideas. One of my favorites, as a female animal behaviorist, is the 'lek paradox' (Borgia 1979). This is the seemingly paradoxical idea that female choice for traits in males—traits that are not associated with any direct fitness benefit (such as paternal care)—persists, even when strong sexual selection might be expected to eliminate genetic variation. The implication is that without genetic variation females would no longer choose. But female choice does not depend on genetic variation (Borgia refers to 'genetic choice'). Instead, it depends on phenotypic variation among males which could persist indefinitely without genetic variation. Sexual selection would not stop. It would simply stop affecting evolution. The lek paradox has had the good result of stimulating a stream of ideas (continuing into the present; e.g., see Dugand et al. 2019) regarding the maintenance of genetic variation in populations. It has also provided theoretical support suggesting the widespread presence of standing genetic variation and therefore for the idea (e.g., in West-Eberhard 2003 and references therein) that selection does not await mutation to affect evolution.

On the origin of novel phenotypes and their reaction norms. There was a void—what Schlichting (2021) in this volume calls a "lacuna"—in our thinking, during the Synthesis Era: there was very little discussion about the origin of the complex phenotypic traits that interested naturalists. Futuyma (2021) raises several important questions about the relevance of developmental plasticity to filling that void.

[Remember, this is a personal account of history so I need to explain why I will pay extra attention to Futuyma's critique of ideas about plasticity in his chapter of this book. Doug Futuyma and I were graduate students and friends at the University of Michigan in the mid-1960s, where we were both steeped in the burgeoning Evolutionary Synthesis. Our paths then diverged, Doug's toward a thesis on *Drosophila* genetics and mine toward a thesis on the natural history and behavior of social wasps—taking us into different branches of the two whose consensus formed the Synthesis. So it is not surprising that we now have somewhat different views about the roles of developmental plasticity and genes in evolution. I will refer to some of them below, in the open spirit of the old Michigan debates.]

Doug (henceforth, Futuyma) raises a question about origins in his discussion of reaction norms (Futuyma 2021). He notes, in a discussion of genetic assimilation, that "there is hardly any challenge to standard theory when derived characters are a fixed state of an advantageous ancestral reaction norm." But, he points out, such discussions always begin with change in reaction norms that are already present in ancestral populations. That is (to insert my own words), they treat evolution as it has been treated in the past, without addressing the old void regarding origins.

In fact, there is a great deal of information about the origin of reaction norms. But to take advantage of it you have to consider the genetic architecture of phenotypic traits. Phenotypic traits are characterized by both continuous variation (i.e., variation in the dimensions and regulation of traits, where environmental variation can be described in terms of reaction norms) and modularity of form (organization of phenotypes and gene expression into semi-discrete units). This means that the origin of a (new) phenotypic trait implies the origin of (new) reaction norms—environmentally influenced variation in the dimensions of the trait. And there is a great detail of information about the origin of new phenotypic traits, as discussed below.

Given the genetic architecture of traits, thinking in terms of plastic and non-plastic genes (as in Scheiner and Levis 2021) is potentially confusing. Mechanistically, the locus of plasticity—of reaction norms and the on-off regulation of traits—does not reside in the nature of individual genes; it is a product of many kinds of condition-sensitive polygenic, quantitatively variable mechanisms, like hormones and other physiological systems (see Ledón-Rettig and Ragsdale 2021 in this volume). So it is not true that "The loss of plasticity requires the appearance of non-plastic genotypes in a population," if that means the advent of a mutation (as in the example following that statement in Scheiner and Levis 2021).

Schlichting (2021) gives a similar answer to Futuyma's question about the origins of reaction norms, concluding that "Non-plasticity (i.e., canalization, robustness) is thus arguably a derived state in most biological systems." Ledón-Rettig and Ragsdale (2021), also in this volume, show how physiological mechanisms can link environmental signals to both continuous and discrete variation in traits, with changes in

physiological systems thus capable of influencing the origin of both novel discrete traits and new reaction norms.

The architecture of phenotypes ties the origin of new reaction norms to the origin of new semi-discrete traits. But how do *those* traits originate? Futuyma (2021) almost, but not quite, answers that question when he goes on to "find most interesting several cases in which the ancestral state seems not to have been an adaptive reaction norm." To illustrate this, he cites studies that document the advent of novel phenotypes, one of them being the "curious case" of a novel resource-use morph found in spadefoot toad tadpoles, induced by a dietary manipulation and resembling a form established in a related species (described in Levis and Pfennig 2021; Pfennig 2021 in this volume). Futuyma notes that "The developmental response seems not to be an adaptation, even though it can have an advantageous effect." That sentence could serve as a definition of a novel phenotype at its origin: it is a developmental anomaly that, like a genetic mutation, can have evolutionary potential; then, if it has an advantageous effect, it may become established (genetically accommodated) under selection in a population.

It is worth asking, along with Futuyma (2021) in this volume, whether anomalies like the diet-induced morph of the spadefoot toads: "Are cases such as these odd, rare 'accidents' of development, rare enough to count for little?" The answer, of course, is that rare accidents of development, like genetic mutations, may count for a lot if they happen to be positively selected and become established traits—that was the point of the spadefoot toad tadpole example and others described in the present book (see especially Scheiner and Levis 2021). It is also shown dramatically by a study (Shubin et al. 1995) of newts, *Taricha granulosa*, where a phylogeny of salamanders was used to show how rare accidents of development can become established phenotypes: numerous anomalies seen in a large sample of that one species appeared in related species as alternative phenotypes and established traits (Figure 19.3 in *Developmental Plasticity and Evolution*). Such a pattern of 'recurrence' has been documented in numerous taxa (*op. cit.*).

The origin of novel phenotypes due to developmental reorganization—a consequence of developmental plasticity—has also been very extensively documented (see Chapters 10–19 in *Developmental Plasticity and Evolution*). In a large collection of examples surveying numerous kinds of organisms, I found no case where it had been shown that a complex phenotype of the kind I was trying to understand was formed beginning with a mutation, followed by a series of mutations modifying it to produce a genetically and phenotypically complex adaptive trait. Although no such collection can be complete, if the successive-mutation hypothesis were a viable explanation for the evolution of complex phenotypes there should have been numerous well-documented examples. What I did find, to my surprise and satisfaction (as an amateur historian), is a very large number of origins by developmental reorganization that were a déjà vu of classical phylogenetic embryology—heterochrony, deletion, reversion, and four other kinds of developmental rearrangement, including correlated change in reaction norms of multiple traits showing extreme responses to stress. It matters little whether these developmental rearrangements were initiated by a mutation or an environmental induction, factors that are developmentally equivalent and easily interchangeable as initiators of phenotypes (see West-Eberhard 2003

on interchangeability). Whatever the initiator, degree of environmental or genetic control can in principle be adjusted upward or downward by selection on polygenic regulatory mechanisms—pathways created in response to the initiator. Given the strength and variety of evidence that now indicates how developmental plasticity can be integrated with genetics to explain the origin and evolution of phenotypic traits, I regard the burden of proof to lie with those who favor some alternative approach.

In sum, since all new phenotypes are subject to environmentally mediated variation in their dimensions and regulation—their reaction norms—as just mentioned, the abundant evidence on how new traits originate via developmental reorganization constitutes abundant evidence regarding the origin of new reaction norms. This should help to assuage Futuyma's (2021) worry that the study of developmental plasticity "seems not to burrow into the origin of … ancestral reaction norms."

On the role of genes in adaptive evolution. Putting the phenotype in its proper place as a product of development as well as the object of selection leads to a deeper and clearer understanding of the role of genes in adaptive evolution than is possible in purely genetic terms. For example, a discussion of developmental plasticity may view gene products as part of the internal environment that affects a condition-dependent developmental response during ontogeny. Regard for gene products as part of the (internal) environment is implied in genetics by the term 'epistasis,' or gene-by-gene interaction, the dependence of a gene's effect on genetic background (the nature of the other genes present). And, similarly, gene-by-environment interaction recognizes the importance of environmental variation for phenotypic form. Given such terms, it cannot be claimed that traditional genetics has ignored the importance of the environment or of the genotype as a whole. But epistasis, like gene-by-environment interaction, describes a statistical interaction, not a mechanistic developmental one. These quasi-causal terms for statistical correlations are potential traps for the innocent. For some, these terms may obscure the fact that research is needed to explain what causes those fundamental genetic phenomena to occur.

The studies described in this book conveniently summarize in one place some of the ways in which looking at plasticity and development deepens understanding of the biology underlying patterns observed in genetics. For example, Goldstein and Ehrenreich (2021) discuss how genetics has moved in the direction of understanding mechanisms, and how it can now contribute to the discovery of underlying processes. And Ledón-Rettig and Ragsdale (2021) deepen evolutionary understanding of pleiotropy by discussing its physiological basis, especially focusing on hormones which have diverse (pleiotropic) effects on complex phenotypes. They discuss both the fundamental nature of pleiotropy and the coordinated origin of complex traits.

During the Synthesis Era, both naturalists and geneticists assumed complex phenotypes to be underlain by sets of particular genes. Including development in the genetic theory gives substance to that assumption, and to Darwin's link between the inheritance and the development of traits. But this link requires showing that the phenotypic traits under selection actually are underlain by coordinated sets of *expressed* genes. Twenty years ago there was indirect evidence for this from studies using electrophoresis and from biochemical analyses of variation in the timing of production of particular enzymes. Now there has been such an explosion of direct

information on the molecular biology of gene expression and its relevance to adaptive evolution (e.g., see Schlichting 2021; Sultan 2021 in this volume) that it may seem strange that this has not always been obvious. This kind of progress in understanding the developmental genetics of conditionally expressed adaptive phenotypes means that the idea of developmental plasticity is permeating the collective understanding of genetics and evolution, whether plasticity is mentioned or not.

It would be a mistake, however, to think that gene expression is the whole story. Developmental plasticity is a manifestation of pathways that connect the environment with the genome. Without these pathways, the genome would be inert. What might be called 'intermediate processes' are crucial—the connections made by biochemical signal-response interactions, hormone systems, and other physiological mechanisms. Physiology and cell biology are areas of mechanism-related biology that, like developmental biology, have been largely estranged from evolutionary biology in the past. They are now crucial contributors to understanding selectable variation and evolution, as evidenced by the discussion in Ledón-Rettig and Ragsdale's (2021) chapter in this volume.

On the Baldwin effect, genetic accommodation, and genetic assimilation. As Futuyma (2021) says, current ideas about plasticity are compatible with those we learned as graduate students. But I am not as graciously forgiving as he is about the arguments that at that time dampened interest in the evolutionary importance of development and plasticity. Compatibility arguments are often preludes to dismissals. For example, Simpson (1953) found the 'Baldwin Effect' fully compatible with modern evolutionary theory but lacking in evidence that "it is a frequent and important element in adaptation." In those days, this was undoubtedly taken by many to mean that the Baldwin effect just wouldn't be worth studying, since, according to this giant of evolutionary biology, "it is seldom assigned an important role in evolution" (p. 110; for discussions of the Baldwin effect in the present volume, see Diamond and Martin 2021; Futuyma 2021; Pfennig 2021, Box 3.2).

The idea of evolution by genetic accommodation was bound to be a target of criticism because it not only accepts but also dares to extend the idea of genetic assimilation. Genetic assimilation was dismissed during the Synthesis as unworthy of special attention (Mayr 1963; see also Box 3.2 in Pfennig 2021 in this volume). It had a reputation among students of my generation as a crackpot idea with Lamarckian overtones. Futuyma (2021), in this volume, treats genetic accommodation and assimilation as he does the Baldwin effect: as having been "subject to debate" but "compatible with the theory that emerged from the Evolutionary Synthesis."

Nevertheless, even prior to the year 2001, when the writing of *Developmental Plasticity and Evolution* was finished, there were abundant data from molecular genetics (electrophoresis) and experiments in quantitative genetics showing the presence of sufficient genetic variation to support selection for virtually any selectively favored trait, including, as required for genetic assimilation, changes in the threshold for expression of conditionally expressed (environmentally induced) ones. Now it is even easier to find evidence for the necessary genetic variation using keywords like 'standing genetic variation' and 'cryptic genetic variation' (for examples in this volume, see Ledón-Rettig and Ragsdale 2021; Levis and Pfennig 2021; Pfennig 2021; Schlichting 2021). Two decades ago there were also numerous examples of transitions

from environmental to genetic determination of traits implying genetic assimilation and supported by phylogenies (e.g., in West-Eberhard 2003). Although Futuyma (2021) found phylogenetic support lacking for the polarity of these transitions, the phylogenies are hidden in plain view in numerous figures (e.g., Figures 5.15, 5.16, 12.3, 17.4, 19.1, 27.4, 28.1, 28.2, and 28.4) in West-Eberhard (2003). Genetic assimilation has survived to be understood as a selection-driven loss of plasticity, an important aspect of evolution, and a worthy topic for future research (see Scheiner and Levis 2021 in this volume).

Evidence for polarity of change (direction of evolution, as from environmental to genetic determination) is essential for evolutionary transition hypotheses. But phylogenetic evidence need not involve mapping onto a phylogeny. Indeed, it is important to value the power of indirect evidence in evolutionary biology. Most of the evidence for natural selection in nature, for example, is an accumulation of indirect evidence that combines models and data testing the many implications of the idea (see also Lister 2021 in this volume on the evidence for plasticity in fossils). The likely polarity of a change can be deduced from various kinds of comparative evidence (see, for example, Schwander and Leimar 2011). For instance, the freshwater phenotype of some large anadromous fish populations, with yearly migrations of individuals between marine and freshwater environments, closely resembles the phenotypes of 'landlocked' non-migratory populations of the same region that are trapped in lakes, where the freshwater form is fixed. The freshwater form can be deduced with a high degree of probability to be derived from the developmentally plastic anadromous form common in the same region. Phenotype fixation can involve purely environmental change, due to an absence of conditions inducing an alternative form. So in this case showing fixation to involve genetic accommodation would require demonstrating reduced ability to switch to the marine form. But the polarity of the change is clear without a formal phylogeny.

Waddington's genetic assimilation can represent quantitative genetic change in the threshold for expression of a trait, moving it to a level where the trait is no longer expressed; or it can result from a mutation of major effect on regulation affecting a threshold (Waddington 1942, 1953). Such mutations of large effect make complex environmentally influenced human diseases like bipolar illness become 'genetic' or characteristic of families (West-Eberhard 2008a). Of the three examples of genetic assimilation described in detail by Scheiner and Levis (2021) in this volume, one involved a mutation (affecting a glycolipid layer on the heterocyst of a bacterium), while another (concerning spadefoot toad tadpoles) likely involved selection affecting standing (i.e., pre-existing) genetic variation. Schlichting (2021) cites reviews of genetic accommodation and describes several exemplary recent studies.

It is difficult to define when genetic accommodation would stop in changing environments. So it could be said that all quantitative genetic change in the dimensions or regulation of established traits that is mediated by natural or artificial selection represents genetic accommodation.

Clearly, then, genetic accommodation is nothing new. The fact that it fits readily with traditional ideas should give the idea a boost. But why give it a new name? First, giving genetic accommodation a new name draws attention to the special role of quantitative genetic change in the establishment of new qualitatively distinctive

(discrete) traits. Second, the term genetic accommodation helps distinguish it from *phenotypic* accommodation (*sensu* West-Eberhard 2003, 2005)—adaptive phenotypic adjustment in the absence of genetic change following a novel or extreme input during development. Both phenotypic accommodation and genetic accommodation can contribute to the establishment of novel traits even though these two processes may be phenotypically indistinguishable without experimental tests. Finally, a third reason to coin the term genetic accommodation is to emphasize that trait establishment need not imply fixation (assimilation) with a complete and permanent loss of plasticity. Many traits show durable adaptive plasticity in their condition-sensitive regulation. For example, the prolific adaptive radiations of African lake cichlids, Hawaiian drosophilids, and Galapagos finches illustrate how marked and durable plasticity in morphology, biochemistry, and learning, respectively, can facilitate rapid evolution in multiple directions (see Chapter 28 in *Developmental Plasticity and Evolution*). Furthermore, some lineages change repeatedly between genetic and environmental control: some of the figures cited above as indicating genetic assimilation show transitions to environmental determination of trait expression as well. A term-lover could invent a silly term, like 'genetic de-assimilation,' or 'genetic plastification,' to contrast genetic assimilation with evolution in the opposite direction. But it seems preferable to use a term like 'genetic accommodation' for both directions of change, emphasizing their similarity. Both involve genetic change that adjusts the degree of environmental influence on trait expression.

On genes as followers in adaptive evolution. According to Futuyma (2021), this "oft-quoted" statement from *Developmental Plasticity and Evolution* may have influenced "The critical reactions [to ideas regarding plasticity and evolution] of some traditional evolutionary biologists." The reactions of traditional evolutionary biologists are of interest for general discussions of phenotypic plasticity like those of this book. Intuition tells me that adverse reactions to genes-as-followers may reflect a strong conviction that genes take the lead in evolution. It also occurs to me that such biologists are unlikely to read books like the present volume that carefully examine an alternative view. The context of the genes-as-followers statement was to say that *if* developmental plasticity plays the role proposed for it in adaptive evolution, *then* genes are followers in adaptive evolution. Within that scheme, the majority of genetic change follows the origin of a developmentally reorganized phenotype: trait initiation is followed by selection and genetic change (genetic accommodation). Accordingly, genes could be leaders in adaptive evolution if a phenotype favored by selection originated due to a genetic mutation or a particular genetic configuration that happened to give rise to a favorable developmental novelty. Still, the majority of genetic change would likely be polygenic modification of the newly expressed trait, either increasing or decreasing the frequency of its expression.

Not all traditional evolutionary biologists have reacted negatively to ideas about developmental plasticity implying a somewhat altered view of the role of genes in evolution. Ernst Mayr (1904–2005) noted in correspondence (17 May 2003) that it is a point that "will sink in only slowly… I remember how daring I felt when in 1963 I bluntly stated 'the phenotype is the target of selection.' At that time we did not yet have the faintest notion how this plasticity was regulated." And (15 June 2004),

"historians have failed to report how gene-centered evolutionary biology was from the 1920, to the 1960s." Earlier Mayr (1991, p. 157) had identified the role of development in evolution as one of the frontiers of evolutionary biology "likely to see the greatest advances in the next ten or twenty years."

Is developmental plasticity universal? It is often said that plasticity is universal, or a universal property of living things (Chenard and Duckworth 2021; Pfennig 2021; Sultan 2021; see also Nijhout 2003). In one sense plasticity seems to be an "intrinsic property of organisms" (*sensu* Sultan 2021). It characterizes evolutionary genetics at its most fundamental level because all gene expression is condition dependent (see Schlichting 2021; see also Nijhout 1990): the genome is inert during its transmission between generations, to become important for development only when activated, a piece at a time, by particular developmental conditions.

Saying that all gene expression is condition-sensitive is just an updated way of saying what has long been axiomatic in evolutionary biology—that organismic traits are products of genes and the environment. But extreme statements regarding universality may invite needless debate, so let's just say that condition-sensitive—i.e., plastic—trait expression is extremely common. One reason for this is that plasticity, when advantageous, can be adjusted up or down under selection to an advantageous level that enables it to persist. That is a hypothesized role of genetic accommodation that sets it apart from genetic assimilation (West-Eberhard 2003), which eliminates, rather than maintains, phenotypic plasticity (see Scheiner and Levis 2021 in this volume). Ledón-Rettig and Ragsdale (2021) and Pfennig (2021) survey a multitude of ways in which condition-sensitive plastic responses get incorporated into development due to selection, helping to explain why plasticity is so common despite the fact that plasticity is not always advantageous and may be costly (Snell-Rood and Ehlman 2021). Plasticity builds upon itself, for it can use established developmental plasticity to yield new environmentally responsive traits via developmental reorganization when it is environmentally induced, a theme developed by Schlichting (2021).

Studies that compare plasticity in different taxa need to refer to particular traits. It is not meaningful to classify species or other taxa as plastic or non-plastic and then compare, say, their rates of speciation or diversification without reference to some specific aspect of their phenotypes. Such enumerative tests of the importance of plasticity may be tempting in the age of meta-analyses of the literature but they are meaningless unless they are explicit about the trait whose plasticity is being compared. It is also tempting to debate whether or not plasticity promotes evolutionary change, as if it were a question of always or never doing so. Plasticity *can* or *may* promote evolutionary change: it can contribute to the phenotypic variation required for organisms to change under selection (see Pfennig 2021; Schlichting 2021 in this volume). However, this does not mean that plasticity *always* promotes evolutionary change (as discussed by Pfennig 2021 in this volume).

The ability for plasticity to facilitate evolutionary change may also tempt thinking that it has evolved under selection for 'evolvability'—the ability to evolve. Many factors, just mentioned as documented in this book, contribute to the commonness of plasticity, obviating the need to seek an explanation in terms of selection above the individual level, as required by the evolvability hypothesis. Similarly, the universal modular aspect of what we call 'traits,' which also contributes to evolvability, should

not be regarded as a product of selection for evolvability per se. Instead, the universal modular (discrete) aspect of 'traits' is arguably due to the role of development in limiting connectedness at the time of trait origin (West-Eberhard 2019).

The present volume indicates that *Developmental Plasticity and Evolution* is fast becoming an antique. In another 10 years, following the usual cycle of amnesia, it will be forgotten, whether due to dismissal or assimilation. The data it cites will endure; I still consult my battered copy of Mayr 1963 to see the examples cited and how they were used. This brings to mind a passage about facts and theories ('views') found in my even more battered copy of Darwin's *The Descent of Man and Selection in Relation to Sex* (1871 [1874], p. 909). Darwin noted that facts endure even when false "but false views, if supported by some evidence, do little harm for every one takes a salutary pleasure in proving their falseness: and when this is done, one path towards error is closed and the road to truth is often at the same time opened."

I do not regard the present-day interest in developmental plasticity and evolution as an extension of the mid-20th-century synthesis as do some authors interested in developmental plasticity and evolution (e.g. see Pigliucci 2007; Laland et al. 2015). Instead it reaches back toward Darwin to rescue lost lines of thinking about the origins of selectable variation. Darwin's integrated view of development and evolution was either sidelined, as in the Synthesis, or actively suppressed, as in the Lysenko era of Russian genetics (Wake 1986; Berg 1988), where there had been a vibrant and broadly integrative evolutionary genetics. The charismatic geneticist Theodosius Dobzhansky, a leader of the Synthesis who was trained in the Russian school, might have imported increased interest in development into the thinking of his time. But that was not his passion. Dobzhansky did bring Schmalhausen's (1949 [1986]) book to the attention of English-speaking biologists, saying that "it supplies…an important missing link in the modern view of evolution" (Dobzhansky 1949 [1986]).

It is gratifying to see the chapters of this book by a diversity of leaders in thinking about plasticity and evolution. I found some of the chapters breathtaking as synthetic summaries of modern findings, full of original thoughts on topics that have interested me for so long. They pinpoint objections, evaluate them, concisely present the authors' latest ideas, and document consequences for evolution. Studies of plasticity bring developmental environments and phenotypes back toward the center of evolutionary biology, with an improved understanding of their relationship to evolutionary genetic change.

ACKNOWLEDGMENTS

I thank Jessica Eberhard, William Eberhard, and David Pfennig for helpful comments. Douglas Futuyma responded rapidly to some last-minute questions.

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Preface

In 1882—the last year of his life—Charles Darwin wrote a short Preface for the English translation of the book, *Studies in the Theory of Descent*. The author was the German biologist August Weismann, an early advocate of the theory of evolution by natural selection. In his book, Weismann sought to explain trait variation through a series of observations and experiments on butterflies and salamanders. He understood that without such variation there could be no natural selection, and without natural selection there could be none of the exquisitely adapted features that characterize living things. Weismann's studies led to an inescapable conclusion: an individual's environment could strongly influence its traits. Indeed, even during normal development, different environmental conditions could produce trait variation as pronounced as that typically seen between distinct species. Darwin was fascinated by Weismann's results. He wrote:

> *Several distinguished naturalists maintain with much confidence that organic beings tend to vary… independently of the conditions to which they and their progenitors have been exposed; whilst others maintain that all variation is due to such exposure, though the manner in which the environment acts is yet quite unknown. At the present time there is hardly any question in biology of more importance than this of the nature and cause of variability.*

Ironically, we still do not fully comprehend "the nature and cause of variability." It's true that two decades before Darwin wrote these words an obscure Moravian monk, Gregor Mendel, had deduced that parents pass to their offspring discrete factors that predictably influenced the traits of their offspring. However, these findings were ignored until they were rediscovered in 1900 (in what one geneticist referred to as "one of the strangest silences in the history of biology"). Shortly thereafter, the Danish biologist Wilhelm Johannsen gave a name to Mendel's factors: 'genes' (from the Greek word *genno*, "to give birth"). Johannsen also introduced the concepts of the 'genotype' (to refer to an organism's genetic makeup) and the 'phenotype' (to refer to an organism's observable characteristics) and in so doing stressed that the phenotype results from an interaction between genes and environment. Around the same time, the Swedish biologist Herman Nilsson-Ehle coined the term 'phenotypic plasticity' to describe the phenomenon that Weismann had observed—where a single individual can produce multiple phenotypes in direct response to different environmental circumstances.

Nevertheless, with the rediscovery of Mendel's work, embryologists and evolutionary biologists began to discount the environment in generating trait variation. Instead, genes became the dominant paradigm for explaining biodiversity. This gene-centric perspective characterized the 'Modern Synthesis' of evolutionary biology (the reconciliation in the 1930s and 1940s between Darwin's ideas and the emerging field of genetics) and was codified further in the so-called 'central dogma' of molecular biology—taught in every introductory biology class—which states

that information in biological systems flows exclusively from nucleic acids, not the environment. This gene-centric perspective has even permeated the public's thinking. For example, in his influential book, *The Selfish Gene*, Richard Dawkins (1976) argued that an organism's phenotype is merely a vessel for propagating its immortal genes. Similarly, in Ken Burns' 2020 film *The Gene: An Intimate History* (based on Siddhartha Mukherjee's 2016 masterful book by the same name), viewers are told that "it is this one tiny molecule, DNA, coiled up in the heart of all living cells, that is the script in which the book of life is written." In short, genes are often given omnipotent powers in dictating how living things develop and evolve.

Yet, as Weismann showed nearly a century and a half ago, genes alone do not determine an individual's phenotype. Genes therefore cannot represent the sole explanation for biodiversity. Indeed, we now know that all organisms have the ability to react to changes in their external environment by adjusting some aspect of their phenotype (as Weismann had observed), even if this aspect is some internal feature that would normally not be noticed by an outside observer, such as a change in the expression of a single gene. At the same time, however, we also know that phenotypic plasticity should not be thought of as 'non-genetic' change. In many organisms, different genotypes vary in whether and how they respond to any particular environmental cue. This suggests that phenotypic plasticity is typically underlain by genetic variation, which further implies that phenotypic plasticity itself can evolve. Indeed, we now know that the evolution of phenotypic plasticity can have important downstream consequences.

But how do we square these facts with modern biology, which continues to treat the phenotype as the actualization of the genotype? If the phenotype is instead the product of an individual's genes and its environment, what are the underlying mechanisms by which this gene by environment interaction produce different phenotypes? How do these mechanisms evolve and how do they, in turn, shape ecological and evolutionary processes? Can phenotypic plasticity actually impact evolution? If so, should the study of phenotypic plasticity alter how we think about evolution?

Such key questions form the bases for this book. To address these questions, this book brings together 21 researchers who study phenotypic plasticity from diverse perspectives to synthesize existing theory and data on the causes and consequences of phenotypic plasticity. In selecting the topics to cover, I sought to convey the excitement surrounding the study of phenotypic plasticity and to describe how phenotypic plasticity unites all of biology. However, the specific emphasis in this book is on the long-running controversies regarding phenotypic plasticity's role in evolution. Therefore, this meant that some vital topics were necessarily left out, such as the implications of phenotypic plasticity for understanding many human diseases as well as organismal responses to global change. Furthermore, the individual chapters are written to appeal to a broad array of readers, especially beginning graduate students in biology. Therefore, the chapters are meant to be as accessible as possible without sacrificing rigor. Finally, at the end of most chapters, the authors provide a text box in which they list key challenges for future research. The hope is that these will provide ideas for future researchers to follow up.

The book consists of four, interconnected sections. The first section introduces concepts, historical antecedents (including, importantly, Darwin's views), and key

questions about phenotypic plasticity that will be addressed throughout the book. As the authors describe, biologists have long recognized that phenotypic plasticity is an intrinsic property of living things; at the same time, biologists have long struggled to incorporate plasticity into their framework for understanding organismal development and evolution. The second section highlights what is known about the causes of phenotypic plasticity. The topics in this section range from the underlying genetic and physiological bases of plasticity to the role that ecology plays in molding the expression of plasticity, including the evolutionary loss of phenotypic plasticity. The third section focuses on the consequences of phenotypic plasticity: for both evolution and ecology. In this section, the authors describe how phenotypic plasticity can impact not only ecological and evolutionary processes occurring within populations, but how it might have impacted such large-scale macroevolutionary phenomena as the origins of multicellularity, including phenomena that might be detectable in the fossil record. Finally, the last section deals with the debates that have swirled around phenotypic plasticity and evolution for centuries but that have resurfaced recently. The goal of this section is to not simply rehash these controversies; rather, it is to explore areas where a consensus regarding phenotypic plasticity's role in evolution has been (or can be) reached.

I thank the many people who made this book possible, starting with the outstanding authors and reviewers of the individual chapters. I especially wish to thank Mary Jane West-Eberhard for preparing the Foreword on short notice. Additionally, I thank my students and colleagues who may not appear as authors in this book, but whose insights have helped shape my thinking about plasticity over the years. I am especially grateful to Nick Levis, Emily Harmon, Andrew Isdaner, and Pat Kelly for their keen insights. I also thank the participants in a National Science Foundation-funded working group that I co-organized in 2009–2010 (with Armin Moczek) at the National Evolutionary Synthesis Center in Durham, North Carolina, where the seeds of many of the ideas in this book were first planted. Some participants appear as authors in this book, but I would like to acknowledge the other members: Armin Moczek, Ehab Abouheif, Tammy Cruickshank, Ian Dworkin, Susan Foster, Fred Nijhout, and Matt Wund. For the funding to make open access of this book possible, I thank the National Science Foundation (and, especially, my program officer, Leslie Rissler) as well as the following authors: Sarah Diamond, Renée Duckworth, Ian Ehrenreich, Adrian Lister, Rick Michod, Erik Ragsdale, and Sonia Sultan. I am also grateful to Brian Hall and Sally Moody for inviting me to prepare this book and to Brian for his insightful comments on early drafts of the table of contents. I also wish to thank Chuck Crumly and Ana Lucia Eberhart of Taylor & Francis Group/CRC Press as well as the team at CodeMantra for their experience, patience, and encouragement in producing this volume. Finally, I thank Karin Pfennig for her continued guidance and unswerving support.

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Section I

Plasticity and Evolution

Concepts, Contexts, Questions

DOI: 10.1201/9780429343001-I

1 Phenotypic Plasticity as an Intrinsic Property of Organisms

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CONTENTS

1.1 INTRODUCTION

It is a familiar biological observation that the traits of an organism will vary to some extent depending on environmental conditions. This individual flexibility is termed 'phenotypic plasticity,' which can be defined as *the ability of a given genotype to express different phenotypes in different environmental circumstances.* This opening chapter of the book provides some essential conceptual and practical tools to engage with this remarkable property of organisms. Starting with a background section that lays out the key observations and ideas, the chapter goes on to examine biological and statistical aspects of genotype-environment interaction; experimental approaches to studying plasticity; and the recently emerged area of transgenerational plasticity, including some new insights regarding multi-generational environmental influences.

1.2 BACKGROUND AND KEY CONCEPTS

Over the past four decades, plastic responses have been documented across the phylogenetic spectrum—in bacteria, fungi, and lichens; algae and land plants; marine and freshwater invertebrates; insects, fish, amphibians, reptiles, and mammals—in response to contrasting states of a broad range of abiotic and biotic factors including temperature and humidity; concentration of O_2 and CO_2, pH, and other aspects of substrate and atmospheric chemistry; spectral quality, quantity, and diurnal pattern

DOI: 10.1201/9780429343001-1

of light; type and availability of food and other resources; population density and social interactions; presence and density of competitors, predators, herbivores, pathogens, or mutualists; even (for both animals and plants) vibration, touch, and acoustic stimuli (references in Sultan 2000, 2007, 2015; Gilbert 2001; Gilbert and Epel 2009; 2015).

Plasticity encompasses all aspects of the phenotype in which expression varies as a result of environmental differences. Depending on the type of organism and the environmental factor, this can include such traits as growth rate and body mass; size, relative allocation, number, shape, or anatomical structure of parts and organs; metabolic rates and other physiological processes; composition and quantity of defensive compounds and other secondary products; behavior; developmental and life-history timing; sex expression; and reproductive output (op. cit.). Correlations among traits may also vary plastically in magnitude or direction (Gebhardt and Stearns 1993; Brock and Weinig 2007; Fischer et al. 2016). Because plasticity causes variation in functional and fitness traits and in traits that contribute to all kinds of species interactions, it is now recognized to be of fundamental importance to both ecology and evolution.

Broadly different types of organisms are characterized by distinctive modes of plastic expression. Microorganisms exhibit characteristic aspects of plasticity such as cellular movement and stress-based aggregation in response to microscale conditions including substrate texture and resource distribution (Seymour et al. 2010; Rivera-Yoshida et al. 2019). As modular organisms, plants express developmental as well as physiological plasticity throughout the life cycle, continuously adjusting to changing conditions at the level of cells and tissues, and altering the morphology, structure, and number of organs produced via multiple meristematic growing points that can respond independently to environmental signals (Gilroy and Trewavas 2001; Trewavas 2015). By contrast, with some exceptions (such as sponges and corals) most animals have fixed body plans with relatively canalized structural traits, and tissue plasticity is generally more limited to juvenile stages (Walbot 1996; Atkinson and Thorndyke 2001). As a result, during adulthood animals may express plasticity largely via behavioral and physiological adjustments (Dufty et al. 2002). However, differences between animals and plants should not be overstated (Huey et al. 2002); even reptiles, birds, and mammals can undergo rapid, reversible changes, plastically altering such adult traits as gut morphology, musculature, and organ size in response to seasonal change or new biotic challenges (Piersma and Lindström 1997; e.g. Van den Hout et al. 2006). And although plants lack the integrated multi-trait responses to their environments made possible by the animal brain and associated neuroendocrine system, confluences and crosstalk among hormonally mediated signaling pathways allow for a surprising degree of response coordination at the level of the whole plant body, just as in animals (Coupe et al. 2006; Hodge 2009).

Researchers have been particularly fascinated by the many cases in which plastic responses are appropriate to the conditions that elicit them, suggesting that plasticity can be adaptive (see Pfennig 2021 in this volume). Familiar examples include the production of broad, thin leaves by plants in low light, the enlarged feeding structures produced by certain invertebrate larvae in response to low food concentration, and the varied structural and chemical defenses induced by predator and herbivore

attack. Yet plasticity is not always functionally adaptive. For instance, an animal or plant growing in resource-poor conditions will inevitably grow less and consequently produce fewer progeny. This would be considered *maladaptive* plasticity. At the same time, this resource-deprived individual may increase its relative biomass allocation to foraging organs or to reproduction, or adjust its rates of nutrient uptake or use, so as to partially compensate for these inevitable growth limits via adaptive aspects of plasticity. Phenotypes produced in response to stressful physical conditions may likewise reflect both inevitable limits and appropriate adjustments: in low temperature, for instance, developmental and metabolic rates will be unavoidably reduced, while plasticity for surface-to-volume ratio, thickness of insulating tissues, or body positioning may mediate the impact of low temperatures by maximizing heat conservation. Because phenotypic expression pathways are shaped by developmental and phylogenetic constraints as well as by natural selection, the phenotype an organism produces in a given environment will reflect both functionally adaptive and developmentally inevitable aspects of plastic response (Sultan and Stearns 2005).

Many authors have published definitions of plasticity, all of which are variations on a single key theme—phenotypic change in individual organisms that is associated with different environments. However, as with other fundamental biological concepts (for example, 'gene;' Fox Keller and Harel 2007; Portin and Wilkins 2017), there is a diversity of opinion about the precise definition of plasticity that reveals underlying tensions surrounding the term's meaning and scope. The definition given at the outset of this chapter—the ability of a given genotype to express different phenotypes in different environmental circumstances—has a long history and is quite widely used. Other definitions shift the ability to change from the genotype to the organism as a whole (e.g., Agrawal 2001). Both of these definitions emphasize that plasticity results from the genotype's or organism's *response to* its environment. A number of authors (such as Stearns 1989) instead define plastic phenotypic changes as *induced by* the environment, implying that the organism is more passive in this process. Although these alternatives suggest opposite directions of causation, they are in fact equally accurate, since plasticity results from the interaction of an organism with its environment (see next section). In her classic 2003 book, West-Eberhard provides a nuanced definition of plasticity that embraces this duality: "Condition-sensitive development *or* the ability of an organism to react to an environmental input with a change in form, state, movement, or rate of activity."

There is also variation in the way the term is applied. Certain authors may consider plasticity to be inherently adaptive (implicitly emphasizing the role of past selection in shaping plastic responses), though others (starting with Bradshaw's foundational 1965 paper) explicitly note that plasticity need not be adaptive, instead emphasizing developmental processes as such and their dependence on physical and chemical conditions. With respect to the scope of plasticity, the term may extend very broadly to encompass all aspects of phenotypic change that reflect an organism's experience (e.g., use and disuse of parts), or be more narrowly confined to "direct response to stimuli or inputs from the environment" (Pfennig 2021 in this volume). Most authors agree that plasticity "covers all types of environmentally induced phenotypic variation" (Stearns 1989). Those who instead use the term 'developmental plasticity' may do so in an equally inclusive way (e.g., West-Eberhard 2003) or may be (explicitly

or implicitly) limiting their scope to structural features and excluding behavioral, biochemical, and metabolic responses (e.g., Piersma and Drent 2003).

Although plasticity is an obvious feature of living systems, environmentally flexible outcomes fit awkwardly into the simplified view of phenotypic causation that dominated both developmental and evolutionary biology in the mid-to-late 20th century (Sultan 1992; Sarkar 2004; and references therein). In this 'gene-for-trait' model, a specific DNA sequence was understood to directly determine a particular phenotypic outcome, and plasticity was considered an odd exception to this rule that posed a 'problem' for evolutionary research (Gilbert 2001; e.g., Stebbins 1980; Bonner 1988). More recently, sophisticated molecular studies have led to the key recognition that environmental conditions participate in gene regulatory pathways, both through direct inputs and indirectly via physiological changes to internal/intracellular states (Lewontin 2000; Nijhout 2003; Schlichting and Smith 2002; Gottlieb 2004; Carroll et al. 2005; Lemos et al. 2008; Gilbert 2012, Stinchcombe et al. 2012; and references therein), resulting in "an incredible degree of plasticity in gene expression in response to diverse environmental conditions" (Wray et al. 2014). The fundamental insight that gene expression is environmentally sensitive provides our starting point: *plasticity is an intrinsic property of organisms.*

With this principle in mind, the central approach to studying plasticity is to characterize each genotype's 'norm of reaction': the set of phenotypes it produces across a given range of environments, for any trait of interest (Figure 1.1; for a historical overview, see Sarkar 2004). The norm of reaction documents a genotype's repertoire of potential outcomes; it specifies that every phenotype results from the genotype's expression in response to a particular environment.

Note that a genotype's responses may be either constant across a specified environmental range ('canalized'), or markedly different from one environmental state to another (whether continuously 'plastic' or discretely 'polyphenic'; Figure 1.1). It is

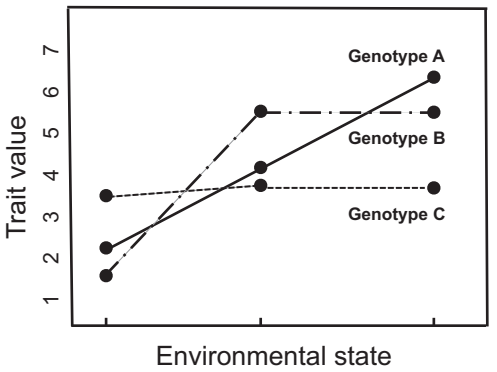

FIGURE 1.1 The norm of reaction. For every genotype, the phenotype for a trait of interest that is expressed in each specified environmental state is measured, and the points are joined to form a response curve (reaction norm). The reaction norm for a given trait may vary continuously (genotype A), consist of a few discrete alternatives (genotype B, polyphenism), or be relatively flat or canalized across the set of test environments (genotype C). (Modified from Sultan 2015.)

important to understand that these alternative patterns do not reflect genetic versus environmental control of the trait. In other words, 'plastic' and 'canalized' are terms that describe different patterns of variation across a certain range of environments—different norms of reaction—but not different biological causes (Sultan 2015). Whether the phenotypes produced by a genotype in alternative treatments are similar or different, each phenotype results from that genome's expression under particular conditions. Note too that a genotype cannot be characterized as entirely 'plastic' versus 'canalized.' For a given trait, the same genotype may have a plastic response to one set of environmental states but a canalized response to other factors or factor levels, and it will express plasticity in some traits but not others. In general, then, norms of reaction for a given species are specific to the genotype, the set of environments, and the trait in question.

Experimentally, plastic responses are determined by generating replicate individuals of each genotype via cloning or inbreeding, and raising the isogenic replicates in two or more different environments of interest, such as contrasting temperature, pH, or resource levels, or the presence versus absence of a biotic signal (such as a kairomone or plant volatile) or interactant (e.g., a competitor or predator; less precise experiments may instead use split sibships or genetically mixed samples from a given population or species as test groups instead of isogenic replicates; Sultan and Stearns 2005). This experimental strategy for characterizing genotypic reaction norms goes back over 75 years (e.g., Dobzhansky and Spassky 1944; Gupta and Lewontin 1982; for even earlier studies, see references in Sarkar 2004). The recently emerged discipline of 'ecological developmental biology' (or 'eco-devo') builds upon and expands this approach to include the underlying mechanisms as well as the outcomes of phenotypic expression in environmental context (Gilbert 2001; Sultan 2007; Gilbert and Epel 2009, 2015). Eco-devo studies examine how different possible environmental conditions may alter the signaling pathways that underlie development, physiology, behavior, and life-history. An eco-devo approach thus contrasts with the conventional study of development in a single—and often highly artificial—'control' environment (Gilbert 2001). Just like earlier norm of reaction experiments, eco-devo studies explicitly consider how genetic *and* environmental factors jointly determine phenotypes, starting at the level of gene expression. This recognition makes it clear that there is no neutral 'control' environment; an organism always develops in response to a specific set of conditions, even if those conditions consist of a petri plate on a lab bench.

1.3 GENOTYPE-ENVIRONMENT INTERACTION

These insights have crucial implications for understanding genetic diversity as well as development, and hence are of central relevance to evolutionary biology. Because DNA sequence differences affect signaling pathways for environmental perception and transduction as well as resulting impacts at the molecular and cellular levels, different genotypes in a given species will express characteristic norms of reaction; these distinct norms are rarely parallel to each other, since (as noted in Falconer's foundational *Introduction to Quantitative Genetics*) certain genotypes are "more sensitive than others to environmental differences" (Falconer 1989, p. 135; see also

Haldane 1946; Gillespie and Turelli 1989; Kruuk et al. 2008; Mackay et al. 2009; Moczek et al. 2011; Des Marais et al. 2013). In quantitative genetics, non-parallel reaction norms are described by the statistical term 'genotype-by-environment interaction' or *GxE* (Falconer 1960, 1989). *GxE* variation comprises the extent to which genotypes differ in their *patterns* of phenotypic change over a given environmental range; it provides the raw material for plastic response patterns themselves to evolve (Via and Lande 1985; Scheiner 1993, 2006; Kruuk et al. 2008; Baythavong 2011; Chevin et al. 2013; see also Goldstein and Ehrenreich 2021 in this volume). As with any aspect of genetic diversity, populations and taxa differ in patterns of *GxE* interaction variance as a result of previous selection, drift, and mutation (see references in Colautti et al. 2017).

Notice that as a result of *GxE* variation—that is, of non-parallel norms of reaction—both the size and the rank order of trait differences among a given set of genotypes may vary from one environmental state to another (as is illustrated by the hypothetical genotypes shown in Figure 1.1). Genotypes may express different phenotypes in certain environments and converge on similar phenotypes in others; a genotype may have high or low fitness relative to others only in certain conditions (Falconer 1960, 1989; Gupta and Lewontin 1982; Conner and Hartl 2004; Scheiner 2006; Van Buskirk and Steiner 2009; Colautti et al. 2017). To the extent that fitness-related traits reflect this pervasive aspect of genetic diversity, the amount and pattern of genetic variation available to natural selection will depend on environmental context (Via and Lande 1987; Barton and Turelli 1989; Falconer and Mackay 1996). Such context-dependent or conditional expression of genetic differences can alter the pace and/or the direction of selective trajectories (Snell-Rood et al. 2010; van Dyken and Wade 2010; Lédon-Rettig et al. 2014). This insight has particular resonance with respect to the altered environments being rapidly created by human activities, since a novel environment can either trigger a selective event in a population by revealing genotypic differences, or buffer selection if genotypes express similar phenotypes in that environment (Sultan 2007).

The statistical quantity genotype-environment interaction reflects a biological fact: environmental conditions influence a genotype's expression, so phenotypic outcomes result from the particular developmental interaction of these two factors (see Lewontin 2000; West-Eberhard 2003; Mackay et al. 2009; Gilbert and Epel 2009, 2015). Although as biologists we are trained to seek single causes or 'main effects,' when the cause of a certain outcome is an interaction between two (or more) factors, the individual factors cannot be analytically separated, because the effect of one factor depends on the level of the other (Sokal and Rohlf 1987; Dodge 2003). In this case, the effect of having a given genotype on an individual's phenotype depends on its environmental state, just as the phenotypic effect on an individual of being in a given environmental state depends on its genotype (Griffiths 2006; Lewontin 2006).

This point has important implications for experimental analysis. In a conventional development experiment, several genotypes of interest (for instance a 'mutant' and a 'wild type') are compared in a single test environment. With this design, any phenotypic differences (other than developmental noise) result from the differences between the genotypes. If genotypes are instead compared in two or more test environments (as in a plasticity experiment), both environment and genotype as well as

their interaction can be tested as potential sources of variation, using ANOVA or a variety of statistical approaches (Falconer 1960; Sokal and Rohlf 1987). Whenever norms of reaction are non-parallel (i.e., genotypes differ in the phenotypic change from one environmental treatment to another), the *biological* interaction that determines phenotypes will result in a *statistical* genotype-by-environment interaction term.

This changes how we analyze—and think about—genotype and environment as causal factors: in any dataset where the *GxE* interaction term is significant, the 'main effects' of genotype and environment cannot be estimated as distinct factors (Lewontin 2006; Vitzthum 2003). As biostatisticians Sokal and Rohlf (1987, p. 198) explain, "many statisticians would not even test [the two main effects] once they found the interaction mean square to be significant, since in such a case an overall statement for each factor would have little meaning." In other words, it is largely meaningless to assess the causal impact of genotype alone on a trait when that impact depends on environmental state, and vice versa.

Accordingly, in the presence of *GxE* variation, main effects that are tested offer only limited insight to trait variation. A main effect of genotype is most accurately interpreted simply as an indicator of an average effect across the study's environmental treatments, and likewise for a main effect of environment. Lack of a significant main effect does not mean that genotype or environment is unimportant. On the contrary, a significant *GxE* term means they do contribute significantly to variation, but in an interactive rather than a fixed, additive way. This is demonstrated in Figure 1.2, where despite the dramatically different phenotypes produced by two genotypes in alternative environments, neither main effect would be statistically significant.

When *GxE* is significant, just as it has "little meaning" to consider genotype and environment as separate phenotypic causes, estimating separate variance

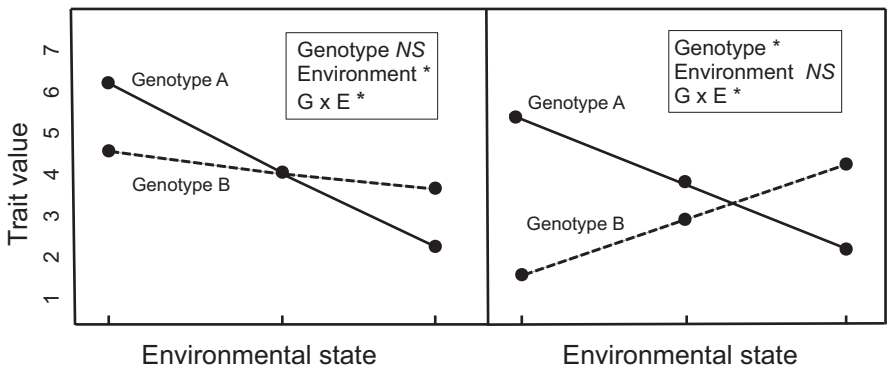

FIGURE 1.2 *GxE* interaction. Whenever norms of reaction are non-parallel (significant genotype-by-environment interaction term in ANOVA), genotype and environment cannot be meaningfully assessed as separate components of variance. This is illustrated here by two hypothetical datasets. On the left panel, there is no main effect of genotype even though the two genotypes clearly differ considerably; on the right, there is no main effect of environment despite a substantial impact of environmental state on trait value for each genotype.

components for these terms is also questionable (Lewontin 2006). A pragmatic solution is to include the interaction variance component within the variance component for one of the main effect terms (Sokal and Rohlf 1987, Falconer 1989). This solution is conceptually problematic, however, since a researcher could equally justify including the interaction as part of the genotype effect, or as part of the environment effect! An alternative that avoids these interpretive problems is to assess the relative magnitudes of the main (i.e., average) effects of genotype and environment and of their interaction as descriptive statistics for a particular dataset rather than explanatory or causal ones, by simply comparing the effect sizes (for example, based on the mean square variance in an ANOVA; R.C. Lewontin, pers. comm).

Because genotype and environment jointly determine individual phenotypes, this developmental interaction shapes the variation expressed in natural populations. In other words, "it is on the product of genotype and environmental influences that natural selection acts" (Nager et al. 2000), so genetic diversity alone does not determine a population's evolutionary potential. Whenever naturally occurring genotypes express *GxE* variation for relevant traits, fitness differences among genotypes—and hence the pace and outcome of selection—will depend on the distribution of environmental states in the field (Via and Lande 1985; Mitchell-Olds and Rutledge 1986; Scheiner 1993; Nager et al. 2000; Snell-Rood et al. 2010; van Dyken and Wade 2010; Lédon-Rettig et al. 2014; further references in Sultan 2015). For this reason, plasticity studies that characterize norms of reaction in response to field-based treatments (see next section) can most accurately test the evolutionary potential of real populations. Precise insight to *GxE* results can be gained through post hoc tests, such as linear contrasts following ANOVA to determine whether genotypes differ significantly within a specific treatment of interest. This kind of detailed analysis can address targeted questions regarding a population's evolutionary potential, for instance under predicted future temperature or CO_2 conditions (see Janes and Wayne 2006; Horgan-Kobelski et al. 2016) or when exposed to an introduced pathogen or other novel stressor.

1.4 STUDYING PLASTICITY: ENVIRONMENTAL CUES AND PHENOTYPIC RESPONSES

An organism's plastic expression—its norm of reaction—results from an underlying sequence of biological events. Depending on the organism's sensory and physiological systems, the individual perceives some aspect of the environment as a cue. This received signal is then transduced to initiate coordinated effects that lead to a characteristic phenotypic response, either by means of signaling molecules such as hormones and metabolites or by epigenetic modification (Dufty et al. 2002; Jaenisch and Bird 2003; Gottlieb 2004; Carroll et al. 2005; Cramer et al. 2011; Badeaux and Shi 2013; Lema and Kitano 2013; Morris and Mattick 2014; details and further references in Gilbert and Epel 2015; Sultan 2015). Such environmentally modulated regulatory pathways can directly alter physiology (for example by up-regulation of heat shock proteins; Queitsch et al. 2002) or can up- or down-regulate gene products including transcription factors and microRNA's so as to shape expression patterns of other genes and gene networks (Carroll et al. 2005; Cramer et al. 2011). In plasticity

experiments, the researcher manipulates cues and documents responses of interest, whether those responses are morphogenetic or life-history outcomes, short-term behaviors or altered metabolic rates, changes to signaling pathways at the biochemical level, epigenomic modifications, or resulting transcriptome impacts.

Interdisciplinary approaches that integrate these layered modes of plasticity can provide important insights into systems of cue perception, transduction, and phenotypic response. In the cichlid fish *Astatotilapia burtoni*, for instance, different social encounters cue males to rapidly and reversibly switch between a brightly colored, aggressively territorial 'dominant' phenotype and a cryptic, nonaggressive, and nonreproductive 'subordinate' phenotype (Burmeister et al. 2005). Underlying these alternative life-history and behavioral phenotypes are neuroendocrine pathways that up- or down-regulate approximately 5% of neural genes studied (Renn et al. 2008) and produce contrasting patterns of brain cell proliferation (Maruska et al. 2012).

A researcher's initial challenge is to identify and implement environmental treatments that will provide useful insight to the study organism's plastic responses. Here, the balance between experimental feasibility and realism is critical. The most informative design will recreate alternative conditions that elicit plastic responses in natural populations, yet do so with sufficient precision for results to be interpretable with respect to specific environmental cues (Miner et al. 2005; Groothuis and Taborsky 2015). This requires sufficient knowledge of the organism's biology and natural history to determine the environmental factors likely to be relevant and to choose naturalistic factor levels; plasticity studies that test easily manipulated but ecologically arbitrary treatments reveal little about the variability and potentially adaptive adjustments that might be expressed in real populations. Previous studies or pilot work with a given system can be invaluable in making these experimental choices.

Potential cues for an organism depend upon its evolved sensory and metabolic capacities—i.e., how it gathers information about its environment. In some cases, identifying a key cue for plastic response seems simple. For example, the amount of photosynthetically usable light is clearly a key component of the environment for plants. Yet more nuanced aspects of this seemingly straightforward environmental factor may substantially influence plant phenotypes, including the precise spectral distribution of available light (Smith 2000; Schlichting and Smith 2002; Franklin 2008; Ballaré 2009). With this awareness, a researcher might devise contrasting treatments that covary light intensity and spectral quality, for instance based on field measurements of alternative types of site or microsite that the species inhabits.

In other situations, the environmental cue that induces an organism's plastic response may be difficult to discern. The specific types of territorial interaction that cue the dramatic plastic responses of male *A. burtoni* cichlids described above provide a case in point. Complex cues have also been studied in several amphibian species known to express a crucial aspect of life-history plasticity. Tadpole larvae that occupy transient pools are well known to hasten their metamorphosis when these ponds begin to dry up, but it is not clear exactly how they perceive this impending change while they are still submerged. For one species of spadefoot toad (*Scaphiopus hammondii*), carefully designed lab studies were required to show that neither the higher water temperature nor the more frequent physical interactions with neighbors

that result from diminishing water volume served as cues to initiate this timing change. Instead, the operative signal to speed metamorphosis consists of two perceived changes: the metabolic feedback effect of restricted foraging in the reduced volume of water, combined with the visual cue of greater proximity to the water's surface (Denver et al. 1998). Note that closely related species may have evolved to utilize distinct plasticity cues even for very similar adaptive responses. For instance, in *S. couchii*, a congeneric spadefoot toad species with similar metamorphic plasticity, larvae sense the imminent drying of ephemeral pools through more frequent physical contact with other tadpoles as the water volume decreases (Newman 1994). These cues (as well as indirect indicators of other potential risks such as predator presence) are perceived through the animal's sensory systems and then transduced via the amphibian neurohormonal stress pathway to shape developmental responses (Denver 2009, 2013).

Having identified a key environmental factor that cues plasticity (whether or not its transduction system is fully known), designing treatments also requires knowledge of its patterns of temporal and spatial variation in the field. Rather than vary the factor mean in alternative fixed treatments, sometimes it is more ecologically meaningful to vary the range, timing, duration, or periodicity of environmental states (Miner and Vonesh 2004). For instance, both the amount and the diurnal distribution of light vary in temperate forests as a result of canopy structure, though the latter is tricky to vary experimentally. In a meticulous field study, Wayne and Bazzaz (1993) showed that birch seedlings given the same total, reduced daily amount of photosynthetically active sunlight expressed very different plastic responses to this shade depending on whether the light was provided at a consistent, moderate level throughout the day or (as in a forest gap) in the form of very low light with a brief interval at full intensity. Treatments that vary temporal patterns for key factors can also show how quickly individual organisms can plastically adjust relevant functional traits, revealing timing differences of potential adaptive relevance among genotypes or taxa (e.g., Sultan et al. 1998; Bell and Sultan 1999). Experimental treatments may also be designed to covary an environmental factor of interest with another aspect of the organism's environment (Gebhardt and Stearns 1993). Research on plastic expression in predicted future environments may test an organism's response to combinations of CO_2 concentration and temperature rather than to each factor alone (e.g., Miller et al. 2012). Such covariation designs can reveal important synergistic effects of factors that may co-occur. In general, field data on environmental variation will provide a robust basis for well-informed experimental design and for contextualizing the results (Miner et al. 2005).

As noted above, plastic responses may be studied at various levels of biological organization; a researcher's expertise and experimental goals will guide the choice of traits. An evolutionary, physiological, or behavioral ecologist might focus primarily on the expression of putatively adaptive plastic traits such as physiological adjustments, anatomical changes to tissues or organs, alternative life-histories or behaviors, and altered whole-body morphologies. As with environmental cues, knowledge of the organism's natural history is the essential guide to trait choice; environmental challenges known to vary temporally or spatially within populations of a given species can suggest candidate plasticity traits to measure. Plastic responses

may be considered as functionally adaptive based on statistical associations with fitness (phenotypic selection analysis), but because environmental conditions affect both trait and fitness values these analyses can be strongly biased (Stinchcombe et al. 2002; see Scheiner et al. 2002 for a path-analytical approach). In cases where plastic trait expression is irreversible or can be experimentally manipulated, fitness benefits can be tested by direct comparing alternative outcomes within each environmental treatment (Schmitt et al. 2003). However, in many well-studied cases of plasticity, trait changes are interpreted as adaptive based simply on ecological or functional considerations; examples include predator- and herbivore-induced structural and chemical defenses; thermoregulatory morphological and behavioral phenotypes that minimize heat and cold stress; and allocational or structural changes to tissues that maximize a limiting resource, such as increased gill surface area of fish in hypoxic water, longer root systems of plants growing in nutrient or moisture-limited substrates, and enlarged or specialized feeding structures that maximize access to available foods (references in Gilbert and Epel 2015; Sultan 2015).

In contrast to studies focused at the level of trait expression, biologists who study genomic, cellular, endocrine, and neural systems might instead seek to determine underlying mechanisms of ecologically relevant trait plasticity by tracking signal transduction pathways, epigenetic marks, or transcription changes. In addition to elucidating plastic cue and response systems as such, mechanistic studies can provide new evolutionary insights by revealing unexpected convergences, shared signaling pathways, and similar epigenetic dynamics among diverse organisms as well as differences that indicate phylogenetic constraint or distinct selective histories (Niederhuth et al. 2016; Adrian-Kalchhauser et al. 2020). Indeed, plasticity is an exceptionally rich area for collaborative investigations that combine traditionally distinct approaches to link molecular mechanisms with ecological and evolutionary consequences.

1.5 TRANSGENERATIONAL PLASTICITY AND MULTI-GENERATION NORMS OF REACTION: A MORE COMPLEX PICTURE

An even more complex picture of phenotypic causation emerges when we consider how environmental conditions encountered during one generation may influence the next. Inherited positive and negative effects of maternal environment ('maternal effects') have long been familiar (in part through the work of plant and animal breeders) as a substantial non-genetic source of phenotypic variation (Falconer 1989). These effects may be transmitted to offspring via changes to the amount and composition of nutritive tissues packed by maternal individuals into seeds or eggs; these 'provisioning' changes often directly reflect resource availability to parent individuals and lead to congruent increases or decreases in offspring size or growth rate (Haig and Westoby, 1988; Fenner and Thompson, 2005; Hafer et al. 2011; Uller et al. 2013; Baker et al. 2018).

A more recent insight is that offspring phenotypes may be influenced by parental conditions in highly specific ways beyond provisioning effects. The precisely cued responses of individual organisms to their environments may extend beyond immediate adjustments to their own development, life-history, and behavior to

include 'transgenerational plasticity'—trait-specific changes to offspring phenotypes induced by parental conditions (reviewed by Mousseau and Fox 1998; Agrawal et al. 1999; Uller 2008, 2013; Bonduriansky and Day 2009, 2018; Mousseau et al. 2009; Herman and Sultan 2011; Salinas et al. 2013; Donelson et al. 2018; see also Pfennig 2021 and Bonduriansky 2021 in this volume). Considerably more debate surrounds this aspect of plasticity, especially regarding its phenotypic impact beyond early life stages and hence its potential adaptive value and evolutionary role (Bossdorf et al. 2008; Badyaev and Uller 2009; Charlesworth et al. 2017; for theoretical investigations see references in Bonduriansky and Day 2018; Uller 2019). Fundamental questions also remain about the transmission mechanisms that underlie transgenerational plasticity, including whether heritable regulatory factors are environmentally induced or stochastic and how long such factors can persist across generations (Haig 2007; Jablonka and Raz 2009; Boyko and Kovalchuk 2011; Grossniklaus et al. 2013; van der Graaf et al. 2015). Below is a quick overview of this fascinating area of plasticity research (for further discussion, see Bonduriansky 2021 in this volume).

Adaptive transgenerational plasticity has been documented at the phenotypic level in a wide range of plant and animal taxa (e.g., Agrawal et al. 1999; Galloway and Etterson 2007; Herman et al. 2012; Miller et al. 2012; Shama and Wegner 2014). In these systems, parent individuals exposed to a particular abiotic or biotic stress altered offspring phenotypes in ways that specifically pre-adapted them to that stress. For instance, juveniles of the common reef fish *Acanthochromis polyacanthus* were able to acclimate to a stressful 3°C increase in water temperature (simulating future ocean conditions) if their parents had been exposed to elevated temperatures, due to heritable methylation changes in a number of oxygen-processing and metabolic genes (Ryu et al. 2018). Although in many cases the regulatory factors that mediate such adaptive transgenerational effects are not known, molecular studies point to several possible mechanisms. A specific parental environment may induce: (1) changes to heritably transmitted regulatory molecules such as hormones and small RNAs that are transferred into the embryo via maternal cytoplasm or sperm, and/ or (2) heritable maternal or paternal DNA or chromatin modifications that epigenetically 'mark' the offspring genome and alter its transcriptional activity (Soubry et al. 2014; Blake and Watson 2016; Quadrana and Colot 2016; Perez and Lehner 2019). Environmentally induced changes to DNA methylation state appear to be a particularly widespread mode of epigenetic change in plants and many animals that in some cases can persist for several descendent generations (Law and Jacobsen, 2010; He et al., 2011; Schubeler 2015; e.g., Dowen et al., 2012; Yu et al., 2013; Skinner, 2014; Colicchio et al. 2015; Baker et al. 2018). 'Adaptive transgenerational plasticity' is predicted to evolve when the parental environment reliably predicts the offspring environment, for instance in cases when offspring are likely to encounter the same specific stresses as the parent(s) (Agrawal et al., 1999; Galloway, 2005; Herman et al. 2014). Yet whether transgenerational effects induced by specific environmental stresses are a major source of adaptive variation across diverse natural systems remains to be robustly established (Uller et al. 2013).

What is already clear, however, is that environmental effects inherited from the previous generation (or possibly generations) can influence an organism's developmental response to its own environment: in other words, its norm of reaction (Miller

et al. 2012; Plaistow et al. 2015; Sultan 2019). For example, herbivore damage to parental plants can 'prime' offspring to express faster or heightened responses if they are attacked, or can alter their threshold for response induction (Holeski et al. 2012 and references therein). In anemonefish, mean juvenile growth rate was sharply reduced in elevated dissolved CO_2 compared with standard conditions, but when parents had been exposed to high CO_2 concentrations their progeny developed normally in both environments, resulting in flatter norms of reaction across CO_2 treatments (Miller et al. 2012). In mammals, parental stress or toxin exposure can lead to substantially different physiological and behavioral responses of juvenile and adult offspring to stresses they encounter (e.g. Crews et al. 2012; Gapp et al. 2014).

This point raises an important question about one of the main tenets of plasticity research. For over a century, the norm of reaction has been defined as a genotype's characteristic repertoire of responses to alternative conditions—"the expected phenotype of a given genotype as a function of the environment" (Chevin et al. 2010). This view guides both experimental design and evolutionary modeling. Yet if inherited environmental effects can alter the responses of a given genetic individual to its current conditions, the norm of reaction cannot be considered a fixed genotypic property. For example, seedling norms of reaction for *Polygonum* plant genotypes in response to sun and shade differ depending on whether their (maternal/paternal) parent had been grown in sun versus shade (Figure 1.3).

An alternative view is that the developing individual integrates environmental factors both past and present in genotype-specific ways, a perspective that suggests

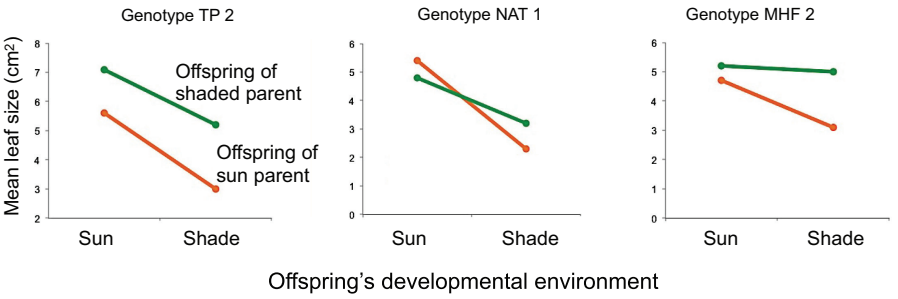

FIGURE 1.3 Transgenerational effects on the norm of reaction. Environmental conditions experienced during the parental generation can alter developmental responses of offspring to their own environments, causing changes in the genotype's norm of reaction. This is illustrated here with data for three highly inbred genotypes of the annual plant *Polygonum persicaria*. Replicate parent individuals of each genotype were grown in greenhouse sun and shade treatments, and their progeny were grown in sun and shade. Offspring of each genotype expressed two different norms of reaction depending on their parent's environment. The parent environment effect on offspring reaction norm was genotype-specific: in TP2 (left), the progeny of shaded parents made larger leaves in both offspring environments, while in MHF 2 (right), the effect of parental shade was significant only for offspring developing in shade, and in NAT 1 (center), parental shade slightly reduced leaf size of offspring grown in sun. Two norms of reaction are shown for each genotype based on means of ten replicates from each parental environment in each progeny treatment. (Modified from Sultan 2017)

several changes to research approaches (English et al. 2015; McNamara et al. 2016; Stein et al. 2018; Sultan 2019). Do transgenerational effects substantially alter norms of reaction, or can we continue to study plasticity as a genotypic property that is generally only slightly influenced by previous environments? Further studies testing multi-generation interactions between genotypes and environments in diverse biological systems are needed to resolve this central question about the causes of phenotypic variation.

1.6 CONCLUSIONS

The recognition that gene expression is environmentally sensitive has put an end once and for all to the misleading idea that genes and environment are alternative causes of phenotypic variation. Instead, it is now widely understood that plasticity—the variable expression of a given genotype in different environments—is an intrinsic property of organisms. As a result of both selective histories and evolved constraints, biological systems will differ characteristically with respect to the environmental signals that induce plastic responses; their transduction pathways; which traits are altered and precisely how; and the resulting context-dependent patterns of genetic variation. Box 1.1 provides some suggestions for future research. Investigating these dimensions of plasticity—either for a single taxon of interest or in a comparative framework—is key to understanding the phenotypic variation relevant to function and fitness, and hence to ecology and evolution.

BOX 1.1 SUGGESTIONS FOR FUTURE RESEARCH

- Identify the environmental factors and factor levels that act as plasticity cues for a given system. To predict variation patterns in natural populations, determine the distribution of the relevant environmental states within and among field sites or, in the case of transgenerational plasticity, from one generation to the next.
- Investigate precise pathways of environmental cue perception, transduction, trait expression changes, and (in the case of transgenerational effects) heritable transmission. This can be done through mechanistic studies of hormonal systems, metabolic feedbacks, or epigenetic modifications.
- Test the adaptive or maladaptive consequences of specific plastic responses in the context of environmental distributions, including predicted future conditions. This can clarify the short-term adaptive impact of both within-generation and transgenerational plasticity, a critical question with regard to rapid adaptive ‘rescue’ of populations under human-altered conditions.
- Assess future evolutionary potential by determining patterns of genotype-by-environment interaction variance for genotypes from natural populations, rather than quantifying allelic (sequence) variation per se.

- Examine (both empirically and theoretically) how multi-generation *GxE* interaction (i.e., environmental influences of two or more successive generations on fitness-related variation) may affect the process and strength of natural selection. Multi-generation studies may also provide exciting new ideas about how organisms integrate genetic factors, current environment, and previous environment to guide real-time phenotypic expression, and how these regulatory integration systems themselves evolve.
- For any of the above approaches, studies focusing on a species of interest can be valuably expanded by comparing populations, congeners or other close relatives, or different taxa from the same ecological community.

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2 There Is Hardly Any Question in Biology of More Importance: Charles Darwin and the Nature of Variation

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2.1 INTRODUCTION

In the earliest known correspondence between Charles Darwin and August Weismann, dated the 22nd of October 1868, the English naturalist thanked his younger German colleague for the intended gift of his "Kleine Schrift"—a printed copy of Weismann's inaugural lecture at Freiburg University—but regretted that the gift appeared to be lost in the post (DCP-LETT-6427; *Correspondence*, 16(2): 808). Darwin was sent another copy of *Über die Berechtigung der Darwin'schen Theorie* ("On the Validity of the Darwinian Theory") and found a kindred spirit, impressed with both Weismann's defense of evolution and his appreciation of the important

DOI: 10.1201/9780429343001-2

outstanding questions. Perhaps foremost among these was the nature of variation, something that Weismann later explored in coelenterates and butterflies, among other organisms. Variation, he declared in his 1868 lecture, represents the "reaction" of the organism to environmental influences, the outcome of which depends "on the quality of the external influences and the nature of the individual" (Weismann 1868, p. 27). This is something that Darwin and many other naturalists at the time agreed with: environment played an intimate role in both stimulating and ultimately selecting variation—phenotypic or trait variation, since for Darwin variation meant variation in characters, great and small, internal and external. But what was variation's source? Was all variation qualitatively the same? What "laws" governed its expression and its transmission? Twelve years later, Darwin addressed the ongoing debate over the nature of variation in his Preface to the English translation of Weismann's *Studies in the Theory of Descent:* "Several distinguished naturalists maintain with much confidence that organic beings tend to vary and to rise in the scale, independently of the conditions to which they and their progenitors have been exposed; whilst others maintain that all variation is due to such exposure, though the manner in which the environment acts is as yet quite unknown." "At the present time," he concluded, "there is hardly any question in biology of more importance than this of the nature and causes of variability" (Weismann 1882, p. vi). Indeed, understanding the elusive "nature and causes of variability" was central to any understanding of heredity, reproduction, and that "mystery of mysteries," the origin of species.

Poignantly, 1882 was also the year of Darwin's death. His comment in the Preface to Weismann's book was his final word in print on the subject, concluding decades of speculation and analysis as a "lifelong generation theorist," as historian Jonathan Hodge (1985) aptly described him. "Generation" for Darwin initially meant reproduction and embryological development—as in, generation of new life—but over time he extended the concept to transmutation and the ever-ramifying tree of life, as he recognized that processes at the level of organismal reproduction and development ultimately bear on the evolutionary process. Indeed, from the time of his earliest species speculations in the late 1830s Darwin recognized that the nature of variation, its origin, transmission, and expression, was central to "generation" and thus to evolution. Over the ensuing 40-plus years he elucidated principles and constructed elaborate hypotheses to make sense of the vast and often conflicting empirical data on the subject. Finding signal in the noise of trait expression when the hereditary process was largely a black box was exceedingly difficult, and Darwin's prefatory comment suggests that he left the determination of inheritance very nearly where he found it, to paraphrase Richard Owen in a critique of Darwin on species (Owen 1860, p. 494).

* * *

To Darwin and his contemporaries, variation was variation. There was no genotype-phenotype distinction, and trait variation evident in morphology, physiology, behavior, or development was presumed to be, by and large, heritable. Similarly, there was no essential difference recognized between what we now term phenotypic plasticity—the capacity to alter phenotype in direct response to environmental variation—and any other expression of variation. Indeed, plasticity per se was not understood until the early 20th century, and even then it took many years to be

studied as such, and recognized as a widespread phenomenon (see Futuyma 2021 in this volume). But while we don't always know the proximate source or cause of the variation that Darwin was talking about, there are clearly cases where he seemed to be discussing environmentally induced phenotypic variation (plasticity).

Darwin's life-long grappling with variation and inheritance is instructive—in particular, his appreciation of environment as an integral component in the generation and expression of variation. It bears pointing out that Darwin and Wallace both recognized that evolution by natural selection requires differential reproduction, which, in turn, requires not simply variation, where individuals differ in some of their characteristics, but *heritable* variation. Inheritance, where parents pass on some of their characteristics to their offspring, is central to the process. This is why understanding the nature and causes of variation was so important to Darwin (and why the first two chapters of the *Origin* were on variation)—and, therefore, why it remains so important today. In this chapter I discuss Darwin as "generation theorist" making sense of variation and inheritance through his career. Along the way I consider Darwin's interests and inspirations in this subject, and explore intersections and departures of his thinking in relation to that of his contemporaries up to the modern period. By necessity my treatment provides only a general overview; readers interested in more in-depth analyses of Darwin on variation and inheritance should consult Bowler (1974), Deichmann (2010), Endersby (2003), Hodge (1985, 2003), Kohn (1980), Olby (1963, 2009), Provine (2001), Sloan (1985, 1986), Vorzimmer (1963), and Winther (2000).

2.2 OF GRANTIAN TRANSFORMISM AND HENSLOVIAN VARIATION

Darwin was a fairly average undergraduate, first at the University of Edinburgh and then Cambridge, by all indications orthodox in his thinking and solid if unremarkable in aptitude. But two aspects of his education are noteworthy in their bearing on his later interest in trait variation and generation. The first is Darwin's passion for beetle collecting as a college student. This consuming interest may seem trivial, but later in life Alfred Russel Wallace (Figure 2.2), co-discoverer with Darwin of evolution by natural selection, pinpointed this as the key trait the two held in common that led them to their joint discovery:

> *First (and most important, as I believe), in early life both Darwin and myself became ardent beetle-hunters. Now there is certainly no group of organisms that so impresses the collector by the almost infinite number of its specific forms, the endless modifications of structure, shape, colour, and surface-markings that distinguish them from each other, and their innumerable adaptations to diverse environments.*
>
> Wallace (1909, p. 8)

Wallace went on describe their passion as "an intense interest in the mere *variety* of living things—the variety that catches the eye of the observer even among those which are very much alike, but which are soon found to differ in several distinct characters." This appreciation for the seemingly endless natural variation of

beetles—the single most species-rich animal taxon—dovetailed with the second noteworthy aspect of Darwin's education, namely his introduction to the burning questions of philosophical naturalism of the day: the nature of the earth and its history, species and varieties, diversity and its geographical distribution, the nature of life itself, and reproduction.

Darwin's introduction to philosophical naturalism began at the University of Edinburgh, where as a medical student he was introduced to the heterodox thinking of Robert Edmond Grant, professor of comparative anatomy whose research focused on marine invertebrates (Figure 2.1). Grant was a "transformist" of the French school, having engaged with the ideas of Jean Baptiste de Lamarck in Paris, studying "philosophical anatomy" under Lamarck's champion Étienne Geoffroy Saint-Hilaire—transformism, or transmutation, being terms of the period for what we call evolution. Grant also admired the famous physician, poet, and speculative philosopher Erasmus Darwin (Figure 2.1), Charles' grandfather, himself an admirer of Lamarck and who promoted transmutational ideas in verse and prose, notably in *Zoonomia* (1794) and *The Temple of Nature* (1803) (Browne 1995, p. 83). Although by the 1820s Erasmus Darwin's poetry, politics, and radical science were decidedly out of favor, his grandson was well aware of his transformist thinking, with reproduction at its heart.

Lamarck (Figure 2.1) developed this philosophical system in three notable works: *Système des animaux sans vertèbres* (A System of Invertebrate Animals, 1801), *Recherches sur l'organisation des corps vivans* (Researches into the Organization of Living Bodies, 1802), and his magnum opus, the *Philosophie zoologique* (Zoological Philosophy, 1809) (see Burkhardt 2013; Turner 2013). Like virtually all thinkers on the subject since antiquity, Lamarck and his followers held to a concept of blending inheritance, the widely held idea that elements of the parents fully and irreversibly mix in the process of reproduction. But this was combined with the interplay of two forces or processes that complicated structure (transmutation) over time: an inherent organizing force (*pouvoir de la vie*) that increases complexity, and an adaptive force (*l'influence des circonstances*) that allows organisms to respond to environmental conditions in an adaptive manner. Between these, species acquired modifications over the generations, slowly transforming in morphology and physiology to adapt to their environmental circumstances. In a sense some variation was thus directed, from within, while most inter-individual or populational variation was noise, unimportant flaws, or developmental fluctuation about the type. The net effect of the accumulation of directed variation is progressive change, corresponding to a "chain of being," the hierarchical arrangement of species along a scale of supposed complexity, one form transmutating into another. With microscopic life constantly generating spontaneously as the source of the chain, myriad parallel lineages of progressively complex species form over time, eventually giving rise to the taxonomic hierarchy seen today from "monad to man" (Burkhardt 1977; Ruse 1996; Bowler 2003). Lamarck is often associated almost solely with the inheritance of acquired characters in transmutation today, but this was but a minor element of his thinking—it should be noted that inheritance of acquired characters was a widely held idea at the time (including by Charles Darwin, as will be evident in this chapter), and one that Lamarck took no creit for originating (Burkhardt 2013).

Lamarck's ideas were highly influential in the more progressive intellectual circles of Paris, Edinburgh, and London; in Britain even Charles Lyell, who was to become the leading opponent of transmutation as epitomized by Lamarck, its standard-bearer, was initially enamored (Corsi 1978; Desmond 1989). But the more materialistic elements of Lamarck's ideas in particular led to opposition on religious grounds by some in France as well as Britain, and by the time Charles Darwin arrived in Edinburgh in 1825 there were few adherents, at least openly. Even Robert Grant was circumspect, and Darwin was perhaps the first person to whom he revealed the extent of his transformist leanings, going even further than Lamarck and Erasmus Darwin in advocating for a fundamental link between plants and animals—best

FIGURE 2.1 (a) Jean-Baptiste de Lamarck (1744–1829), an inspiration to (b) Erasmus Darwin (1731–1802) and (c) Robert Edmond Grant (1793–1874), held that internal "complexifying" and "adaptive" forces of organisms led to variation in response to environmental challenges and transmutation. (d) Rev. John Stevens Henslow (1796–1861), Charles Darwin's esteemed botany professor, explored the limits of variation in the context of species fixity.

(Image credits: J.-B. de Lamarck: Stipple engraving by A. Tardieu (1821), after J. Boilly. Courtesy of Wellcome Library, image no. 5219i. E. Darwin: portrait after Joseph Wright of Derby (1770s). Courtesy of Birmingham Museum and Art Gallery, Wikimedia Commons. R. E. Grant: portrait by T. H. Maguire (1852), courtesy of Wellcome Collection, Attribution 4.0 International (CC BY 4.0). J. S. Henslow: portrait by T. H. Maguire (1851). Courtesy of U.S. National Library of Medicine Digital Collections, image no. B014334.)

exemplified by the "zoophytes," or animal-plants, a name he coined for certain marine invertebrates (now recognized as polyzoans).

Darwin's work with Grant certainly piqued his interest in the reproduction of zoophytes and their relatives. This became the subject of Darwin's first scientific paper, to a local scientific society, and it was working with Grant that he was introduced to the term "gemmae" or "gemmules," used by Grant and others to describe mysterious motile reproductive "ova" in *Flustra* and other marine invertebrates (e.g., Grant 1827, p. 110). But Grantian transformism was far from Darwin's mind in the 1820s, and he did not accept the idea of transmutation or Grant's argument for the union of the plant and animal kingdoms. He would return to these ideas a decade later and see them in a new light, but at the time his orthodoxy was reinforced on his move to Cambridge in 1829, having resolved that a medical career was not for him. At Cambridge Darwin came under the tutelage of the botanist Rev. John Stevens Henslow (Figure 2.1) who, while no transformist, introduced Darwin to philosophical questions about species and varieties and the limits of variation, albeit from the perspective of species fixity.

Kohn et al. (2005) showed that Henslow, apparently uniquely among botanists of the time, was keenly focused on individual variation in plants, an appreciation impressed upon Darwin—the pupil that spent so much time assisting the professor that he became known as "the man who walks with Henslow." Henslow's herbarium teaching sheets often featured multiple specimens of phenotypically variable species mounted side by side for direct comparison—some cases undoubtedly reflecting phenotypic plasticity, others populational variation, but these distinctions were not made at the time. The project related to the pressing question of the variational limits of species, and surely resonated with Darwin's ardent beetle-collecting going on at the same time. But if beetles exemplified abundant species variation to Darwin, Henslow's treatment of botany presented more philosophical questions about variation. The extent and manifestation of *trait* variation was a central issue in the debates surrounding transmutation at the time: variation was manifest, yes, but must have its limits if species are permanent, fixed entities with no possibility of change. What were the limits? Henslow (1830) further investigated this question experimentally, confirming the conclusion of renowned plant breeder and clergyman Rev. William Herbert, for example, showing that primrose, oxlip, cowslip, and polyanthus—all very distinct from one another—are but "local varieties depending on soil and situation" (Herbert 1822, p. 19). Henslow's lessons from such investigations into species, varieties, and variation were underscored and reinforced for Darwin in his subsequent reading of Charles Lyell's *Principles of Geology* while on the *Beagle* voyage a few years later.

The second (1832) volume of *Principles* included perhaps the most damning critique of transmutation of the day, including the argument that species could only vary so far, denying that variability could continue in any one direction long enough to produce transmutation. Lyell cited Herbert, Henslow, and others in an argument for the immutability of species based on the development of horticultural varieties, which to Lyell demonstrated not only the inherent variability and plasticity of plants of a single species under cultivation, but how that variability can mislead naturalists into concluding that they were witnessing the development of new species when in fact these were only varieties fluctuating about a permanent form or type.

Another compelling case Lyell presented was that of the common hydrangea: "Some curious experiments recently made on the production of blue instead of red flowers in the Hydrangea hortensis, illustrate the immediate effect of certain soils on the colours of the petals" (Lyell 1832, 2: 34). This species, popular today in landscaping and gardening, provided Lyell with a dramatic example of environment inducing a striking color variation. Some unknown qualities of soil induced variation in a trait, and cases where environment induced many variations simultaneously still represent limited variation about a fixed species "type."

Darwin and Wallace were much later to argue that variation is virtually unlimited, and proposed a mechanism—natural selection—by which heritable variation could be "accumulated" over generations and lead to lineage divergence. Rather than serving to delineate the bounds of species and varieties, to Darwin and Wallace trait variation reflected divergence in action. So-called "doubtful forms"—confusing species boundaries, the bane of botanists' identification manuals—became evidence not for the limits of variation, but for transmutation (Costa 2014, pp. 135–139, Appendix 3). Heritable trait variation was thus the stuff of transmutational change for Darwin and Wallace, but prior to the development of this theory, Darwin considered another approach to understanding trait variation and the variability of species and varieties.

2.3 *GENERATION HERE SEEMS A MEANS TO VARY…*

At Henslow's recommendation, right out of Cambridge Darwin ultimately joined the expedition of HMS *Beagle* on a worldwide naval voyage, where, as a gentleman companion to the Captain and *de facto* naturalist, he was encouraged to conduct broad-ranging investigations and make extensive biological and geological collections. Long stretches at sea lent themselves to the study of marine invertebrates, where Darwin was once again struck by the intricate structure and reproduction of zoophytes and corallines (Keynes 2000). And he contemplated the nature of species and varieties, notably on the return trip home while trying to make sense of his Galápagos Islands collections (Hodge 2010):

> *When I recollect, the fact that [from] the form of the body, shape of scales & general size, the Spaniards can at once pronounce, from which Island any Tortoise may have been brought. When I see these Islands in sight of each other, & possessed of but a scanty stock of animals, tenanted by these [mockingbirds], but slightly differing in structure & filling the same place in Nature, I must suspect they are only varieties…If there is the slightest foundation for these remarks the zoology of Archipelagoes will be well worth examining; for such facts would undermine the stability of Species.*
>
> Barlow (1963, pp. 261–262)

When Darwin became a committed transmutationist some 6 months following his return from the *Beagle* voyage (Sulloway 1982), making sense of such variation loomed large. In the fruitful period of 1837–1838, in which he initiated the series of private notebooks on the "species question" and its implications (now known as the "transmutation notebooks;" Barrett et al. 1987), it is no coincidence that

reproduction and variation are central to his initial theorizing. The term "variation" and cognates (variety, varieties, vary) appear some 350 times in the transmutation notebooks, "generation" another 93 (Weinshank et al. 1990). Significantly, the B Notebook opens with five pages of notes on the key section "Of Generation" in his grandfather's treatise *Zoonomia* (Darwin 1794–1796, pp. 478–533). In what may be considered Darwin's first theory of transmutation, prior to his discovery of natural selection, he posited that sexual reproduction is itself the driver of evolutionary change (Kohn 1980, pp. 81–87). In these notes "generation" is synonymous with sexual reproduction, and he contrasts this with asexual reproduction, as by budding or cuttings. New individuals produced asexually are "constant," while sexual reproduction "here seems a means to vary or adaptation" [sic]. In other words, sexual reproduction is the means by which variation is generated—permanent, adaptive, heritable variation, which he also states is directly induced by the environment: "We know world subject to cycle of change, temperature & all circumstances which influence living beings—We see the young of living beings, become permanently changed or subject to variety, according to circumstance—seeds of plants sown in rich soil, many kinds, are produced though new individuals produced by buds are constant." Environmental change is thus directly connected to adaptation and transmutational change: "therefore generation [sexual reproduction] to adapt & alter the race to *changing* world" (B2–B4; Barrett et al. 1987, p. 171; emphasis Darwin's).

At this stage of Darwin's thinking, in July 1837, sexual reproduction was thus intended to be an explanation for organic change, in particular gradual, linear, Lamarckian evolution (Kohn 1980). He did not discuss *how* he thought sexual reproduction engendered variation, but he was already beginning to formulate the only obvious model for transmitting hereditary information consistent with blending inheritance: he posited that reproductive material of some kind, derived from throughout the body (perhaps circulated in the blood) concentrate in the reproductive organs. Here we see the gemmules, so to speak, of one aspect of Darwin's later Pangenesis theory (although at that time he saw a fundamental distinction between the powers inherent in sexual "germs" versus asexual buds). Indeed, in the 3rd edition of *Zoonomia*, published in 1801, Erasmus Darwin postulated precisely such a process, anticipating and perhaps inspiring his grandson's theory (discussed in Section 2.5).

BOX 2.1 ERASMUS DARWIN'S THEORY OF GENERATION

Excerpt from Section XXXIX (On Generation), Part 9.1 of Erasmus Darwin's *Zoonomia* (3rd edition, 1801, pp. 296–297). Such mechanistic and particulate theories of inheritance, with the central concept of derivative germ cells—information "particles" of some kind—from tissues and organs throughout the body gathered and communicated through the reproductive organs, were commonplace since antiquity. Some theories were remarkably similar to Darwin's Pangenesis, notably that of Herbert Spencer, which was likely an inspiration for Darwin's theory (Zirkle 1946, Geison 1969).

> IX. 1. The foregoing remarks on vegetable generation are chiefly transcribed from my work on Phytologia, Sect. VII. and may be applied to animal reproduction; since from this analogy to the lateral propagation of vegetable buds, if we suppose, that redundant fibrils with formative appetencies are produced by, or detached from, various parts of the male animal, and circulating in his blood, are secreted by adapted glands, and constitute the seminal fluid; and that redundant molecules with formative aptitudes or propensities are produced by, or detached from, various parts of the female, and circulating in her blood, are secreted by adapted glands, and form a reservoir in the ovary; and finally that when these formative fibrils, and formative molecules, become mixed together in the uterus, that they coalesce or embrace each other, and form different parts of the new embryon, as in the cicatricula of the impregnated egg; we may more readily comprehend some circumstances, which are difficult to understand on any other system of generation.

The transmutation notebooks contain many entries concerning environmentally induced variation, blending, and adaptation. In August of 1838, prior to his discovery of the principle of natural selection, Darwin stepped back and marveled at the grand vision of physical environment inducing variation, adaptation, and endless species change:

> *What a magnificent view one can take of the world…cause changes in geography & changes of climate superadded to change of climate from physical causes—these superinduce changes of form in the organic world, as adaptation. & these changing affect each other, & their bodies, by certain laws of harmony keep perfect in these themselves—instincts alter, reason is formed, & the world peopled [with Myriads of distinct forms] from a period short of eternity to the present time, to the future—How far grander than idea from cramped imagination that God created.*
>
> D36–37; Barrett et al. (1987, pp. 342–343)

Following his discovery of natural selection in the fall of 1838 Darwin saw variation in a new light. He abandoned his "sexual theory" of transmutation when he realized that asexual reproduction can also yield considerable variability, and while he formerly thought of most variation as adaptive, in light of natural selection he recognized that much of it is not, and that selection sorts out adaptive and non- (and mal-) adaptive variation. Developing the now-famous metaphor of the wedges (later given in *On the Origin of Species*, p. 67), he imagined natural selection like a hammer striking so many wedges (species, varieties, variations) into a yielding surface of only so much space (ecology, environment): "One may say there is a force like a hundred thousand wedges trying force into every kind of adapted structure into the gaps ‹of› in the oeconomy of nature, or rather forming gaps by thrusting out weaker ones [sic]," where "the final cause of all this wedgings [sic], must be to sort out proper structure and adapt it to change" (D135; Barrett et al. 1987, pp. 375–376). Natural selection required an abundance of heritable variation. Where did it come from?

2.4 THE ENVIRONMENTAL BASIS OF TRAIT VARIATION

2.4.1 From *Essay* to *Origin*

Environment, in a broad sense, was the ultimate source of all variation for Darwin, and he tended to see most variation as heritable—again, there was no genotype/phenotype distinction. In Darwin's 1842 "Sketch" of his theory, and in greater detail in the "Essay" that followed in 1844 (Darwin 1909) — a 200-page forerunner to *On the Origin of Species*—Darwin distinguished between the "direct" cause of variation from known and unknown external agencies and the "indirect" cause from what he called the "laws of embryonic growth" and, most importantly, of reproduction. The reproductive process still played a central role in association with environment in engendering variation. That variation cannot stem from external conditions alone is clear from what might be termed the "peas in a pod" problem: plants grown up from seeds developing in the same pod or capsule (or pups in the same litter) vary, yet they were clearly exposed to precisely the same parentage and environmental conditions in development.

Domesticated varieties and breeds presented Darwin with a powerful analogy for understanding the generation of natural species and varieties by selection (Secord 1981; Bartley 1992), beginning with the manner in which variations are generated by perturbation of the reproductive system in the domestication process — which entails exposing the organisms to new conditions of life from climate to food. "Probably the indirect effects of domestication in making the organization plastic," he wrote in 1844, "is a much more efficient source of variation than any direct effect which external causes may have…" (Darwin 1909, p. 63)—an argument repeated in the *On the Origin of Species* 15 years later (Darwin 1859, p. 8). Note his use of the term "plastic" in much the same way that we use it in evolutionary biology today: changeable trait expression. He also generalized from domestication to natural species, maintaining that in both cases exposure to altered conditions generates heritable variation. For domesticates, altered conditions include exposure to (being brought to and bred in) new environments, and abundant food. For natural species it is exposure to altered conditions over longer timespans, as by geological and climatic change. But for Darwin "altered conditions" could be internal as well: inter-breeding of individuals of different varieties also stimulated variation, owing to the sensitivity of the reproductive system to "any change in the condition of life," as he put it in the *Origin* (1859, p. 273). This helped explain how hybrids (F1s in modern terms) tended to show far less variability than second-generation offspring (F2s).

Darwin thus initially distinguished between two forms of environmentally induced variation: variation stemming from environment acting on the reproductive system he termed "indirect" variation, because it is expressed in the individual's offspring, in the next generation. He referred to such variation as "individual differences"—ubiquitous, minute, continuous populational variation. In contrast, rarer, discontinuous, "single variations," also referred to as sports or monstrosities, pop up in some individuals here and there; this is "direct" variation, because it is expressed in the organism immediately, in the current generation. Winther (2000) suggested that Darwin's indirect and direct forms of variation can be thought of as "germinally-induced" (acting on the reproductive system) and "somatically-induced" (acting on

a given body part or trait), respectively. Of the two, Darwin held that "indirect" variation or "individual difference" was by far the most important form of variation for transmutation—the abundant, minute, and indeed limitless quality of "indirect" variation was more consistent with his view of evolutionary change as a slow, gradual, and continual process (see Darwin 1909, pp. 108–111).

Bowler (1974) argued that Darwin further envisioned a third category of variation, intermediate between these first two. These can be thought of as "small sports"—greater in magnitude than the small-scale ("insensible") individual variations but smaller than the larger-scale sports. This third way of envisioning variation is developed in the *Origin*. Continuously distributed "individual differences" are there too, but the small-sport version of what Darwin called "single variations"—somewhat discontinuous, rare, favorable variants—take center stage. Although Darwin could be vague about whether he thought favorable variants were rare or relatively common, several passages in the *Origin* trend toward rarity, as Bowler (1974) points out. For example, describing how individuals with useful variations "should sometimes occur in the course of thousands of generations" (Darwin 1859, p. 80) implies both extreme rarity and that it is the occasional *individual* that is favored—neither altogether consistent with mere insensible, continuous, populational variation. Wallace took Darwin to task over this view. He held firm to a concept of minute and abundant variations, plenty of raw material for selection to act upon, and argued that Darwin was painting himself into a corner by claiming that favorable variations are rare accidents. Critics can and did seize on this, Wallace pointed out, to claim that timely and favorable variations are too vanishingly rare to be of any real use, rendering selection intermittent at best. "I think it would be better to do away with all such qualifying expressions," he urged Darwin, "and constantly maintain (what I certainly believe to be the fact) that variations of every kind are always occurring in every part of every species, —& therefore that favourable variations are always ready when wanted" (2 July 1866, DCP-LETT-5140; *Correspondence* 14: 229).

Darwin's concept of variation had important implications for his model of transmutational change. While early on Darwin was struck by the potential for evolutionary divergence of small populations in isolation, as on islands, he largely abandoned this view in the *Origin* because of the sheer rarity of favorable variants cropping up in the odd individual. He always envisioned transmutation to be slow, but this would be far *too* slow. He thus argued that, far more important than isolation was just the opposite: large populations occupying large (and ideally heterogeneous) ranges, as in continental areas. The chances of favorable, single variations arising for selection to efficiently act upon increase with population size, and all the more so with large populations occupying large ranges since in his view exposure to the varied environmental conditions of a large range will induce more variations. The point was underscored in the context of Darwin's familiar analogy of domestication:

> *I must now say a few words on the circumstances, favourable, or the reverse, to man's power of selection. A high degree of variability is obviously favourable, as freely giving the materials for selection to work on; not that mere individual differences are not amply sufficient, with extreme care, to allow of the accumulation of a large amount of modification in almost any desired direction. But as variations manifestly useful or pleasing to man appear only occasionally, the chance of their appearance will be*

> *much increased by a large number of individuals being kept; and hence this comes to be of the highest importance to success. On this principle Marshall has remarked, with respect to the sheep of parts of Yorkshire, that as they generally belong to poor people, and are mostly in small lots, they never can be improved.*
>
> Darwin (1859, pp. 40–41)

Two points are noteworthy here. First, Darwin's distinction between the two kinds of variation: "mere individual differences" would suffice, if selected by a breeder "with extreme care," for improving or developing a breed, but rarer single variations, larger in magnitude and "manifestly useful or pleasing," are even better. Second, the odds of getting more of the latter are increased with population size—this is agriculturist William Marshall's point about improving sheep: smaller lots, i.e., smaller flocks, yield fewer favorable variations for the breeder to select. In modern terms, larger numbers of individuals afford more opportunities for mutation.

In making this argument for the importance of large populations in generating the middling-magnitude favorable "single variations" for selection to act upon, Darwin shifted his speciation model from allopatry to sympatry, to use the modern terms. This left him open to a serious problem, which was soon pointed out in perhaps the most important critique of his theory, by Scottish engineer Fleeming Jenkin.

2.4.2 Fleeming Jenkin and the Swamping Conundrum

In early 1869 Darwin wrote to botanist Joseph Hooker: "Fleming Jenkins [sic] has given me much trouble, but has been of more real use to me, than any other Essay or Review" (16 January 1869, DCP-LETT-6557; *Correspondence* 17: 21). He was in the midst of revising the *Origin* for the 5th edition, one important change to which was a response to Jenkin's critical review in the *North British Review* (Jenkin 1867) arguing that natural selection was incapable of generating sustained evolutionary change on the basis of either continuous "individual differences" or discontinuous and rarer "single variations," regardless of how advantageous these variations may be. With respect to minute continuous variations, Jenkin maintained that selection could only tweak existing structure, not lead to a new structure, reasserting the old argument about limits of variation. And equating Darwin's rare "single variations" with sports and monstrosities, he further argued that such variations would soon be swamped by reproductive blending, going nowhere (Vorzimmer 1963; Bowler 1974).

The problem of swamping by blending inheritance—the prevailing model of inheritance at the time—was not new to Darwin. He acknowledged swamping in so many words in his early private writings (e.g., in the *Sketch* of 1842 he wrote: "...if varieties allowed freely to cross, except by the *chance* of two characterized by the same peculiarity happening to marry, such varieties will be constantly demolished;" Darwin 1909, p. 2). He also briefly considered the issue in the *Origin* (p. 103), but it is telling that he did so in the section "Circumstances favourable to natural selection." He apparently believed at the time that since intercrossing would mainly take place between members of the same emerging variety, more or less confined geographically, swamping was not an issue and it had the benefit of keeping individuals of the same species or variety "true and uniform in character."

He never maintained that evolution proceeds by selection acting on true sports or monstrosities, but he evidently later realized that even the kind of rare discontinuous variants he had been invoking would soon be lost by blending, as Jenkin argued. He acknowledged the force of Jenkins' argument in the new (5th) edition of *Origin* (pp. 104–105). His response was to return to the importance of "individual differences" (minute, abundant, continuous, populational variation) over discontinuous "single variation." As he put it to Wallace: "I always thought individual differences more important than single variations, but now I have come to the conclusion that they are of paramount importance, & in this I believe I agree with you. Fleming Jenkyn's [sic] arguments have convinced me" (22 January 1869, DCP-LETT-6567; *Correspondence* 17: 37). Such variations were shared among many individuals in the population, and any potential swamping effect on *them* is further minimized through physical and ecological isolation, plus selection purging the population of less favorable variants and so reducing relative numbers. This solution reasserted the central role of abundant, minute variations in the evolutionary process—though in moments of doubt Darwin came to question whether even this was sufficient for the evolutionary process—while striking a balance between free intercrossing and (relative) isolation.

2.5 THE PROVISIONAL HYPOTHESIS OF PANGENESIS

We have seen that Darwin held to a model of ubiquitous, abundant, minute, and heritable variations in providing the material basis for evolution by natural selection, and that environment engendered these variations by acting mainly on the reproductive system—the first mechanism proposed directly linking environment with heritable variation and plasticity (see Bonduriansky and Day 2018; Bonduriansky 2021 in this volume). But how, exactly? Darwin tried to address this question in *The Variation of Animals and Plants Under Domestication* (1868), one of three treatises he had planned building upon the key sections of the *Origin*, and the only one realized (the other two included a book-length treatment of the *Origin*'s deductive core of natural variation, struggle, and selection; and the third was to expand upon the explanatory power of the theory as a *vera causa*, a "true cause" that illuminates empirical patterns in a range of fields, from comparative anatomy and embryology to paleontology to biogeography; see Costa 2009a). *Variation*'s very title signals that it is more than a mere account of artificial selection and the development of novel varieties and breeds: the nature, expression, and inheritance of *variation* is central to the book. Bartley (1992) suggested that Darwin's work on domestication beginning in the 1850s stemmed directly from his need to address long-standing questions concerning inheritance, which are in essence questions concerning the origin and fate of variation.

Variation reverses the argument structure of the *Origin* in that it treats empirical patterns first (expression of variation in various domesticated animal and plant groups), then attempts to make sense of these by exploring principles of inheritance, culminating in Darwin's "Provisional hypothesis of Pangenesis" which constitutes Chapter XXVII of the second volume. His chapter opening bears quoting in full:

IN the previous chapters large classes of facts, such as those bearing on bud-variation, the various forms of inheritance, the causes and laws of variation, have been discussed; and it is obvious that these subjects, as well as the several modes of reproduction, stand in some sort of relation to each other. I have been led, or rather forced, to form a view which to a certain extent connects these facts by a tangible method. Every one would wish to explain to himself, even in an imperfect manner, how it is possible for a character possessed by some remote ancestor suddenly to reappear in the offspring; how the effects of increased or decreased use of a limb can be transmitted to the child; how the male sexual element can act not solely on the ovule, but occasionally on the mother-form; how a limb can be reproduced on the exact line of amputation, with neither too much nor too little added; how the various modes of reproduction are connected, and so forth. I am aware that my view is merely a provisional hypothesis or speculation; but until a better one be advanced, it may be serviceable by bringing together a multitude of facts which are at present left disconnected by any efficient cause. As Whewell, the historian of the inductive sciences, remarks:—'Hypotheses may often be of service to science, when they involve a certain portion of incompleteness, and even of error.' Under this point of view I venture to advance the hypothesis of Pangenesis, which implies that the whole organisation, in the sense of every separate atom or unit, reproduces itself. Hence ovules and pollen-grains, —the fertilised seed or egg, as well as buds, —include and consist of a multitude of germs thrown off from each separate atom of the organism.

Darwin (1868, 2: pp. 357–358)

Darwin began sorting his notes for *Variation* in the early 1860s, in particular trying to make further sense of pattern and process in trait expression and heredity—hybridism, reversion, prepotency, telegony, and more (see Box 2.2)—problems and puzzles that had concerned him since the late 1830s, several of which were discussed in the species "Sketch" and "Essay" of the 1840s, and later in the *Origin*. From early on Darwin recognized that domestic varieties were not only instructive analogs for the origin of species and varieties in nature, but could provide insight into heredity since breeders pay close attention to the expression of traits of interest. He gathered data in his usual way: from extensive readings, far-flung correspondents, distributing a 21-item questionnaire on animal breeding, and conducting his own breeding experiments, notably with pigeons (Vorzimmer 1969; Barrett et al. 1987; Bartley 1992). In 1865, he wrote out his ideas in manuscript form, "Hypothesis of Pangenesis," for T. H. Huxley (CUL-DAR51; Olby 1963; see also Darwin's letter to Huxley of 27 May 1865, DCP-LETT-4837; *Correspondence* 13: 150). This manuscript became the basis for the "Provisional hypothesis of Pangenesis" in *Variation*.

BOX 2.2 HEREDITARY PHENOMENA OR PROCESSES INVESTIGATED BY DARWIN AS PART OF HIS DECADES-LONG RESEARCH PROGRAM ON VARIATION AND INHERITANCE, AND WHICH HIS PANGENESIS THEORY WAS AN ATTEMPT TO EXPLAIN

Reversion: The re-appearance of characters after a few to many generations, or **Atavism** the tendency for individuals to express ancestral traits, interpreted

in Darwin's day as lying latent in the bloodline. Can be understood in terms of complementary gene action today.

Prepotency: In crosses between two races or varieties, one prevails over the other in the expression of traits in the offspring. Can be understood in terms of dominant/recessive expression today.

Telegony: Effect of the male element on the female reproductive system such that long-past mates still influence the offspring of later mates.

Hybridism: Cross between species, varieties, or races, a process that Darwin believed could induce variation by perturbation of the reproductive system.

Yarrell's law: In crossing two different varieties or breeds, the older of the two produces the greatest effect on the offspring. Considered invalid or scientifically discredited today.

2.5.1 Foundations of Pangenesis

Pangenesis was an intuitive model that sought to connect some puzzling dots. One of the most puzzling was "reversion"—the reappearance of an ancestral trait after seemingly disappearing for several to many generations (Box 2.2). This strongly suggested to Darwin that the trait, or information for it so to speak, is still present, passed down through the generations dormant until something triggers its expression once again. Thus while most hereditary material was derived from the immediate reproducing individuals, their heritable material could be supplemented with a bit from ancestors. Besides combing the literature for cases of reversion, Darwin verified the phenomenon with crossing experiments conducted with the help of poultry fancier and bee-keeper William Tegetmeier: "I want to try the following little experiment," he wrote Tegetmeier in 1858; "viz to get a cock & some Hens of several breeds, which never have red feathers in them; & then let them cross, & their mongrel children cross again & see whether red birds will not appear" (16 November 1858, DCP-LETT-2362; *Correspondence* 7: 200). By the following September he had exciting results: one young cock produced by the cross initially had jet black feathers, but soon developed "*splendid red Hackles* on neck & on back over tail; so that it will make in this first generation some approach to wild Gallus" (Darwin's emphasis; 13 September 1859, DCP-LETT-2491; *Correspondence* 7: 333). Tegetmeier repeated the experiment with similar results. Darwin, reporting the experiment in *Variation* (1: 240–243 and 2: 40), interpreted this as a case of reversion to the red ancestral coloration of the jungle fowl (*Gallus bankiva*), from which domestic chickens were derived. Reversion was discussed extensively in the *Origin* as well, notably in chapter V, "Laws of Variation," though this experiment was not reported.

The concept of discrete packets in some way bearing traits made good sense in explaining reversion, as well as the related phenomenon of "telegony"—where past mates appear to influence the traits of offspring sired by later ones. Telegony was famously illustrated in Darwin's time by the case of Lord Morton's mare (Burkhardt 1979). George Douglas, the Earl of Morton, communicated "a singular fact in natural history" to the Royal Society (Douglas 1821): a mare once mated to a quagga

apparently produced offspring from later sires that continued to bear the striped markings of the quagga. This suggested some heritable element from the quagga became a part of the mare's reproductive constitution, an idea reinforced by similar reports by others, and consistent with the widely held belief that the "male element" acted directly upon the female reproductive system and even somatic tissue in animals and plants. Consistent with prevailing theory and substantiated by similar observations reported by other authorities (e.g., Giles 1821), Darwin cited the case of Lord Morton's mare in both the *Origin* (p. 165) and *Variation* (1: 403-404; 2: 42, 366). (In the wake of the Modern Synthesis of the 1940s, telegony was widely considered invalidated, but telegony-like effects have recently been reported; see, e.g., Crean et al. 2014.)

Pangenesis was further intuitive in that it resonated with the long-standing "particulate" theories of environmental influence on organisms back to at least the school of Hippocrates in antiquity (Zirkle 1946). The various pre-pangenesis theories of heredity differ in the details, but all posited that one way or another an organism's traits are encapsulated and transmitted in reproduction, and that these traits are more or less influenced and modified by the organism's environment, from climate to the food they consume (Zirkle 1946; Glass 1947; Bowler 1973, Sandler 1983; Olby 2009; reviewed by Bowler 1989, 2003). Darwin's theory resembled some of these earlier models in broad terms, but is much closer in detail to the more contemporary theories of Richard Owen, Charles Naudin, and especially Herbert Spencer (Figure 2.2; see Geison 1969, pp. 396–409). Olby (1963) and Hodge (1985) pointed out that an important theoretical insight by Darwin laying the groundwork for pangenesis was

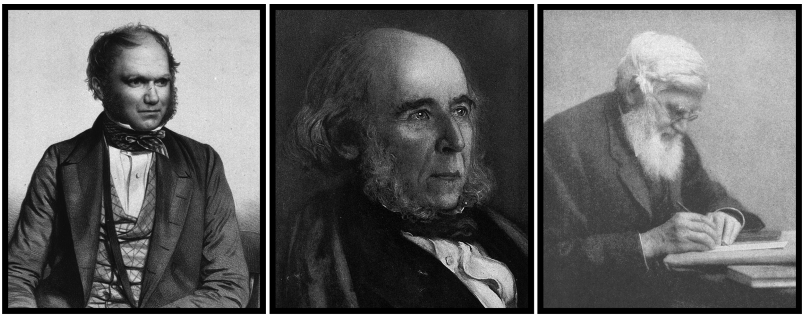

FIGURE 2.2 (a) Charles Darwin (1809–1882) and (c) Alfred Russel Wallace (1823–1913) saw that species and varieties have an unlimited capacity for variation, the foundation for transmutational change by natural selection, their joint discovery. Darwin was more of a theorist of variation than Wallace, putting forth his gemmule-based "Provisional hypothesis of Pangenesis" in 1868, a theory likely inspired by (b) philosopher Herbert Spencer (1820–1903).

(Image credits: C. Darwin: Lithograph of portrait by T. H. Maguire (1849). Courtesy of U.S. National Library of Medicine Digital Collections, image no. B05050. H. Spencer: Photogravure, 1901, after Sir H. von Herkomer. Courtesy of Wellcome Collection, Attribution 4.0 International (CC BY 4.0). A. R. Wallace: portrait from H. F. Osborn (1924), *Impressions of Great Naturalists,* C. Scribner's Sons, p. xxxii.)

seeing the identity of sexual and asexual reproduction—sexual "germs" and asexual "buds" are essentially the same, and indeed a common underlying form of generation underlies reproduction (including asexual forms such as binary fission and parthenogenesis), as well as growth, wound healing, and regeneration.

2.5.2 Mechanics of Pangenesis

As for exactly how variation is generated, Darwin postulated that all cells of the body at all stages of development give off minute, sub-cellular bodies dubbed gemmules, capable of self-replication, and which diffuse throughout the body—but not circulated in the blood, as Darwin (1871) pointed out. Gemmules were envisioned to undergo a development of sorts, starting out immature (and so more "impressionable"). They directly contribute to growth in development or tissue regeneration by binding to nascent cells of the same kind of tissue that gave rise to them—a point that Darwin clarified in a letter to the magazine *Scientific Opinion* (Darwin 1869). A mature organism is thus permeated with gemmules, corresponding to all of its constituent parts at all of its stages of development. Those required for a given somatic or reproductive tissue or structure, at a given stage, come together at that time as needed (Figure 2.3).

Besides the variation imparted to gemmules by the environment and even the very act of reproduction (see Section 2.4), the combination of gemmules from each parent further introduces variation to offspring. Gemmules of one or the other parent may more or less predominate in shaping the eventual trait of the offspring. Whatever the nature of the "information" inherent in gemmules, environment somehow shakes this up, inducing modifications to the gemmules. Recall Darwin's externalist model: environment induces "indirect" variation, individual differences, by acting on the reproductive system, and can also act "directly" on the body to generate larger-magnitude "single variations." Use and disuse of organs also have an effect, through increase or diminution of gemmules for that organ's tissues over generations of sustained use (or the lack thereof), and he further maintained that hybridization could induce variation by perturbing the reproductive system, a combined internal and external influence. By the time he wrote *Variation* he had refined his views, believing that the nature of the organism itself was more important than the nature of the environmental conditions in determining the kind and extent of variation generated. In the chapter on "Causes of Variation," he noted that "organic beings, when subjected during several generations to any change whatever in their conditions, tend to vary; the kind of variation which ensues depending in a far higher degree on the nature or constitution of the being, than on the nature of the changed conditions" (Darwin 1868, 2: 250). He further stressed the secondary importance of environment with a clear analogy: "We are thus driven to conclude that in most cases the conditions of life [environment] play a subordinate part in causing any particular modification; like that which a spark plays, when a mass of combustibles bursts into flame—the nature of the flame depending on the combustible matter, and not on the spark" (Darwin 1868, 2: 291–292).

Nonetheless, pangenesis provided a mechanism for the inheritance of acquired characters (i.e., characters induced by the environment). Darwin thus envisioned an

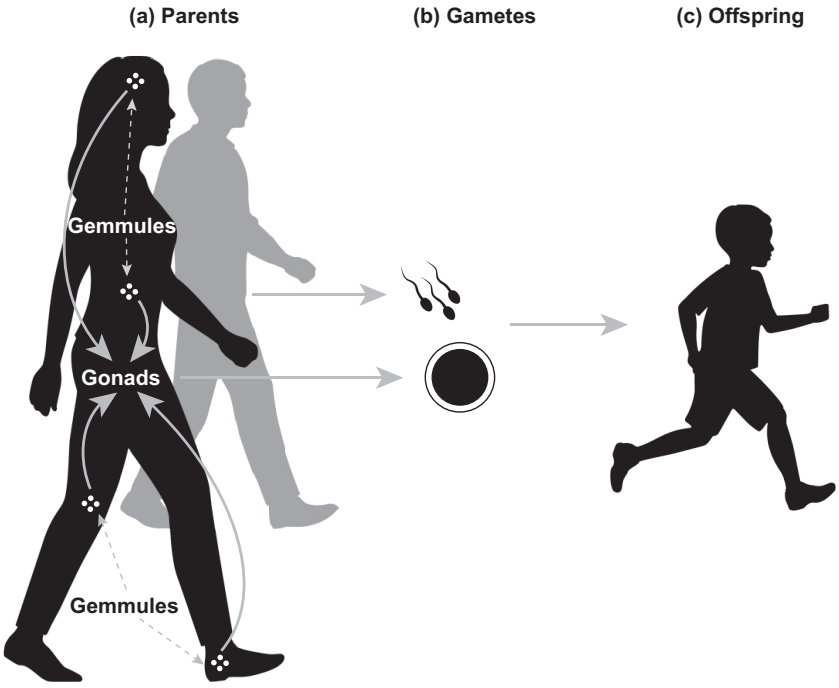

FIGURE 2.3 Schematic representation of Darwin's Provisional hypothesis of Pangenesis. Cells from throughout the body emit minute gemmules which, in sexual reproduction (as shown here), concentrate in the gonads and are transferred to offspring where they interact with and contribute to the growth and maturation of cells in development. They were envisioned to function similarly in asexual reproduction and to interact with developing somatic cells in such processes as wound healing and tissue regeneration. Darwin held that internal and environmental factors can act upon the reproductive system, inducing variation in the gemmules. (Figure courtesy of D. Pfennig.)

interplay of external and internal factors, environment inducing variation largely by perturbing the reproductive system, but attributes of organisms themselves somehow determining its character and extent. He was necessarily vague about these internal attributes, typically writing of the "nature of the organisation" or "constitution of the being." But in any case, environmental conditions provided the trigger, and he was explicit that without varying conditions, there is no variation: "These several considerations alone render it probable that variability of every kind is directly or indirectly caused by changed conditions of life. Or, to put the case under another point of view, if it were possible to expose all the individuals of a species during many generations to absolutely uniform conditions of life, there would be no variability" (Darwin 1868, 2: 255). (Blending by intercrossing or reversion can both result in variation, but by mixing up what is already there, rather than generating new variation.) Darwin's theory of pangenesis, then, was grounded in principles he held as early as the 1840s, notably the environmental basis of variation, generated in both direct and indirect ways in the organism. The theory seemed to explain such phenomena as reversion,

pre-potency, telegony, the ability of one parent to predominate over the other, and the heritable effects of use and disuse of organs.

2.5.3 Reception, Difficulties, Alternatives

Pangenesis met a mixed reception from the beginning from both naturalists and the popular press (see Holterhoff 2014). Huxley evidently seemed sufficiently negative about it that Darwin wondered if he should refrain from publishing the theory. Huxley urged him not to refrain, writing tongue-in-cheek: "Somebody rummaging among your papers half a century hence will find Pangenesis & say 'See this wonderful anticipation of our modern Theories—and that stupid ass, Huxley, prevented his publishing them'... I am not going to be made a horrid example of in that way" (16 July 1865, DCP-LETT-4875; *Correspondence* 13: 203). Hooker, too, was not favorably disposed (although encouraging), but Wallace and Asa Gray were more enthusiastic. Gray summed it up perhaps the most fairly: "pangenesis seemed to strike all of us as as good an hypothesis as one can now make" (24 February 1868, DCP-LETT-5921; *Correspondence* 16(1): 168).

Darwin presented his theory as "provisional" for good reason. He was well aware that the theory was purely inferential, but felt that insofar as it seemed to shed light on several more or less unrelated hereditary phenomena, he was on sound philosophical ground. More problematic philosophically was the fact that the theory was based on virtually unobservable phenomena. No one had seen a gemmule, so Darwin drew analogies with pathogens, and minute organisms "barely visible under the highest powers" of the latest microscopes:

> *An analogy more appropriate is afforded by the contagious particles of certain diseases, which are so minute that they float in the atmosphere and adhere to smooth paper; yet we know how largely they increase within the human body, and how powerfully they act. Independent organisms exist which are barely visible under the highest powers of our recently-improved microscopes, and which probably are fully as large as the cells or units in one of the higher animals; yet these organisms no doubt reproduce themselves by germs of extreme minuteness, relatively to their own minute size. Hence the difficulty, which at first appears insurmountable, of believing in the existence of gemmules so numerous and so small as they must be according to our hypothesis, has really little weight.*
>
> Darwin (1868, 2: p. 403)

More problematic scientifically, perhaps, was the fact that his theory was based on an increasingly dated model of organismic physiology, reproduction, and inheritance. For example, sub- and extra-cellular gemmules would appear inconsistent with principles of cell theory introduced by Rudolf Virchow's *Cellularpathologie* (1858; Darwin read the 1860 English translation), with its famous dictum *Omnis cellula e cellula* ("All cells from cells"). Darwin argued, however, that gemmules derived from and stimulating cell growth could be viewed as compatible with cell theory—cells from cells, but via gemmules (Geison 1969, pp. 392–393).

Potentially more serious were the transfusion experiments of Darwin's cousin Francis Galton, directly testing for the effects of circulating "pangenes" (Bulmer

1999). Using a rabbit breed with silver-gray fur, Galton (Figure 2.5) first transfused blood taken from different colored common lop-eared rabbits, positing that circulating gemmules from the donor rabbits would manifest themselves in the subsequent offspring of the recipients. He also surgically crossed the carotid arteries of the rabbits. The results were negative, leading Galton to conclude that pangenesis was invalidated (Galton 1871a). When Darwin protested that he never claimed that gemmules are borne in the blood per se, Galton took him to task over being sent on a "false quest" owing to the imprecision of Darwin's language in *Variation*, which strongly suggests that gemmules are indeed diffused through the circulatory system (Darwin 1871; Galton 1871b). Undaunted, Galton then attempted experiments with rats, surgically "siamesing" them to permit a fuller exchange of fluids. These results being negative as well, Galton largely abandoned Darwin's model of pangenesis, although he borrowed elements for his own inheritance theory based on what he termed "stirps" (from Latin *stirpes*, root), collections of hereditary gemmules or "germs" inherent in cells, *not* circulated through the body. These gemmules are either active ("patent") or latent in varying combinations and ratios. Most importantly, he eventually adopted the view that patent and some latent gemmules develop into somatic tissue, while the remaining latent gemmules giving rise to germ cells (Bulmer 1999)—the hereditary element was restricted to the reproductive organs.

These studies, together with his analyses of twins, led Galton to posit that hereditary material is insulated from such "environmental" effects as use and disuse or habit (Galton 1876), confirming his earlier (1865) position that acquired characters cannot be inherited. Darwin had a difficult time understanding both Galton's complex arguments and mathematical treatment, but flatly rejected his strong "hereditarian" view: "If this implies that many parts are not modified by use & disuse during the life of the individual, I differ widely from you, as every year I come to attribute more & more to such agency" (7 November 1875, DCP-LETT-10245; *Correspondence* 23: 436). Darwin never gave up his view of heritable environmental influence, and in fact increasingly invoked the heritable effects of use and disuse and of habit as he alluded in his letter to Galton. With the efficacy of natural selection as the primary agent of evolutionary change under attack on several fronts, notably in terms of whether there is sufficient variation in natural populations, and whether there is sufficient time available (the age of the earth), Darwin's reliance on such Lamarckian processes to speed up evolution only increased over time, as reflected in the 5th (1869) and 6th (1872) editions of the *Origin* (see discussion in Costa 2009b, pp. 491–495). But by the 1880s this was becoming increasingly untenable—uncertainty that he acknowledged in his Preface to Weismann's *Studies in the Theory of Descent* when he admitted that "the manner in which the environment acts is as yet quite unknown" (Weismann 1882, p. vi).

Galton's view that the hereditary material is confined to the reproductive organs was borne out by Weismann's germ plasm theory (1883). Initially accepting of inheritance of acquired, environmentally induced variation, like virtually all other naturalists at the time, Weismann first publicly rejected this view in a lecture given in 1883, *Über die Vererbung* ("On inheritance"), articulating the difficulties and contradictions of the view that environmental influences on the cells of the body can become heritable. Weismann followed with his theory of the "continuity of the germ

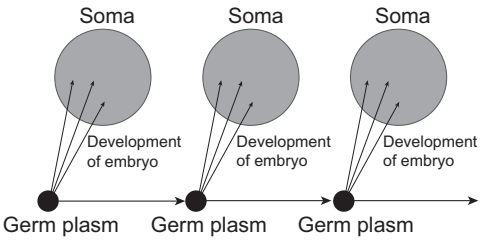

FIGURE 2.4 Schematic representation of the germ plasm theory of August Weismann (1882). The hereditary material, the germ plasm, is restricted to the gonads. Somatic cells develop anew each generation from the germline. Hereditary variation occurs in the germ plasm only; changes induced in somatic cells are not heritable. (Courtesy of I. Alexander, kindly redrawn by D. Pfennig.)

plasm" (*Keimplasma*) (Weismann 1885, 1892), arguing that the germline through the generations is wholly sequestered from the body (soma), so that no alteration of somatic cells can be communicated to the germ cells (Figure 2.4). He built upon this idea in a series of essays, published as a collection (Weismann 1889), and culminating in *Das Keimplasma: eine Theorie der Vererbung* (published in English the following year as *The Germ Plasm: A Theory of Inheritance*; Weismann 1892, 1893). In that work Weismann also addressed critics, notably the Dutch botanist Hugo de Vries, whose book *Intracellulare Pangenesis* (1889) attempted to improve upon Darwin's pangenesis and Weismann's germ-plasm theory. De Vries (Figure 2.5) coined the term "pangens," mutually independent hereditary characters most closely associated with what came to be called "unit-characters," alleles. Every germ cell or bud contained a set of pangens, collectively representing the hereditary material of the whole organism. He rejected the idea that all cells of the body give off pangens that concentrate in the reproductive organs. While he thus agreed with Weismann's germline/soma distinction, and wrote that Weismann had "shaken [*shattered*, in the English translation of 1910] the generally accepted doctrine of the inheritance of acquired properties" (de Vries 1889, p. 4), de Vries argued that the barrier is not as absolute as Weismann maintained, pointing to how somatic cells give rise to germ cells in plants (Darden 1976; Stamhuis 2003).

An in-depth treatment of Weismann's ideas is beyond the scope of this chapter (see Churchill 1968; Mayr 1985; Winther 2001). Suffice it to say here that Weismann's sequestered germline idea resonated with and built upon contemporary discoveries in cell biology, from Meischer's investigations into the cell nucleus and its phosphorus-rich nucleoproteins (first published in 1871) to the discoveries of mitosis and meiosis by Flemming, van Beneden, Strasburger, and others in the 1880s, discoveries that had shifted the discussion of the origin of trait variation. Weismann himself had, in 1887, recognized the significance of reduction division in gametogenesis and its role in generating variation. This insight is further resonant with Darwin's earliest thinking on sexual reproduction and transmutation (Section 2.3), where he posited that sex is responsible for generating variation, fueling

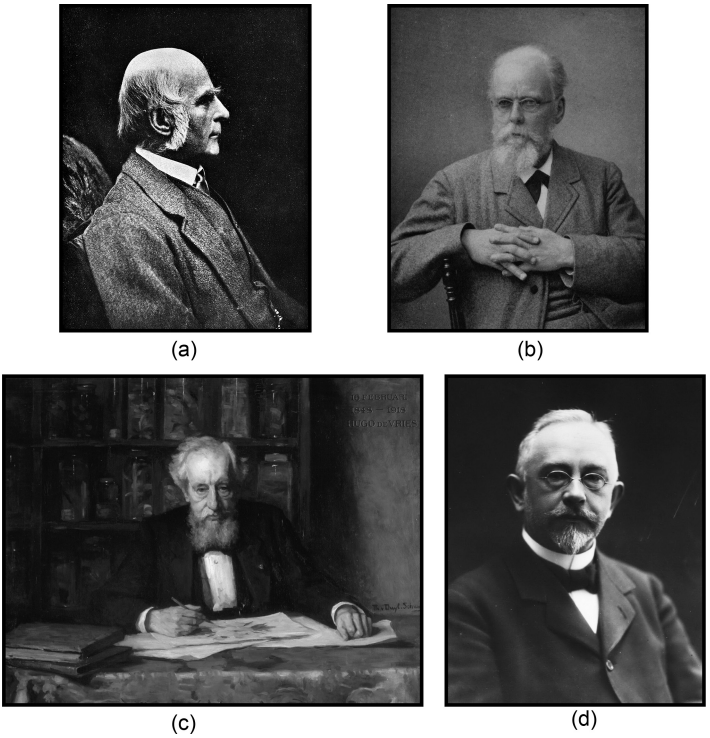

FIGURE 2.5 (a) Darwin's cousin Francis Galton (1822–1911) rejected pangenesis after failing to empirically confirm its central feature of gemmules, developing his "stirp" theory which anticipated Weismann's later germ plasm-soma distinction. (b) August Weismann (1834–1914) posited that the germline cells ("germplasma") are wholly sequestered from environmental influences on the soma. (c) Hugo de Vries (1848–1935) developed a theory of "pangens" to improve upon pangenesis, and later developed the Mutation theory of discontinuous variation and saltational evolution. Mutation theory grew out of de Vries' experimental work with *Oenothera* (evening primrose), the plant he is depicted drawing in the portrait. (d) Wilhelm Johannsen (1857–1927), one of the "rediscoverers" of Gregor Mendel's work, originated the terms genotype and phenotype in the course of controlled crossing and hybridization experiments with garden peas, helping clarify the nature of variation and paving the way for understanding the phenomenon of phenotypic plasticity.

(Image credits: F. Galton: Portrait courtesy of Wellcome Collection, Attribution 4.0 International (CC BY 4.0). A. Weismann: From E. G. Conklin (1915), *Proceedings of the American Philosophical Society* 54:216. [Plate A]. H. de Vries: Portrait by Thérèse Schwartze (1918) courtesy of Universiteitsmuseum Amsterdam, Wikimedia Commons. W. Johannsen: Portrait (1911) courtesy of The Royal Danish Library, Copenhagen, neg. 47358, SKAN 515243. Billedsamlingen, Danske portraetter.)

transmutation. Although Darwin always saw a link between sexual reproduction and variation (inducing gemmule variation by perturbing the reproductive organs), he later recognized that sexual reproduction can have the opposite effect as well, through the homogenizing effects of blending.

2.6 PARTIAL ECLIPSE

Historian Peter Bowler (1983, 2005) described the waning enthusiasm for natural selection as the primary agent of evolutionary change in the late 19th and early 20th centuries as the "eclipse of Darwinism." While Darwin and Wallace's ideas were never wholly eclipsed, of course—it was more of a partial eclipse—there emerged several competing schools of thought that differed dramatically in their view of the nature and evolutionary significance of variation—and which in varying degrees diverged from the Darwinian vision of environmentally induced variation (Provine 2001). A new appreciation of the interplay of environment and variation grew out of these debates.

The *neo-Lamarckian* school argued that the abundant, small-scale, random variations that Darwin relied upon were too trivial to be of importance for evolution. This school, prevalent among American paleontologists such as Edward Drinker Cope and Alpheus Hyatt, held that evolution was driven by ontogenetic or developmental changes (Cope's "law of acceleration") and the inherited effects of use and disuse (Bowler 1977). Cope's theory of "kinetogenesis" held that repeated, habitual movements aided in the alteration and development of anatomical structures, in a process reminiscent of Lamarck's internal organizing and adaptive force (see Section 2.1)—though unlike Lamarck, Cope saw this as a divinely guided process. This was a return to the view of variation as so much noise, largely irrelevant to an inwardly driven and purposeful evolutionary process (Cope 1868, 1896).

The *mutationist* or *saltationist* school, in contrast, put variation front and center. Not Darwin's ubiquitous and minute "individual differences," however, but more akin to his larger, discontinuous "single variations" (see Section 2.4.1). Darwin, recall, had long argued against saltational evolution, as reflected in the phrase *Natura non facit saltum*—"Nature does not take leaps"—which he repeated no fewer than six times in the *Origin*. The mutationist school became philosophically aligned with the emerging Mendelian school in the early 20th century—the common denominator being a focus on discontinuous variation. Mendelism was initially seen as incompatible with Darwinian evolution largely on that basis. Huxley was one of those skeptical of the efficacy of selection acting on minute continuous variation, but perhaps the most notable skeptic was Francis Galton, who essentially became the father of the saltationists. Impressed with the stability of striking "sports" and the problems posed by regression to the mean with continuous variation, Galton addressed "Evolution Not By Minute Steps Only" in his book *Natural Inheritance* (1889), and 5 years later published "Discontinuity in evolution" (Galton 1894). William Bateson, greatly influenced by Galton's arguments, became a champion of de Vries and his mutation theory (Provine 2001; Bowler 2003).

Through the decade following *Intracellulare Pangenesis* in 1889, Hugo de Vries conducted extensive experimental studies on what he termed "fluctuating variability" (essentially Darwin's "individual differences") and atavism (his preferred term for reversion) (Darden 1976). De Vries postulated that continuous "fluctuating variability" was caused by changes in the relative numbers and ratio of pangens, in contrast to "mutability" or "mutation" (a term he coined), describing *discontinuous* variation caused by gain and loss of pangens. His plant breeding experiments led

him to independently discover the principle of segregation, which Mendel had found in 1866, and he was subsequently one of the three scientists to "rediscover" Mendel's work in 1900. In *Die mutationstheorie* (1901–1903), de Vries presented the results of his studies of discontinuous variation, especially of the evening primrose *Oenothera lamarckiana*, and tried to explain differences in trait expression in terms of active and latent pangens. He was especially interested in "progressive" mutation, his term for the formation or appearance of completely new or long-latent traits, which he suggested could greatly accelerate evolution. De Vries' discontinuous variation in *O. lamarckiana* was later recognized to stem from a combination of genetic factors such as ring chromosomes, lethal alleles, and polyploidy. Mutationism enjoyed a brief resurrection in the 20th century, in the form of the hypothesis dubbed the "Hopeful Monster" (Goldschmitdt 1940).

The *biometrician* school, led by bio-statistician Karl Pearson, Walter Weldon, and their followers, held most closely to the Darwin-Wallace model of gradual evolution by natural selection acting on ubiquitous, continuous, heritable variations. Its founders had been inspired by Galton's statistical methods, yet arrived at opposite conclusions from Galton regarding continuous versus discontinuous variation. As Pearson expressed it, Galton's dismissive attitude toward "fluctuating variations" "left him practically in the ranks of the mutationists—a strangely inconsistent position for one who has been looked upon as the Founder of the Biometric School!" (Pearson 2011, 3A: 86).

The competing empirical and theoretical investigations of the Mendelian and biometrician schools focused, then, on questions of variation: its magnitude, heritability, and distribution were central to understanding what selection can or cannot effect, and therefore how evolution proceeds. It was in the course of these investigations that Wilhelm Johannsen (Figure 2.5) undertook a series of experiments that helped crystallize the genotype-phenotype distinction—terms he coined—and paved the way for an appreciation of the nature of phenotypic plasticity (Churchill 1974; Provine 2001; Meunier 2016; see Futuyma 2021 in this volume). In 1901 and 1902 Johannsen undertook experiments with self-pollinating garden beans. He first planted one set of beans representing the population average (in weight and dimensions) and other sets representing the largest and smallest size classes. He then compared the offspring beans and found that while those from the large and small sets deviated from the mean in the expected direction, they did so to a lesser degree than the parents. This was interpreted as consistent with Galtonian regression to the mean and variability within limits. Johannsen next developed a number of "pure lines" by planting sets of offspring beans, each set derived from one parental bean from the original population. This permitted the analysis of variation in the "pure lines" as well as the population as a whole. The results underscored the limited, fluctuating nature of variation, but more importantly underscored that the observed variation in each of his "pure line" sets was not heritable (Johannsen 1903):

> *The general results of this work would form a not unimportant support for the modern concepts of Bateson and de Vries on the great significance of "discontinuous" variations, or "mutations," for the theory of evolution. For a selection in cases such as mine is effective only in so far as it selects out representatives of an already*

> *existent [genotype]. These [genotypes] would not be successively originated through the retention of those individuals which vary in the desired direction; they would merely be found and isolated.*
>
> Johannsen (1903; translated in Peters 1959, pp. 24–25)

Johannsen next undertook experiments entailing the crossing, or hybridization, of pure lines, experiments that led to insights into the effects of crossing in generating variation, extending his analysis of the relationship between genotype and phenotype and the ways in which environment can interact with genotype to yield phenotypic variation (Meunier 2016). Johannsen recognized the evolutionary potential for this kind of interaction, greatly exceeding that afforded by mere fluctuating variability.

The year 1909—the year that Wilhelm Johannsen coined the terms "genotype" and "phenotype"—also saw the publication of Richard Woltereck's analysis of defensive polyphenism in *Daphnia* and *Hyalodaphnia* water fleas (Figure 2.6), the first published study of direct environmental induction of phenotypic variation of a given genotype, what we term adaptive phenotypic plasticity, and in which the term "reaction norm" (*Reaktionsnorm*) was coined (Woltereck 1909). Woltereck did not fully understand the significance of his findings, but Johannsen recognized that his results demonstrated how genotypes can react to particular environmental circumstances—each genotype has its own reaction norm. Together with Johannsen's work, Woltereck's studies set the stage for the evolutionary study of phenotypic plasticity, a phenomenon now recognized to be widespread (Sarkar 1999; see Bonduriansky 2021 and Sultan 2021 in this volume). Darwin would have been fascinated by the modern concept of genetic assimilation (see Scheiner and Levis 2021 in this volume), resonant with his belief that long-repeated habit or learned behavior—another form of environmental influence—can become heritable. This idea was first proposed by J. Mark Baldwin in the 1890s (Baldwin 1896, 1897) and is now known as the "Baldwin effect" (see, e.g., Simpson 1953; Crispo 2007; Scheiner 2014).

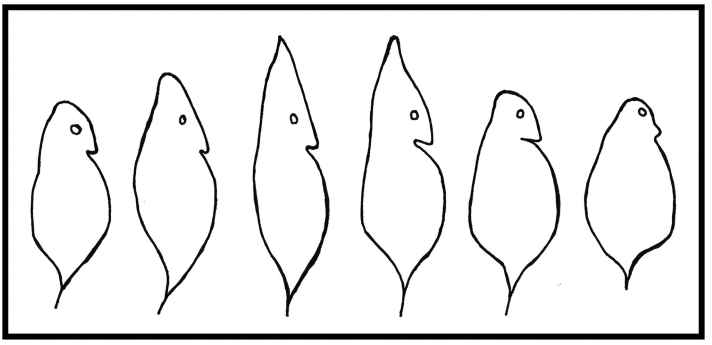

FIGURE 2.6 Head-shield phenotypic plasticity in *Hyalodaphnia* water fleas. (Figure redrawn from figure 5 in Woltereck [1909, p. 122]).

2.7 CONCLUSION

As it happens, August Weismann had a special interest in *Daphnia*, publishing several papers and a monograph on the genus (Weismann 1879). He gifted Darwin copies, and the two exchanged letters regarding *Daphnia*, Weismann commenting on the "complex circumstances" of their biology and Darwin wondering if they might exhibit sexual selection (17 June 1876, DCP-LETT-10335; *Correspondence* 24: 209). Darwin was aware of the alternating sexual and asexual stages of water fleas and their morphological variation. He would surely have been intrigued to learn of the capacity of *Daphnia* for morphological change in response to environment, but perhaps not surprised. Throughout his life as a "generation theorist," Darwin always held that trait expression has an essential and intimate environmental basis. The modern genetic and developmental framework for understanding this differs greatly from Darwin's framework, of course, and greater still perhaps from Lamarck's before him, but their intuition was basically correct. In broad terms, the environmental basis of trait variation is clear, from environmentally induced mutations and transposon activity to pheromones, epigenetics, genetic assimilation, and the signaling that triggers polyphenism. Nearly 140 years after Darwin penned his preface to Weismann's book, it remains largely true that "there is hardly any question in biology of more importance" than the nature and causes of variability—phenotypic plasticity foremost among them.

ACKNOWLEDGMENTS

I am grateful to David Pfennig for the invitation to contribute to this volume, and to Leslie Costa and David Pfennig for their kind assistance in preparing the figures. Leslie Costa, Emily Harmon, Andrew Isdaner, Pat Kelly, Nick Levis, and an anonymous reviewer provided helpful comments, corrections, and suggestions. I also gratefully acknowledge Highlands Biological Station and Hunter Library of Western Carolina University for research support during the writing of this chapter.

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3 Key Questions about Phenotypic Plasticity

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CONTENTS

3.1 INTRODUCTION

Phenotypic plasticity—the ability of an individual organism to change its phenotype in direct response to variation in its environment—has long fascinated and perplexed biologists. Indeed, its widespread existence demands that we confront such vexing questions as: Are an organism's features determined solely by its genome, or does its environment also play a role? Can environmentally induced features be passed on to the individual's offspring? And, does plasticity impact evolution? Many biologists would likely respond to these questions by arguing that an organism's genes are the *primary* cause of variation and the *exclusive* cause of inheritance and that plasticity therefore cannot influence evolution. So pervasive is this perspective that it has permeated much of science and has even become entrenched in the public psyche. Consider, for instance, the following statement from one of the world's leading biological research organizations:

> *Each genome contains all of the information needed to build and maintain that organism.*
>
> **U. S. National Institutes of Health (2020)**

Yet, an increasing number of scientists have begun questioning these fundamental precepts that minimize the environment's role in development and evolution. Behind this change in perspective is a growing realization that phenotypes emerge from

DOI: 10.1201/9780429343001-3

the interplay of genes and environmental factors; that a change in an individual's environment can generate pronounced trait variation through plasticity (Figure 3.1); that, under certain circumstances, some such environmentally modified traits can be passed on to offspring; and that plasticity might influence evolution and leave an indelible imprint on the history of life. Indeed, although phenotypic plasticity is increasingly appreciated for its practical applications (Box 3.1), it remains especially controversial in evolutionary biology.

In this chapter, I explore these issues by examining five key questions about phenotypic plasticity. These questions are: (1) Does plasticity confer a unique evolutionary advantage? (2) What are the proximate mechanisms of plasticity? (3) When should plasticity evolve, and what form should it take? (4) Can plasticity

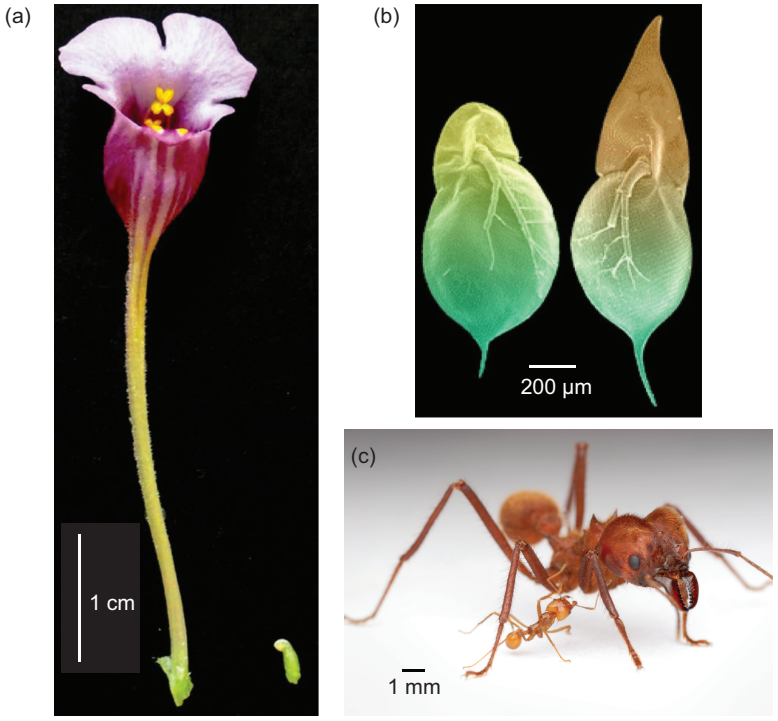

FIGURE 3.1 Spectacular examples of phenotypic plasticity occur in organisms that display 'polyphenism,' in which multiple, discrete phenotypes arise from a single genotype as a result of differing environmental conditions. For instance, (a) monkeyflower plants (*Mimulus douglasii*) produce large open ('chasmogamous') flowers (left) or, in response to longer day lengths, small closed ('cleistogamous') flowers (right); (b) a single clone of water fleas (*Daphnia cucullata*) can produce either a normal morph (left) or, in the presence of predators, a helmeted morph (right); and (c) depending on the nutrition they receive as larvae, leafcutter ants (*Atta cephalotes*) develop into either a large major worker or a small minor worker. Based on: (a) Barnett et al. (2018); (b) Agrawal et al. (1999); (c) Wheeler (1986). (Photos reproduced with the kind permission of: [a] Laryssa Barnett; [b] Christian Laforsch and Ralph Tollrian; [c] Alex Wild.)

influence evolution? and (5) Does plasticity fit within existing evolutionary theory? Of course, these five questions do not constitute all of the interesting questions one could possibly ask about plasticity. Instead, this list is intended to provide an overview of what we know—and what we do not know—about the causes and consequences of plasticity, especially as they pertain to evolution. Moreover, these questions will set the stage for the remainder of the book, where they will be considered in greater detail. After discussing the five questions, I conclude with suggestions for future research.

BOX 3.1 SOME PRACTICAL APPLICATIONS OF PLASTICITY

- **Predicting evolutionary responses to climate change**. The capacity of individual organisms to display plasticity, coupled with the capacity of populations to undergo adaptation, will likely determine which species will 'win' and which will 'lose' under anthropogenic environmental change (Diamond and Martin 2016).
- **Optimizing agricultural yields**. Understanding plasticity is important for knowing how to *reduce* its effects. For example, in developing crops, it is essential to reduce plasticity to ensure that the same crop produces high yields in different parts of the world, despite differences in environment. Fisheries and animal husbandry could similarly benefit from understanding how to select on phenotypic plasticity (de Jong and Bijma 2002).
- **Understanding the causes of nonheritable birth defects (teratogens) in both humans and nonhuman animals**. The environment is a font of information for normal development; however, it can also disrupt development. Indeed, 2%–5% of human infants are born with an anatomical abnormality, as are an increasing number of other animals. Although some abnormalities have genetic causes, many are triggered by environmental factors (Gilbert and Epel 2015).
- **Clarifying the evolutionary causes of nutrition-related disease in humans**. Nutrition-induced plasticity is common in humans (Gluckman et al. 2009), and it can lead to obesity and obesity-related diseases (Bateson et al. 2004). For example, obesity affects more than 300,000,000 people worldwide. The most dangerous form is exaggerated development of visceral adipose tissue (VAT). It has recently been proposed that selection favored increased investment in VAT among individuals that were food deprived when young as adaptive anticipatory plasticity to mitigate malnourishment in adulthood (West-Eberhard 2019).
- **Understanding the human brain**. In response to changes in the environment, brains can 're-wire' synaptic interactions. Such 'neuroplasticity' can even allow neurons to compensate for injury and disease (Shaw et al. 2001).

3.2 QUESTION 1: DOES PLASTICITY CONFER A UNIQUE EVOLUTIONARY ADVANTAGE?

Phenotypic plasticity is ubiquitous (Nijhout 2003; Palacio-López et al. 2015). Indeed, when confronted with environmental variation, *all* organisms can modify some aspect of their phenotype or adjust their internal conditions to maintain a stable equilibrium (Whitman and Agrawal 2009; Gilbert and Epel 2015; Sultan 2015). These adjustments range from conspicuous changes in morphology (Figure 3.1) to subtle changes in gene expression, which are generally invisible to an outside observer but which are found across all taxa. Thus, *there is no such thing as a non-plastic organism*. Plasticity can therefore be viewed as a defining feature of life (Nijhout 2003).

But why is plasticity ubiquitous? One obvious answer is that plasticity confers a unique evolutionary advantage. Although some forms of plasticity arise as an unavoidable consequence of fundamental laws of chemistry or physics and are therefore not necessarily beneficial (for instance, poor nutrition leads to stunted growth in most organisms, which is generally not beneficial; Monaghan 2008), many forms increase the bearer's fitness. For example, when attacked by herbivores, certain plants greatly increase the production of defense chemicals (glucosinolates) in their leaves, which deters further attacks (War et al. 2012). Similarly, predation is reduced following predator-induced increases in body length in *Daphnia* (Agrawal et al. 1999; see Figure 3.1b) and shell thickness in snails (Auld and Relyea 2011). More generally, numerous studies have shown that plasticity can be adaptive (for examples, see Watt 1968; Pfennig 1990; Warkentin 1995; Van Buskirk et al. 1997; Denver et al. 1998; Wells and Pigliucci 2000; Kishida and Nishimura 2004; Lyytinen et al. 2004), although not necessarily for all traits or all environmental conditions that the organism might encounter (Caruso et al. 2006; Auld and Relyea 2011). Why is plasticity often beneficial? Here, I describe how plasticity bestows on its bearer a unique evolutionary advantage: it provides a mechanism whereby an individual organism can modify its phenotype to match its *current* environment, including rapidly changing and even novel environments.

Consider that every natural environment varies, whether spatially or temporally, and whether owing to abiotic factors, such as climate, or biotic factors, such as predation. Moreover, these fluctuations often occur within the lifetimes of individual organisms (Levins 1968). Such changing environmental conditions can be harmful because they erode the match between the organism's phenotype and its environment (Levins 1968). Although adaptive evolution can help maintain this match (Meyers and Bull 2002), evolution can only occur (by definition) *between generations*. Consequently, adaptive evolution is always one (or more) generation(s) behind in responding to environmental variation. Even rapid evolution (Reznick et al. 2019) cannot keep pace with environmental change that occurs more rapidly than an organism's generation time.

Plasticity, by contrast, generates phenotypic change *within generations*. Plasticity can therefore potentially keep up with rapid environmental change. Essentially, plasticity allows organisms to adjust their phenotypes in *developmental time* to match *current* conditions, which (as noted above) contrasts with evolution, where there is

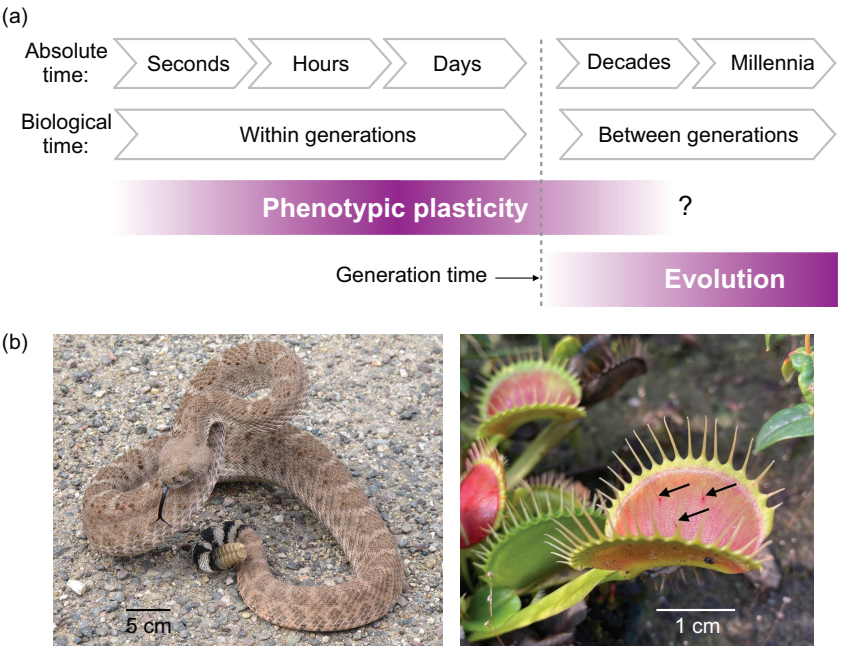

FIGURE 3.2 (a) Plasticity enables organisms to respond to environmental variation within a generation. In contrast, evolution occurs between generations only (some forms of plasticity can also be transmitted between generations). (b) Some plastic responses can occur within seconds of a change in the environment, as when a rattlesnake vibrates its rattle upon being threatened. Even some plants can react to environmental change rapidly. For instance, the Venus flytrap (*Dionaea muscipula*) can close its leaves and thereby ensnare its prey within 40 milliseconds of its trigger hairs being touched (arrows). More generally, most organisms can alter patterns of gene expression within seconds of a shift in their environment. (Photos: David Pfennig.)

always an intergenerational time lag between when conditions change and when an adaptive response can occur (Figure 3.2).

Moreover, the phenotypic variation wrought by plasticity is often adaptive (see citations above). This widespread adaptiveness of plasticity arises, in part, because plasticity can often be repurposed for another, related environmental context. For instance, recall from above that predation induces increases in body length in *Daphnia* (Agrawal et al. 1999; see Figure 3.1b). As it turns out, this form of plasticity can be triggered by many different predators, even though the chemical signals that elicit this response likely differ. This predator-resistant phenotype can even be induced by chemicals released by dead *Daphnia* inside a fish's gut (Stabell et al. 2003). Similarly, many species of plants and animals can respond adaptively to interspecific competition through plasticity-induced niche shifts (see Table 2.1 in Pfennig and Pfennig 2012). These abilities are presumably common because the same response has also evolved as an adaptive solution to *intra*specific competition, and the stimuli or cues that trigger the response to one form of competition can also

trigger the response to the other (Pfennig and Pfennig 2012). Hence, many organisms can generalize environmental stimuli from one context to another, which helps account for plasticity's widespread adaptiveness. Furthermore, as I describe below, because plasticity (like most other traits) is often underlain by genetic variation, it can undergo adaptive evolution such that a particular plastic response becomes better at matching current conditions.

Even in instances where the maintenance or expression of plasticity is costly (DeWitt et al. 1998; and such costs have proved difficult to detect; Auld et al. 2010; Murren et al. 2015; see also Snell-Rood and Ehlman 2021 in this volume), plasticity may still be an optimal strategy. Although an individual with a fixed phenotype might achieve higher fitness than an individual with a plastic phenotype in the environment for which the fixed phenotype is specialized, this fixed phenotype would likely have much lower fitness in a different environment. An individual with a plastic phenotype, by contrast, should have higher fitness *overall* when averaged across multiple environments (Figure 3.3). Given that environments vary, all organisms are likely to experience such diverse selective regimes, which provides an overall advantage to plasticity.

Of course, not all plasticity is adaptive (Ghalambor et al. 2007; Nettle and Bateson 2015; Palacio-López et al. 2015; Acasuso-Rivero et al. 2019; see also Sultan 2021 in this volume). Indeed, some plasticity appears to be maladaptive, as when organisms confront increasingly stochastic change. For example, climate change is characterized by increased environmental stochasticity (IPCC 2013). Consequently, existing environmental cues for influencing phenology (that is, the timing of any seasonal biological event, such as when plants and animals reproduce, migrate, or go dormant) will likely become less accurate predictors of seasonal progression. As a result, previously adaptive plasticity is more likely to produce mismatches between phenotype and environment. Such maladaptive phenological shifts have been observed in many species (Diamond and Martin 2016; Thackeray et al. 2016).

In sum, plasticity can confer a unique evolutionary advantage by enabling organisms to respond appropriately and immediately to environmental variation. Because environmental variation is commonplace, phenotypic plasticity is also commonplace.

3.3 QUESTION 2: WHAT ARE THE PROXIMATE MECHANISMS OF PLASTICITY?

Given that plasticity is ubiquitous, and often adaptive, how does it come about? Biologists have long known that phenotypes can be modified by a diverse array of environmental factors (see Costa 2021 in this volume). Here, I begin our discussion of the mechanisms of plasticity by briefly summarizing how selected environmental factors can induced plastic changes (for a more comprehensive discussion, see Gilbert and Epel 2015; Sultan 2015).

A common trigger of plasticity is temperature. This is hardly surprising given that temperature influences nearly all biochemical and biophysical processes. Although temperature's effects on development are often subtle, these effects can also be profound. For instance, in many turtles, crocodilians, and fish the temperature at which an embryo develops determines its sex. In turtles, researchers recently uncovered

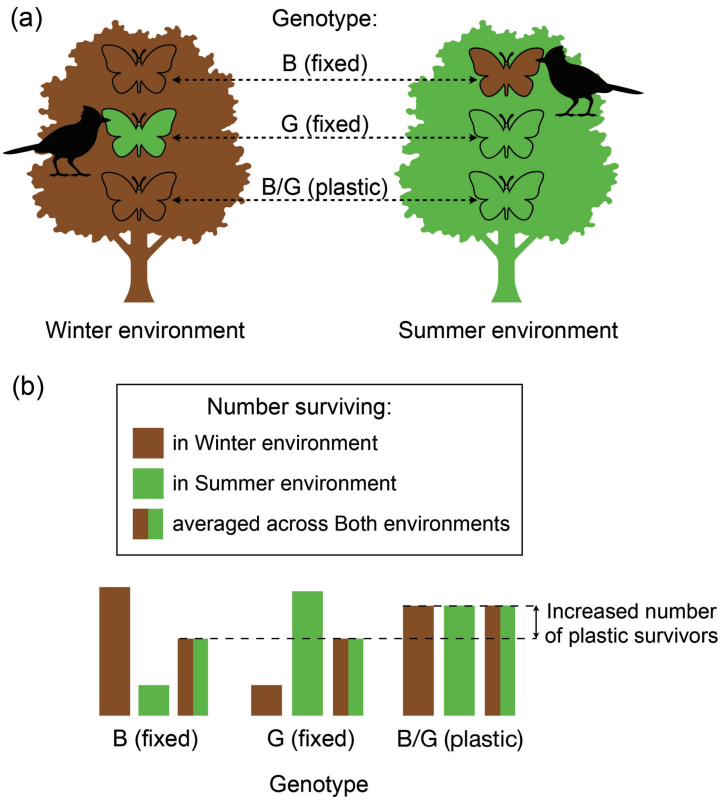

FIGURE 3.3 A fitness advantage of plasticity. Although individuals with fixed phenotypes might achieve higher fitness than individuals with plastic phenotypes in any one environment, plastic individuals should have higher fitness across multiple environments. (a) For example, in the presence of visually oriented predators, camouflaged individuals should suffer less predation. In this hypothetical data set, genotypes that produce fixed green and brown individuals are more likely to be detected by predators in winter (brown) and summer (green) environments, respectively. In contrast, genotypes that produce plastic individuals (that is, individuals that can be either brown or green, depending on their background) are less likely to be detected by predators in either environment. (b) Consequently, plastic genotypes should have higher survival than fixed genotypes when averaged across *both* environments (for a real-life example, see Noor et al. 2008).

how temperature determines sex: they identified a temperature-sensitive gene that lies in the pathway influencing testes development (Weber et al. 2020).

Another environmental factor that commonly causes plasticity is food. Again, this is not surprising given that food is taken internally and often contains potent chemicals that can induce dramatic phenotypic change. For example, depending on their larval diet, female honeybees develop into either queen or worker castes (castes are a form of discrete plasticity known as a 'polyphenism' [see Figure 3.1c] consisting of behaviorally and [often] morphologically distinct groups within a single colony; Wilson 1971). If fed for most of its larval life a protein-rich diet ('royal

jelly'), a female will become a queen; otherwise, she will become a sterile worker (Maleszka 2008). This switch happens because royal jelly is rich in both nutritive proteins and a small protein ('royalactin') that increases levels of juvenile hormone (Kamakura 2011). Both increased nutrition and juvenile hormone levels increase the developing honeybee's size, and larger individuals are much more likely to become queens than workers.

Finally, a frequent trigger of plasticity are signals produced by other organisms. For instance, as noted in Section 3.2, many species can detect the presence of predators (sometimes by sensing 'kairomones'; chemicals released by another species) and respond by changing their behavior, physiology, or morphology. Such predator-induced plasticity is widespread in both plants and animals (Agrawal et al. 1999; see also Figure 3.1b). Other species can sense competitors and respond through adaptive plasticity. For example, in the presence of competitors, colonies of the ant *Pheidole pallidula* increase the production of distinctive soldier castes to ward off foreign invaders (Passera et al. 1996). Even more impressively, many species of plants and animals can distinguish kin from nonkin and alter their phenotype accordingly (Pfennig and Sherman 1995). For instance, the larvae of western tiger salamanders (*Ambystoma mavortium*) are less likely to develop into a distinctive cannibal morph (Figure 3.4) when surrounded by kin (Pfennig and Collins 1993), presumably by detecting in the water chemical cues associated with their genetic relatives (Pfennig et al. 1994).

As this brief review makes clear, adaptive plasticity typically involves two main stages: assessment and response (West-Eberhard 2003; Sultan 2015; Levis et al. 2020). Assessment begins when an individual acquires information about its environment. For example, animals have evolved numerous specialized cells and tissues that can receive stimuli from their environment and then translate these stimuli into electrical or chemical signals that the nervous system can decipher (Stevens 2013). Plants have also evolved sophisticated systems to detect changes in their environment and then relay the information to effector systems (Kiss 2006). Once information about the environment is acquired and processed, the individual must then determine how to use that information. More precisely, the individual must 'decide' which of the plausible phenotypes that it could produce will likely yield the highest fitness, *given both its environment as well as its own condition* (for instance, its energy reserves, health status, age, sex, growth rate, or body size). It is important to emphasize that such assessment need not involve an active decision and may instead be 'passive' (as in the case of temperature-dependent sex determination discussed above). Indeed, even organisms lacking brains can perform sophisticated feats of environmental assessment. Many bacteria, for example, have evolved 'quorum sensing' (Miller and Bassler 2001), where they can gauge the density of bacterial cells in their immediate vicinity and produce (through plasticity) different phenotypes at high population densities. Essentially, natural selection should favor individuals that can gather and use whatever information is available to them to evaluate their environment accurately and effectively.

Understanding how organisms process information during environmental assessment is a largely overlooked frontier in plasticity research. One reason this topic may have been overlooked is longstanding skepticism over whether

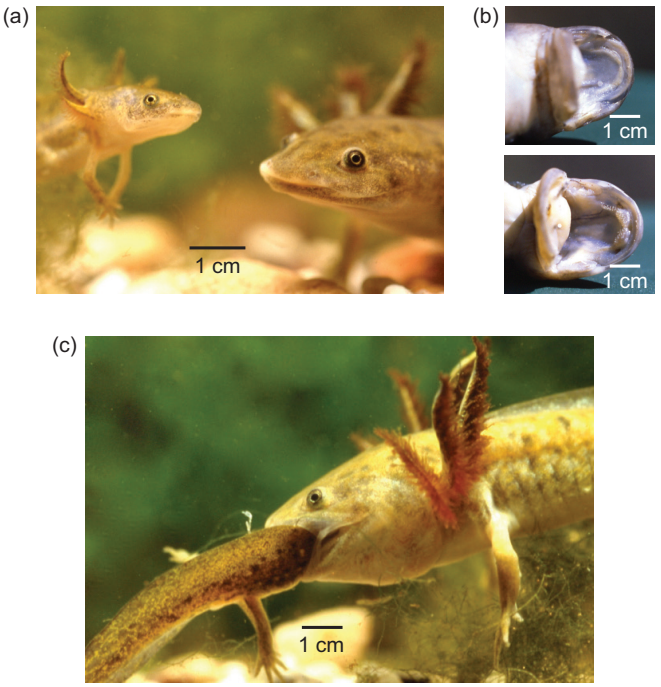

FIGURE 3.4 The presence of genetic relatives suppresses environmentally induced cannibal production in salamanders. (a) Larvae of western tiger salamanders (*Ambystoma mavortium*) normally develop into a small-headed form that feeds on invertebrates. When crowded, however, some individuals transition from this (b) small-headed, small-toothed form (upper photo) into a large-headed, large-toothed form (lower photo) and become exclusively (c) cannibalistic. Individuals are less likely to produce this cannibal morph when crowded by kin than nonkin. (Photos: David Pfennig.)

nonhuman organisms can evaluate environmental conditions well enough to make adaptive decisions. Yet, we now know that many organisms can carry out complex acts of adaptive environmental assessment, such as optimally choosing what to eat, which sex to become, when to reproduce, with whom to reproduce, and toward whom to allocate aid (Davies et al. 2012). Moreover, like other aspects of the phenotype, assessment mechanisms can undergo adaptive evolution when selection refines pre-existing elements that are sensitive to different environmental circumstances (West-Eberhard 2003).

Once assessment has taken place, the second main stage of adaptive plasticity is a response. Essentially, for adaptive plasticity to occur, assessment must be followed by a developmental change that results in production of a (putatively adaptive) phenotype. As with assessment, this response stage often involves a complex set of mechanisms. For example, considerable research effort has gone into identifying the molecular changes that mediate plastic responses (Lafuente and Beldade 2019). Although these mechanisms are still poorly understood, the best-studied model comes from research into how cells within the same multicellular organism can take

on different forms and functions, despite being genetically identical. These studies have revealed what role a cell assumes depends on the presence in the cell of special proteins called 'transcription factors' (Gilbert and Epel 2015). Essentially, transcription factors act as switches that turn genes 'on' or 'off,' thereby ensuring that each gene is expressed in the correct cell at the correct time and with the correct level of activity. Importantly, different signals from the external environment can ultimately enlist different transcription factors. Thus, one proposed model for how plasticity comes about is that different environmental stimuli or cues cause different transcription factors to be present within the organism's cells, which causes different genes to be turned on (or off). This process thereby ultimately results in different phenotypes being produced in response to variation in the environment.

Such changes in gene expression may underlie many examples of phenotypic plasticity (Nijhout 2003), but these changes are not the sole way that plasticity might come about. Indeed, there are several means by which environmental signals can modify the patterns of gene expression (Gilbert and Epel 2015). For example, environmental signals might trigger differential gene expression *directly*, such as when bacteria in an animal's gut induce changes in its intestinal gene expression.

Lastly, when assessing and responding adaptively to their environment, a key challenge all organisms face is distinguishing signal from noise (that is, relevant information from irrelevant information). Generally, natural selection should favor sensory and neural systems that can detect and discriminate a stimulus of importance from background noise. Signal detection theory suggests how adaptive evolution might produce systems that can perform such feats (Wiley 2015). According to this theory, the response threshold (that is, the threshold at which the organism produces one phenotype as opposed to another) should be set where sensory and effector systems are sensitive enough that they correctly detect and respond to a relevant signal but not so sensitive that they respond too frequently to noise. The optimal 'acceptance threshold' (sensu Reeve 1989) is where signal and noise overlap, but where the benefits of responding to the signal (and not to noise) exceed the costs of failing to respond to the signal (or incorrectly responding to noise; Figure 3.5a). This threshold should shift depending on the balance between the aforementioned benefits and costs (Figure 3.5b). Indeed, there is evidence of such threshold shifts in natural populations (Moczek and Nijhout 2003).

In short, studies of the mechanisms of plasticity illustrate how the phenotype emerges from a complex series of developmental, physiological, and even behavioral processes that are influenced by environmental factors as well as by genes (Nijhout 1999). Thus, any particular example of plasticity likely entails many steps, potentially encompassing numerous genes and environmental and physiological factors. This complexity provides copious targets on which selection can act, from the types of signals that an individual's sensory system can detect to the threshold amount of a particular hormone needed to trigger a phenotypic response (Moczek et al. 2011). Clarifying these underlying mechanisms remains an important aim of plasticity research and will be discussed in subsequent chapters of the book (see especially Chenard and Duckworth 2021; Goldstein and Ehrenreich 2021; Ledón-Rettig and Ragsdale 2021 in this volume).

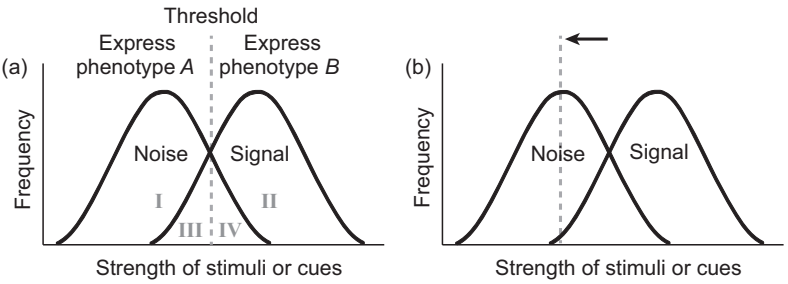

FIGURE 3.5 Signal detection and the expression of adaptive plasticity. (a) When assessing and responding adaptively to their environment, all organisms must discriminate between relevant versus irrelevant information (that is, signal versus noise, respectively). Generally, there should be a threshold value of strength of stimuli or cues (dashed line), above which the organism responds by switching from producing one phenotype (or range of phenotypes; in this case, phenotype *A*) to producing an alternative phenotype (or range of phenotypes; in this case, phenotype *B*). The optimal location of this threshold will depend on the relative costs and benefits associated with: correctly detecting and responding to the signal (a 'hit': zone II in roman numerals); correctly not responding to noise (a 'good call': zone I); incorrectly failing to respond to a real signal (a 'miss': zone III); or incorrectly responding to noise as a signal (a 'false alarm': zone IV). If these costs and benefits vary, the threshold should shift accordingly. (b) For example, with predator-induced plasticity, failing to detect and respond to an actual predator is often costlier than sometimes falsely responding to a predator that is not actually present. In such cases, the optimal threshold should shift to a lower strength of cues to minimize the chances of a miss (zone III). Theory based on Reeve (1989); figure based on Stevens (2013).

3.4 QUESTION 3: WHEN SHOULD PLASTICITY EVOLVE, AND WHAT FORM SHOULD IT TAKE?

As noted above, phenotypic plasticity is probably ancestral to all organisms (Nijhout 2003). Environments have always changed, and every organism appears capable of responding to changes in diverse abiotic and biotic environmental factors. However, not all traits or taxa show similar levels of plasticity (West-Eberhard 2003). In particular, some traits in some lineages are relatively sensitive to environmental influences, whereas others are relatively insensitive (Figure 3.6). What conditions favor increased versus decreased plasticity? Moreover, once plasticity evolves, it can assume different forms (see below). What determines the form that plasticity assumes?

First, greater plasticity (that is, greater environmental influence on the production of a particular trait; Figure 3.6a) should evolve when: (1) organisms confront environmental variation; (2) no fixed trait is best suited for all environmental conditions; (3) individuals can reliably assess their environment; (4) the fitness benefits of expressing plasticity outweigh its costs; and (5) heritable variation for plasticity is present (reviewed in Berrigan and Scheiner 2004; Scheiner 2020; see also Snell-Rood and Ehlman 2021 in this volume).

Consider, for example, environmental sex determination, a common form of plasticity in certain crustaceans, annelids, fish, and reptiles, where the environment

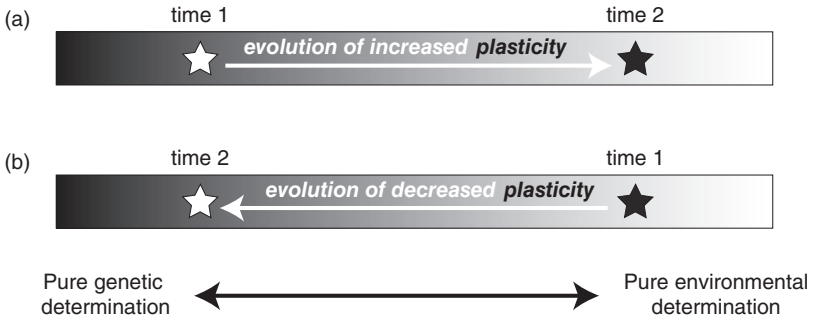

FIGURE 3.6 A schematic depicting the evolution of plasticity, which emphasizes the continuum—and interchangeability—between genetic and environmental influences on phenotype production. Individuals might vary phenotypically either because they carry alternative alleles or because they experienced different environments and their traits' differences arose through phenotypic plasticity. These two proximate mechanisms are best thought of as occupying different positions along a continuum in which strict genetic determination of trait production resides at one end and pure environmental induction resides at the opposite end. However, most (if not all) traits lie between these two extremes (this is why it is meaningless to refer to traits being 'genetically determined' or 'environmentally determined'; nearly all traits are shaped by both genes *and* the environment). Moreover, a trait's position along this continuum can change over evolutionary time. Specifically, a trait may evolve (a) increased environmental sensitivity (that is, increased plasticity), or (b) decreased environmental sensitivity (that is, decreased plasticity). Such evolutionary shifts in plasticity may, in turn, have important evolutionary consequences. (Modified from Pfennig et al. [2010], with kind permission of the publisher.)

in which an embryo develops determines its sex (Bull 1983; see also the previous section). Charnov and Bull (1977) hypothesized that environmental sex determination should supplant strict genetic determination of sex if the environment in which an individual develops has different fitness consequences for males versus females and if individuals can assess their environment during development. For instance, imagine that a population experiences both 'good' and 'poor' environments, where developing in the good environment is much more beneficial to males than females. As long as individuals can assess which environment they are in, selection should favor environmental sex determination, with (in this hypothetical example) males being produced in the good environment and females in the poor environment.

Support for the Charnov and Bull (1977) model comes from studies of the amphipod crustacean, *Gammarus duebeni*, which occurs in temperate coastal marshes. This species has evolved environmental sex determination; specifically, an individual's sex is determined by the photoperiod (the length of daylight), with males being produced early in the mating season and females later. Being produced early in the mating season allows more time to grow, and male fitness improves more than female fitness with size (McCabe and Dunn 1997). Thus, because males benefit from larger size more than females, and because individuals can assess their environment (specifically, photoperiod), environmental sex determination—that is, phenotypic plasticity—is adaptive in this system, presumably explaining why such plasticity has evolved.

Once increased plasticity has evolved, the resulting phenotypes can be distributed continuously or discontinuously, with the former being more common than the latter. Continuous plasticity is referred to as a 'norm of reaction' or simply 'reaction norm' (for an example, see Figure 1.1 in Sultan 2021 in this volume). Reaction norms can allow individuals to finely tune their phenotypic response to the strength of the environmental stimulus. For instance, tadpoles of many species of frogs respond to the presence of predators by developing deeper tails, which enhances their survival. The greater the risk of predation (as measured by the amount of chemicals that predators secrete), the deeper the tail that tadpoles develop (Relyea 2004).

The second main form of plasticity—the occurrence of environmentally induced discrete phenotypes—is referred to as 'polyphenism' (sensu Michener 1961; Mayr 1963, p. 670). Examples of polyphenisms include environmentally influenced sexes (see above); different leaf forms on the same plant ('heterophylly'; Wells and Pigliucci 2000); castes in social insects (Figure 3.1c); seasonal forms produced at different times of the year (Shapiro 1976); alternative reproductive forms in organisms ranging from viruses (Ptashne 2004) to plants (Figure 3.1a) to animals (Pienaar and Greeff 2003; Moczek 2005); certain predator-induced forms (see Figure 3.1b); and alternative resource-use forms found in many organisms (for example, see Figure 3.4). Polyphenisms have long fascinated evolutionary biologists because polyphenisms are thought to represent a key phase in major, lineage-specific innovations (Mayr 1963; Shapiro 1976; West-Eberhard 1989; Nijhout 2003; West-Eberhard 2003). If these alternative phenotypes are subject to independent selection—such that selection acting on one phenotype does not impact the other—then the evolution of a novel phenotype as part of a polyphenism could permit elaboration of that new form without affecting the established phenotype (Snell-Rood et al. 2010; Van Dyken and Wade 2010; Levis and Pfennig 2019; de la Serna Buzon et al. 2020). Thus, clarifying the conditions that favor polyphenism is crucial for understanding the origins of novel phenotypes.

Before discussing the conditions that favor polyphenism, however, it is important to note that most polyphenisms are thought to originate from continuously plastic phenotypes through two different proximate mechanisms (Nijhout 2003). First, polyphenism might emerge from a reaction norm when the environment is discontinuous (or effectively discontinuous, as when the organism experiences it only at discrete times or locations). In such cases, the environment induces only portions of a continuous reaction norm. For example, in bivoltine insects (insects that have two generations per year), each generation develops in a different season and thus experiences different environmental conditions, which can induce different phenotypes (interestingly, when such insects are exposed to intermediate environmental conditions, they often develop intermediate phenotypes not normally seen in nature; Nijhout 1994). Second, polyphenism might arise from a developmental 'switch.' For example, males of the dung beetle *Onthophagus taurus* have a horn length polyphenism, in which small males are hornless whereas large males have well-developed cephalic horns (Moczek 1998). However, horn length varies allometrically with body size (it increases faster than body size), and the relationship is highly nonlinear. This results in a bimodal distribution of horn sizes, even though body size is normally distributed (Moczek 1998). But what conditions favor the evolution of a polyphenism, especially when the alternative phenotypes co-occur?

Generally, polyphenism is thought to be favored when disruptive selection acts on continuously varying plasticity (a reaction norm) and molds it into different morphs (where a 'morph' is an individual expressing a distinct morphology or behavior). Disruptive selection occurs when two or more modal phenotypes have higher fitness than the intermediate phenotypes between them because, for example, they are better suited to specific ecological circumstances (Levene 1953; Levins 1968). However, disruptive selection often acts in a frequency-dependent fashion (Bolnick 2004; Rueffler et al. 2006), which means that the fitness of each morph depends on its relative abundance in the population (Sinervo and Calsbeek 2006). Such frequency-dependent dynamics typically favor just two morphs (these dynamics can also favor three or more morphs, but this is rare; Sinervo and Lively 1996).

Frequency-dependent disruptive selection is often caused by competition for access to resources or mates (O'Donald 1977; Day and Young 2004). During competitive interactions, the more similar any two individuals are to each other, the more intense the competition between them (Martin and Pfennig 2009). Thus, because each morph competes more against itself than against the alternative morph, frequency-dependent selection favors each morph only when it is rare, thereby preventing any one morph from becoming so common that it supplants the other(s). Consequently, such selection will maintain alternative morphs in a population at frequencies where each morph has, on average, equal fitness. In fact, many polyphenisms are associated with resource or mate acquisition. Figure 3.7 shows how competitive interactions for resources or mates can foster the evolution of a resource polyphenism. Figure 3.8a–d provides an example in which a reaction norm appears to have been refined by selection into a polyphenism.

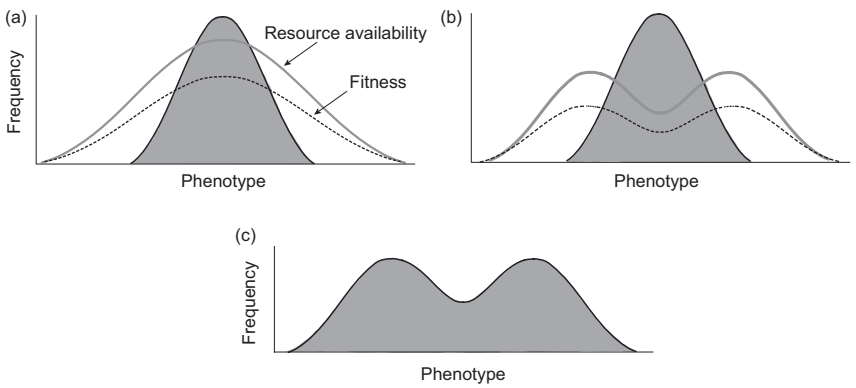

FIGURE 3.7 How disruptive selection can act on continuously varying plasticity in resource use and mold it into a polyphenism. (a) In a population that exploits a range of resource types that are normally distributed (e.g., a range of prey sizes), selection should initially favor those individuals that use the most common resource type (e.g., prey of intermediate size). (b) However, as more individuals exploit this resource type, it becomes depleted over time. Therefore, individuals that use this intermediate resource type will experience more severe competition (and hence, have lower fitness) than those that use extreme, but underexploited, resource types (e.g., very small or very large prey items). (c) Such disruptive selection can eventually promote the evolution of bimodally distributed phenotypes: that is, a polyphenism.

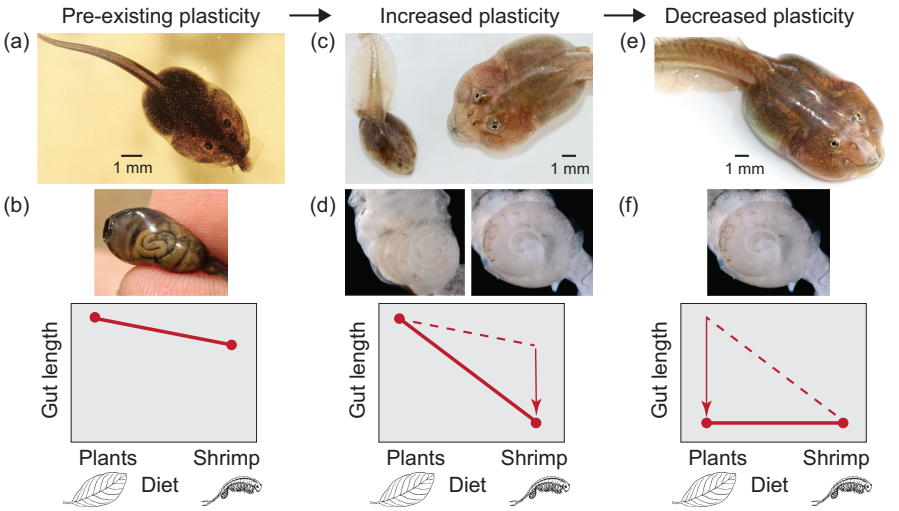

FIGURE 3.8 The evolutionary gain and loss of plasticity, as illustrated in tadpoles of New World spadefoot toads (genera *Scaphiopus* and *Spea*). (a) *Scaphiopus* tadpoles—like the tadpoles of many frogs and toads, appear to harbor pre-existing, diet-induced plasticity. Although they normally develop into a generalized 'omnivore' morph, if they eat large animal prey (such as freshwater shrimp or other tadpoles), (b) they develop a slightly shorter gut. (c) In *Spea*, this diet-induced plasticity has been enhanced as part of a polyphenism, in which individuals that eat meat deviate from the default omnivore morph (left) and develop into a distinctive 'carnivore' morph (right), which (d) produces a dramatically shorter gut (the dashed line indicates the presumed ancestral condition; the arrow indicates the direction of the evolutionary change in plasticity). (e) Finally, this diet-induced plasticity has been lost in certain derived populations of *Spea*, which always produce the carnivore morph (f) with its short gut, regardless of their diet. Based on Ledón-Rettig et al. (2008; 2010), Levis et al. (2018), and Levis and Pfennig (2019). (Photos: David Pfennig.)

As the above discussion makes clear, selection can act on pre-existing plasticity to extend and refine it. Indeed, there is abundant evidence from diverse group of plants and animals that plasticity has undergone evolutionary change, even among closely related lineages (Schwander and Leimar 2011; Murren et al. 2014). However, not only can a particular lineage evolve an increase in plasticity, it can also evolve a *decrease* in plasticity, where a trait becomes *less* responsive to a specific change in the environment (Figure 3.6b). Such decreased plasticity might evolve for at least two reasons. First, when plasticity is costly (Snell-Rood et al. 2010; Murren et al. 2015; see also Snell-Rood and Ehlman 2021 in this volume), selection can actively eliminate it, leading to the fixation of the favored phenotype through an evolutionary process known as 'genetic assimilation' (Waddington 1942; sensu Waddington 1953; for examples from natural populations, see Figure 3.8e, f as well as Scheiner and Levis 2021 in this volume). However, plasticity might also be lost through mutational degradation or genetic drift (Masel et al. 2007), as may occur when non-favored phenotypes are seldom expressed and thereby experience relaxed selection (Kawecki 1994; Whitlock 1996; Van Dyken and Wade 2010).

In sum, plasticity can evolve and assume different forms. Plasticity's evolution may, in turn, have important downstream consequences for evolution more generally, which is the topic I turn to next.

BOX 3.2 PLASTICITY AND EVOLUTION: A BRIEF HISTORY

Prior to the 20th century, most scholars held that: (1) an individual's environment influenced its traits, and (2) environmentally altered features could be passed to offspring (Zirkle 1946; see also Costa 2021 in this volume). These beliefs stemmed from two common observations: (a) that both plants and animals are modified by environmental change (in modern terms, they display plasticity), and (b) that offspring resemble their parents. Although point 2 above—the so-called 'inheritance of acquired characters'—is often associated with the French biologist Jean-Baptiste Lamarck (1744–1829), Lamarck never actually regarded it as his seminal contribution (Burkhardt 2013). Instead, Lamarck merely treated a concept that had been around for at least two millennia as self-evident (Zirkle 1946). Even Charles Darwin (1809–1882) accepted these ideas when he developed his grand theory of 'pangenesis' to explain how the inheritance of acquired characteristics might operate (Galton 2018; Costa 2021).

The person credited with 'disproving' the inheritance of environmentally induced traits was the German biologist August Weismann (1843–1914). In a lecture delivered in 1883 (entitled "On Inheritance"; translated in Mayr 1985), Weismann made a compelling argument against the inheritance of acquired characters. In doing so, he discussed case after case in which variation could not be explained by use and disuse. For example, Weismann asked: How can the numerous special adaptations of the workers and soldiers of ants be inherited by use, when these individuals do not reproduce? From these case studies, Weismann concluded that "the improvement of an organ in the course of generations is not the result of a summation of the result of practice of individual lives, but of the summation of favorable genetic factors" (1883, p. 26). Following this sweeping rejection of the inheritance of acquired characters, the idea never regained full credibility (it is worth noting, however, that Weismann's views on this subject were actually more nuanced than is often depicted; Winther 2001).

Two decades after Weismann's lecture, the field of genetics took off when three scientists independently 're-discovered' the work of the Moravian monk Gregor Mendel (1822–1884). In a paper published in an obscure journal in 1866, Mendel had deduced how parents pass to their offspring discrete, invisible 'factors'—now called genes—that predictably influence the traits of their offspring. In the 1930s, Mendel's insights were merged with the mathematical models of R. A. Fisher, J. B. S. Haldane, and Sewall Wright to form the basis for the 'Modern Synthesis' of evolutionary biology (Huxley 1942 [2009]). One consequence of this synthesis was that the phenotype was thereafter

viewed as a direct readout of the genotype. With few exceptions (Haldane 1946), any effects of the environment on the phenotype were treated as 'noise' not worthy of attention (Falconer 1952). To the degree that any such variation was acknowledged, it was given a genetic explanation by such new terms as 'penetrance' and 'expressivity' (Sakar 1999). Skepticism about plasticity's role in evolution likely stemmed from the tendency to invoke the inheritance of acquired characters whenever discussing plasticity (see example in main text). Plasticity was also scorned in the mid-20th century because of the highly influential—but dangerously flawed—work of the Soviet agronomist Trofim Lysenko (Graham 2016).

At the same time, five scientists were critical in suggesting that plasticity can play an important role in evolution (see also Futuyma 2021 in this volume). The first is the American psychologist James Baldwin (1861–1934; Figure 3.9a), who, in 1896, developed an idea that came to be known as the 'Baldwin effect' (sensu Simpson 1953). According to Baldwin (1896): (1) when the environment changes dramatically, selection favors those individuals that are behaviorally or morphological flexible; (2) the descendants of these individuals breed with each other in the new environment; and (3) eventually, any behavior or morphological change brought about by the new environment becomes congenital (or, in modern terms, genetically fixed). Although Baldwin's ideas remain controversial, he was among the first to link plasticity among individuals to evolution by suggesting that environmentally induced traits can evolve (Gottlieb 1992; Robinson and Dukas 1999; Webber and Depew 2003; Crispo 2007; Badyaev 2009; Scheiner 2014).

Baldwin's ideas were extended by the Russian biologist Ivan Schmalhausen (1884–1963; Figure 3.9b). In his book, *Factors of Evolution: The Theory of Stabilizing Selection*, Schmalhausen (1949 [1986]) argued that a new morphological adaptation starts out as an environmentally induced form following a change in the environment. Later, the adaptation comes under internal control ('stabilizing selection') and is genetically fixed. As empirical support, Schmalhausen provided numerous examples of discrete, environmentally induced alternative phenotypes (polyphenisms) that appear to have become genetically fixed in other lineages.

Shortly thereafter, the British geneticist Conrad Waddington (1905–1975; Figure 3.9c) demonstrated experimentally that an environmentally induced trait could indeed become genetically fixed as a result of persistent selection. In his most famous experiment, Waddington (1953) subjected a group of fruit flies to a heat shock during embryonic (pupal) development (a separate control group was not heat shocked but treated the same otherwise). As adults, some of these flies exposed to heat shock developed wings with few or no crossveins. Waddington selected these crossveinless flies for breeding and subjected their offspring to the heat shock, once again selecting crossveinless flies for breeding (and, once again, maintaining a control group). After repeating

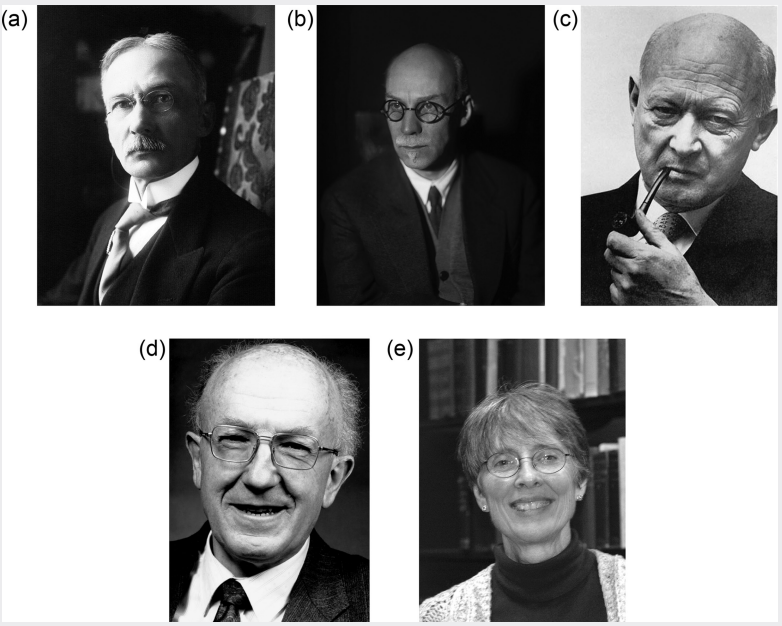

FIGURE 3.9 Key figures in the study of plasticity and evolution. (a) James Baldwin; (b) Ivan Schmalhausen; (c) Conrad Waddington; (d) Anthony Bradshaw; and (e) Mary Jane West-Eberhard. (Photo credits: [a] public domain; [b] Alamy, reproduced with permission; [c] from Robertson [1977], reproduced with the kind permission of the Royal Society; [d] from Fitter [2010], reproduced with the kind permission of the Royal Society; [e] courtesy of Mary Jane West-Eberhard.)

this procedure for 14 generations, Waddington noted that a small proportion of flies in the *control* group expressed the crossveinless phenotype. This was surprising because, although the ancestors of these control flies had been exposed to heat shock in earlier generations, they had not. Thus, a trait that was originally triggered by the environment had lost this environmental sensitivity and become fixed, a process Waddington (1953) dubbed 'genetic assimilation.'

Waddington's experiments were pathbreaking for he had shown that: plasticity can be underlain by heritable variation (he later argued that different genotypes of flies likely differed in how they responded to the heat shock); selection could act on this variation to promote evolution; and such selection could even cause plasticity to be lost (see Scheiner and Levis 2021 in this volume). Yet, Waddington's experiments were largely ignored. Indeed, prominent evolutionists such as Mayr (1963) and Dobzhansky (1970) misinterpreted genetic assimilation as an attempt to resurrect the (by now largely discredited) idea of the inheritance of acquired characters (see above; this misunderstanding

is perhaps only natural given the title of Waddington's seminal 1953 paper: *Genetic Assimilation of an Acquired Character*).

The modern era of plasticity research did not begin, however, until 1965. The key event was the publication of a paper by the British ecologist Anthony Bradshaw (1926–2008; Figure 3.9d). In his paper, Bradshaw (1965) made two important points. First, rather than being simply noise, environmental effects on the phenotype were as important as genetic effects. Second, these effects were themselves under genetic control and could therefore undergo adaptive evolution in response to environmental variation. Indeed, Bradshaw emphasized how different genotypes within the same population typically vary in whether and how they respond to a particular change in environment (as Waddington had proposed and as some early architects of the Modern Synthesis had actually initially suggested; see, for example, Haldane 1946). Such genetic variation in plasticity is now dubbed 'genotype-environment interaction' or simply 'G×E' (see Sultan 2021 in this volume). Following the publication of Bradshaw's paper, researchers began to view phenotypic plasticity as a trait that could undergo adaptive evolution. Bradshaw's paper was also instrumental in bringing plasticity to the attention of a new generation of researchers, especially ecologists and field biologists, who were then motivated to study plasticity in natural populations.

Finally, in 2003, the American behavioral ecologist Mary Jane West-Eberhard (1941–; Figure 3.9e) published a highly influential book, *Developmental Plasticity and Evolution*, in which she argued that most phenotypic evolution begins with environmentally mediated developmental change; that is, plasticity (see also West-Eberhard 2021 in this volume). Specifically, West-Eberhard (2003) proposed that adaptive evolution involves four steps. First, a distinctive developmental variant is produced when a mutation or an environmental stimulus triggers a change in phenotype. Second, a process of 'phenotypic accommodation' occurs when plasticity improves the functioning of the novel phenotype through immediate, additional changes in development. Third, the initiating factor recurs, producing a subpopulation of individuals that express the trait. Finally, a process of 'genetic accommodation'—a sort of adaptive refinement—follows, in which gene frequencies change due to selection on the regulation and/or form of the novel trait (Waddington's concept of genetic assimilation is one extreme form of genetic accommodation, in which the regulation of plasticity evolves to the point at which plasticity is lost and the trait becomes fixed; another extreme is polyphenism, where environmental responsiveness is accentuated and adaptively refined; see Figure 3.1 and Section 3.4). Notably, West-Eberhard marshaled an impressive array of evidence to support these arguments. Consequently, her book stimulated considerable discussion and research (as can be gleaned from many of the chapters in this volume).

3.5 QUESTION 4: CAN PLASTICITY INFLUENCE EVOLUTION?

Although many early naturalists were well aware that the environment could alter an organism's traits—and some even viewed this plasticity as a potent driver of evolution—these ideas were largely swept aside by the emergence of genetics in the 20th century (see Box 3.2; see also Costa 2021 in this volume). Indeed, with the development of the 'Modern Synthesis' of evolutionary biology (the reconciliation of Darwin's theory with Mendelian genetics; see Box 3.2), plasticity came to be regarded as irrelevant to the evolutionary process. According to this view, plasticity cannot affect evolution because evolution requires heritable change, and (the argument went) plasticity cannot be inherited (see, for example, Dobzhansky 1970, p. 32). However, there are two problems with this argument. First, as I describe below, plasticity can impact evolution even when the specific plastic response itself is not inherited. Second, as I also describe below, some plastic responses can, in fact, be inherited.

However, saying that plasticity can influence evolution is not tantamount to saying that it *promotes* evolution. Indeed, because plasticity enables a single genotype to produce multiple phenotypes in response to different environmental conditions, any further genetic change may not be required to adapt to new conditions. In such situations, plasticity might dampen diversifying selection and, hence, evolution (Wright 1931). Or, as Mayr (1963, p. 147) put it:

> *The ability of the phenotype to respond to the demands of the environment without mutation greatly reduces selection pressure.*

Consistent with this idea, there are cases in which plasticity appears to have slowed evolution (Bogert 1949; Huey et al. 2003; Oostra et al. 2018).

However, not everyone agrees that plasticity always impedes evolution. Indeed, some have long hypothesized that plasticity promotes (and in some cases even supplants) genetic evolution. Although various hypotheses have been proposed for how plasticity might facilitate evolution (including 'the Baldwin effect,' 'stabilizing selection,' 'genetic assimilation,' and 'genetic accommodation;' see Box 3.2), all such hypotheses assume that adaptive evolution unfolds when environmentally induced phenotypes appear first, followed by adaptive refinement of this plasticity.

Here, I examine three, non-mutually exclusive routes by which plasticity can facilitate evolution (summarized in Table 3.1; see also Wund 2012). First, plasticity can facilitate evolution *indirectly* by promoting population persistence in novel environments, thereby allowing populations to remain viable until adaptive evolution can occur (the 'buying time' hypothesis). Second, plasticity can facilitate evolution *directly* by exposing formerly unexpressed genetic variation to selection, thereby fueling adaptive evolution (the 'plasticity-led evolution' hypothesis). Lastly, plasticity can facilitate evolution *directly* by forming the basis for an alternative inheritance system on which adaptive evolution can unfold (the 'non-genetic evolution' hypothesis). Below, I discuss each route in turn.

First, plasticity can facilitate evolution by promoting population persistence in novel environments. This hypothesis traces to the late 19th century—most notably,

TABLE 3.1
Alternative Ways That Plasticity Has Been Hypothesized to Impact Evolution

1. Plasticity *does not affect* evolution because it cannot be inherited.
2. Plasticity *impedes* evolution by shielding genotypes from selection.
3. Plasticity *facilitates* evolution by:
   a. promoting population persistence in novel environments (the 'buying time' hypothesis).
   b. exposing 'cryptic' genetic variation to selection, thereby fueling adaptive evolution (the 'plasticity-led evolution' hypothesis).
   c. forming an alternative inheritance system on which adaptive evolution can unfold (the 'non-genetic inheritance' hypothesis).

to the psychologist James Baldwin (1896)—who proposed that, in a rapidly changing environment, selection will favor individuals that are plastic, because these individuals will be more likely to adapt to such changing circumstances (see Box 3.2). This idea, which came to be known as the 'Baldwin effect' (Simpson 1953; see recent reviews in Crispo 2007; Scheiner 2014), was stated succinctly by the paleontologist E. D. Cope (1896, p. 174), who maintained that the most successful lineages in evolution were invariably those that:

> *... presented a combination of effective structures with plasticity, which has enabled them to adapt themselves to changed conditions.*

Plasticity promotes population persistence in rapidly changing environments because of its unique ability to generate phenotypes that match current environmental conditions (see Section 3.2). If, instead, the production of these phenotypes requires favorable mutations, the waiting time can be prohibitively long (Charlesworth 2020), which increases the chances that a population under stress will go extinct. By enhancing population persistence, plasticity fosters evolution *indirectly* by 'buying time' until genetically based adaptations to accommodate any new conditions can evolve (Diamond and Martin 2016; Scheiner et al. 2017; Fox et al. 2019; see also Diamond and Martin 2021 in this volume). Essentially, plasticity can promote 'evolutionary rescue,' which occurs when evolution by natural selection prevents a population from going extinct (Gomulkiewicz and Holt 1995; see also Snell-Rood et al. 2018 for a discussion of the related concept of 'plastic' rescue). As evidence that plasticity might reduce extinction risk, birds that exhibit higher levels of behavioral plasticity (as measured by a higher propensity to innovate) are at a lower risk of extinction—that is, they are more likely to have increasing or stable populations—than birds that exhibit lower levels of such plasticity (Ducatez et al. 2020). Generally, most researchers probably view this buying time hypothesis as the primary way that plasticity facilitates evolution (Pennisi 2018; Fox et al. 2019).

The second main route by which plasticity can facilitate evolution is through 'plasticity-led evolution' (sensu Levis and Pfennig 2019) (sometimes referred

to as 'plasticity-first evolution'; sensu Schwander and Leimar 2011; Levis and Pfennig 2016). In contrast with the traditional process of mutation-led evolution, where a new phenotype first appears following a change in the genome (Carroll 2008), with plasticity-led evolution a new phenotype first appears following a change in the *environment*. To understand how this process works, consider that most natural populations contain a large reservoir of genetic variation that is not expressed phenotypically under normal conditions (Lewontin 1974). This 'cryptic' genetic variation can be expressed phenotypically, however, when organisms experience novel (or stressful) conditions, such as a change in the environment (Gibson and Dworkin 2004; Ledón-Rettig et al. 2014). The phenotypic expression of this variation is crucial because selection acts on phenotypes; not genotypes. However, selection can only act on those phenotypes that are actually expressed.

Once these phenotypes are expressed, selection can act on them and essentially favor the underlying genotypes that are associated with phenotypes that are well adapted for the current environment. As long as the altered environment persists, selection could continue to promote quantitative genetic changes and mold the environmentally induced phenotype into a new adaptive form. Such a process of 'genetic accommodation' can cause a change in both the regulation of plasticity and the form of the phenotype, leading to a better match between phenotype and environment (West-Eberhard 2003). Of course, as with any adaptive evolution, the speed and magnitude of this response depend on the strength of selection and how much genetic (more generally, heritable) variation for plasticity is present. The key point is that the heritable variation that fuels this response is revealed to selection by *plasticity*.

Ultimately, plasticity-led evolution may result in a new phenotype that was not present in the ancestral population, at least not in a well-adapted form (for an example, see Figure 3.8; see also Suzuki and Nijhout 2006). This process might play an underappreciated role in evolutionary innovation and diversification/speciation (see Levis and Pfennig 2021 in this volume). Plasticity-led evolution has also been implicated in triggering adaptive radiation (West-Eberhard 2003; Wund et al. 2008; Pfennig and McGee 2010; Schneider and Meyer 2017), promoting the origins of multicellularity (see Davison and Michod 2021 in this volume), and mediating various other macroevolutionary events (see Lister 2021 in this volume).

Lastly, a third (and by far most controversial) route by which plasticity can facilitate evolution is when environmentally induced responses form an alternative inheritance system on which adaptive evolution can unfold rapidly. In recent years, it has become increasingly clear that vital information can be acquired from parents (that is, inherited) not only through genes but also through various non-genetic factors (factors not encoded by the genomic DNA sequence; reviewed in Bonduriansky and Day 2018). Like genes, such factors can influence the phenotypes of offspring and possibly even mediate evolutionary change (Bonduriansky and Day 2009; Danchin et al. 2011). For example, in many Eukaryotes (the group that includes plants, animals, fungi, and protozoans), the addition of a methyl group (CH_3) to a DNA strand

can influence when, where, and how much the genes on that strand are expressed (Jones 2012; Aliaga et al. 2019). Although some such methyl 'tags' are encoded by the genomic DNA sequence itself, others can be induced by environmental triggers, such as diet or stress (Dominguez-Salas et al. 2014; Miryeganeh and Saze 2020). Once induced, these 'epigenetic' factors can even be transmitted across generations independently of DNA sequence changes. (As an aside, the meaning of the term 'epigenetic' has changed over the years. It was coined by Conrad Waddington in the 1940s to refer to the interaction between genes and environment that results in the production of a phenotype. Now 'epigenetic' often refers to changes in a phenotype caused by modification of gene expression rather than alteration of the genetic code itself.) For example, in the case of DNA methylation, special enzymes (methyltransferases) can copy a methyl tag from the parent strand onto the daughter strand during DNA replication (Wang et al. 2017; Bonduriansky and Day 2018). In this way, an environmentally induced change in gene expression (and, hence, potentially in phenotype) can be inherited.

As additional examples of non-genetic factors that can influence the phenotypes of offspring and possibly even mediate evolutionary change, parents of many species often differentially endow their seeds, eggs, or offspring with acquired information or materials (cytoplasm, hormones), which can then influence their offspring's phenotype (Mousseau and Fox 1998). Although experiences acquired during an individual's lifetime are typically transmitted to offspring only through 'somatic' cells (any cell forming the body of a multicellular organism other than the gametes ['germline']), in some cases, such information can be transported to the germline, where it may then become more permanently encoded (O'Brien et al. 2020). Through this process, an environmentally induced phenotype can be inherited.

Although it is unclear for how many generations, or how reliably, such 'transgenerational plasticity' can be inherited in natural populations, these effects can arise rapidly (because they can be induced within a generation by a change in the environment) and are known to mediate adaptive change in the wild (Galloway and Etterson 2007). However, even if transgenerational plasticity can be passed down for only a few generations, it might still be important in increasing the chances that a lineage will persist until more permanent genetic changes evolve (in which case, this route merges with the buying time hypothesis above). Bonduriansky (2021) discusses transgenerational plasticity in greater detail later in this volume.

In sum, contrary to widespread claims to the contrary, there are circumstances under which plasticity might actually promote evolution. Specifically, plasticity might facilitate evolution by buying time until adaptive evolution can occur, by uncovering genetic variation that fuels evolution, or by forming an alternative inheritance system on which evolution can unfold. As we will see in subsequent chapters, each of these three routes has both theoretical and empirical support. At the same time, more research is needed. For now, having established a possible role for plasticity in evolution, I turn to our final question: does plasticity fit within the existing evolutionary theory?

3.6 QUESTION 5: DOES PLASTICITY FIT WITHIN EXISTING EVOLUTIONARY THEORY?

As noted in Box 3.2, evolutionists have long struggled with whether and how to incorporate plasticity into their framework. Recently, some have suggested that fully integrating plasticity into evolutionary theory will require revamping the Modern Synthesis of evolutionary biology (Pigliucci 2007; Laland et al. 2014, 2015; Pigliucci and Finkelman 2014).

Later chapters will explore this controversy in greater detail (see especially Futuyma 2021; Levis and Pfennig 2021; Schlichting 2021 in this volume), so I will be brief here. Specifically, I emphasize for now that plasticity fits comfortably within existing evolutionary theory. For example, all three routes by which plasticity can facilitate evolution outlined in Table 3.1 are consistent with modern evolutionary theory: each involves selection acting on *heritable* variation, which is the standard model of how adaptive evolution works that has held for over 150 years.

At the same time, knowledge of plasticity can enhance our understanding of evolution by emphasizing how the environment can both select on phenotypic variation and help *generate that variation in the first place* (Matsuda 1987, p. 53). Consequently, incorporating plasticity into evolutionary thinking can illuminate a broader array of evolutionary phenomena, such as how evolution can unfold rapidly, why similar phenotypes are often produced in similar environments, and how novel complex traits evolve.

In sum, while the existence of plasticity does not challenge Darwin's fundamental insight that natural selection, coupled with inheritance, propels adaptive evolution, it does provide a richer view of the evolutionary process.

3.7 CONCLUSIONS

Phenotypic plasticity has long perplexed biologists, and an increasing number of researchers have recently sought to clarify its causes and consequences. As we have seen, plasticity is often beneficial: it can uniquely enable individual organisms to respond appropriately to environmental change during their lifetime. In this way, organisms can potentially track rapidly changing environments, such as those that many natural populations experience. Recent studies have also begun to uncover the mechanisms of plasticity. These studies have revealed that plasticity typically involves numerous genetic, physiological, behavioral, and environmental factors, which provide plentiful targets on which selection can act when promoting either increased or decreased plasticity.

As we have also seen, although plasticity can sometimes impede evolution, it is thought to facilitate evolution through at least three main routes: (1) by promoting population persistence in novel environments, thereby allowing populations to remain viable until adaptive evolution can occur (the 'buying time' hypothesis); (2) by exposing cryptic genetic variation to selection, thereby fueling adaptive evolution (the 'plasticity-led evolution' hypothesis); or (3) by constituting an alternative

inheritance system on which adaptive evolution can unfold (the 'non-genetic evolution' hypothesis). Incorporating these routes more fully into evolutionary thinking will provide a richer perspective on how adaptive evolution works by emphasizing that the environment can both select on phenotypic variation and help generate that variation in the first place.

More generally, a broader understanding of plasticity will likely impact all fields of biology, as it will necessarily require that researchers confront two facts about biological systems: first, that most traits emerge from an interaction between an individual's genes and its environment, and second, that individual variation and flexibility are the rule rather than the exception. Although more research on plasticity is needed (Box 3.3), future studies promise to provide important new insights into how organisms develop, function, interact with each other, and evolve.

BOX 3.3 SUGGESTIONS FOR FUTURE RESEARCH

- Clarify the underlying mechanisms of plasticity. More studies are needed to determine how organisms assess and respond to environmental variation.
- Perform more empirical tests of the theoretical conditions that favor plasticity's evolution. Ideally, such studies should be conducted in both natural populations and experimental evolution studies (Garland and Kelly 2006).
- Determine if, in variable environments, lineages that express higher levels of plasticity are more likely to persist and evolve more rapidly than those that express lower levels. Again, such studies should be conducted in both natural populations and experimental evolution studies.
- Develop the theory, and conduct additional empirical tests of that theory, on whether and how plasticity impacts evolution. More theoretical and empirical work is needed to identify the conditions under which plasticity fails to impact evolution, impedes it, or facilitates it.
- Evaluate whether and how plasticity affects ecology. Because plasticity can enable organisms to respond to other species (competitors, predators, mutualists) rapidly and adaptively, it might thereby influence the composition of ecological communities as well as the selective agents that impinge on its members. More studies are needed, however, to establish whether and how plasticity affects ecology (for example, see Agrawal 2001; Fordyce 2006; Pfennig and Pfennig 2012; Hendry 2016)

ACKNOWLEDGMENTS

For commenting on earlier drafts of this chapter, I thank Karin Pfennig, Nick Levis, Emily Harmon, Andrew Isdaner, Pat Kelly, Sonia Sultan, and Mary Jane West-Eberhard.

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Section II

Causes of Plasticity

From Genes to Ecology

DOI: 10.1201/9780429343001-II

4 Genetic Variation in Phenotypic Plasticity

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CONTENTS

4.1 INTRODUCTION

Most traits of human interest vary among individuals within a population due to a combination of genetic and environmental factors, meaning individuals' phenotypes will depend on both their genotypes and environments. This relationship between genotype, environment, and phenotype is a major area of research that is central to our understanding of genetics in the real world (Falconer and Mackay 1996; Lynch and Walsh 1998). Indeed, whether the goal be to breed crops that maximize yield in particular climates (Kang 1997; Gage et al. 2017; Lowry et al. 2019), to predict, prevent, and treat hereditary disorders (Hunter 2005; Baye et al. 2011; Manuck and McCaffery 2014), or to understand the mechanisms underlying adaptation to changing environmental conditions (Bradshaw 1965; Via and Lande 1985; Scheiner 1993), all of these topics require knowledge of how genotype and environment jointly produce phenotype.

Scientists can learn about the relationship between genotype, environment, and phenotype by studying phenotypic plasticity, the ability of a genotype to produce different phenotypes in response to different environments (Bradshaw 1965; Scheiner 1993; Pigliucci 2001; West-Eberhard 2003). Within genetically diverse populations, distinct genotypes will commonly show differences in plasticity (Ehrenreich and Pfennig 2016). Geneticists dissect this heritable variation in plasticity in order to better determine the impact of environment on the relationship between individuals' genotypes and phenotypes (Debat and David 2001; Rockman 2008; Mackay et al. 2009).

DOI: 10.1201/9780429343001-4

Here, we review genetic research on variation in plasticity. In addition to summarizing key concepts and methods, we also attempt to synthesize current, empirical work in this area and point to key future directions.

4.2 THE GENETICIST'S VIEW OF PHENOTYPIC PLASTICITY

Plasticity arises when the environment alters how physiological and developmental processes impact phenotype (Bradshaw 1965; Dufty Jr. et al. 2002; Schlichting and Smith 2002; Aubin-Horth and Renn 2009; Beldade et al. 2011; Lafuente and Beldade 2019). However, genetically distinct individuals may respond differently to the same environments (Bradshaw 1965; Via and Lande 1985; Scheiner 1993). This heritable variation in plasticity is caused by environmentally responsive genetic polymorphisms (or 'loci') that segregate among individuals (Falconer and Mackay 1996; Lynch and Walsh 1998). Individuals carrying distinct alleles of such loci will exhibit different phenotypic responses to the same conditions (Hunter 2005). Depending on the alleles present, the phenotypic differences between individuals that have distinct genotypes at an environmentally responsive locus may increase, decrease, or change in sign from one condition to another (Figure 4.1b). Because their effects depend on the environment under study, these loci are said to show 'gene-environment interactions' (Figure. 4.1a) (Matsui and Ehrenreich 2016; Wei and Zhang 2017; see also Sultan 2021 in this volume).

Populations usually harbor many loci that interact with the environment (Mackay 2001; Mackay et al. 2009). Thus, variability in plasticity among individuals must be viewed as a result of 'genotype-environment interaction'—i.e., interactions between the environment and individuals' entire complement of environmentally responsive loci (Lee et al. 2016; Matsui and Ehrenreich 2016). Each of these loci may contribute to an overall genotype-environment interaction either 'additively,' 'epistatically,' or both (Gerke et al. 2010; Lee et al. 2016, 2019; Matsui and Ehrenreich 2016; Wei and Zhang 2017). Additive loci have the same effects regardless of the alleles present at other loci (Figure 4.1c). Epistatic loci, on the other hand, participate in genetic interactions with other loci. Consequently, they show different effects depending on the alleles with which they co-occur (Figure 4.1d) (Mackay 2014; Ehrenreich 2017). Genotype-environment interactions may arise due to combinations of purely additive or epistatic loci, or a mixture of the two (Figure 4.1e) (Scheiner 1993; Gerke et al. 2010; Lee et al. 2016, 2019; Matsui and Ehrenreich 2016; Wei and Zhang 2017).

A complete characterization of genotype-environment interaction should also encompass information about the specific genes and genetic variants that contribute to plasticity (Mackay 2001; Mackay et al. 2009). Although most studies do not achieve this degree of precision, it is important to recognize that loci affecting traits represent genetic variants that alter how particular genes function (Schlichting and Pigliucci 1993). At the molecular level, this may involve genetic variants altering transcription, splicing, protein activity, or other aspects of gene function. Of course, the functional impact of a particular genetic variant will depend on the exact nature of the variant at a locus, as well as the identity of the specific gene directly affected by this variant (Gerke et al. 2010; Lee et al. 2016, 2019; Matsui and Ehrenreich 2016).

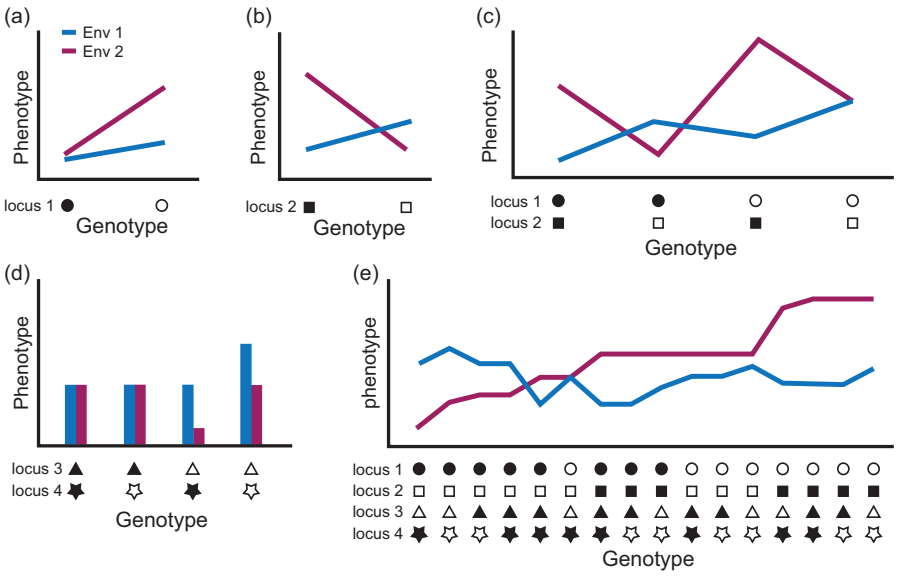

FIGURE 4.1 Examples of gene- and genotype-environment interactions. (a) Locus 1 shows a larger phenotypic effect in response to environment 2 than to environment 1. (b) Locus 2 exhibits a change in effect sign across the two environments. (c) Loci 1 and 2 contribute additively to phenotype across environments. (d) Loci 3 and 4 contribute epistatically to environmental response. Their genetic interaction has a positive effect in environment 1 and a negative effect in environment 2. (e) The relationship between all four loci and phenotype across the two environments. Genotypes are sorted by the rank order of their phenotypes in environment 2. Note that the small effects of each involved locus lead to complicated genotype-phenotype relationships across environments, even within the simplified examples presented here. The different symbols represent distinct loci, while the filled and unfilled versions of each symbol correspond to the two different alleles of each locus.

Because genes usually act in pathways and networks, a genetic variant may also modify the functional relationships between the impact gene and other genes (Ayroles et al. 2009; Civelek and Lusis 2014). There are likely many different mechanisms that can cause loci to show gene-environment interaction, and the continued characterization of loci that cause plasticity can improve our comprehension of these mechanisms.

The above concepts collectively serve as the foundation for understanding the genetic architecture of plasticity. Here, genetic architecture refers to all loci involved in a plastic phenotypic response, as well as their individual effects and interactions with each other and the environment (Hansen 2006; Flint and Mackay 2009). For a given trait, a complete description of the genetic architecture of plasticity would provide a predictive map relating individuals' genotypes and environments to their phenotypes (Gerke et al. 2010; Lee et al. 2016, 2019; Matsui and Ehrenreich 2016; Wei and Zhang 2017). We discuss the methods involved in such work later in this manuscript. We note though, that for a variety of biological and technical reasons mentioned below, obtaining such a complete portrait is difficult in most organisms.

However, large-scale studies in model organisms are beginning to achieve a comprehensive understanding of the genetic architecture of plasticity for certain traits.

4.3 MODIFICATION OF HERITABLE PHENOTYPIC VARIATION BY THE ENVIRONMENT

In this section, we discuss additional concepts that are often used to think about how the genotype-environment interactions that cause plasticity impact the amounts and patterns of heritable phenotypic variation within a population. 'Environmental robustness'—the ability of an organism to express the same phenotype despite environmental perturbation—is of particular importance (Kitano 2004; Felix and Barkoulas 2015). Plasticity and robustness represent opposite extremes of a spectrum, respectively corresponding to states of high and low environmental sensitivity (Figure 4.2) (Ehrenreich and Pfennig 2016; Nijhout et al. 2017; see also Pfennig 2021 in this volume). This view of plasticity and robustness as having an inverse relationship holds in many circumstances, but it is not universally true. For example, some species may evolve to be robustly plastic, e.g., it may be adaptive to express a specific polyphenism in response to particular, repeating environmental cues (Bateson and Gluckman 2011). While we acknowledge this special case, it is not our emphasis here.

Because most organisms live in dynamic environments, they have evolved a wide range of molecular and physiological mechanisms to buffer themselves from

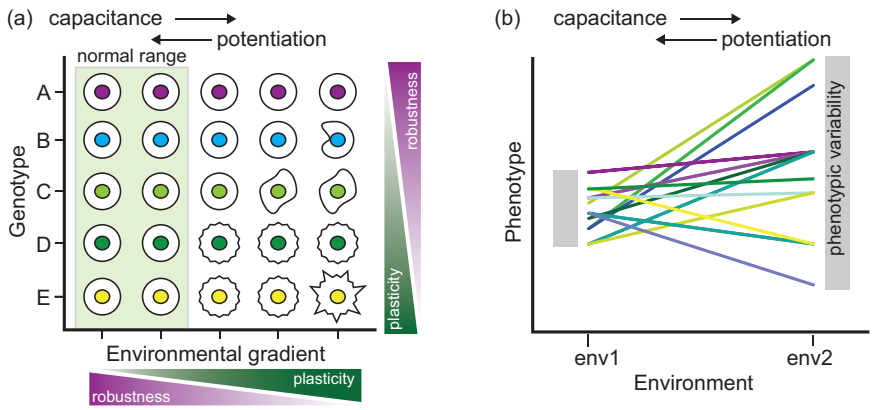

FIGURE 4.2 Robustness and plasticity in qualitative and quantitative traits. (a) Robustness and plasticity can be considered at the levels of a population or different individuals within that population. The *x*-axis demonstrates robustness and plasticity on a population scale. The phenotypic variability (number of external shapes) within the population increases from left to right. On the *y*-axis, individual genotypes show differential sensitivity (i.e., robustness or plasticity) to environmental perturbation. (b) The genotype-environment-phenotype relationships presented in Figure 1e are shown in a different form. Each colored line represents one of the genotypes in Figure 1e. A change from environment 1 to environment 2 results in phenotypic capacitance, an increase in heritable phenotypic variation. The reverse change would result in potentiation, a reduction in phenotypic variability.

common environmental perturbations (de Visser et al. 2003; Felix and Wagner 2008; Levy and Siegal 2008; Masel and Siegal 2009; Frankel et al. 2010; Lempe et al. 2013; Siegal and Leu 2014). However, exposure to novel environments or highly stressful conditions may disrupt the robustness conferred by these buffering mechanisms. This can have consequences for the manifestation of heritable phenotypic variation because the molecular mechanisms that provide robustness to environmental perturbation may also buffer the effects of new mutations and standing genetic variants (Masel and Siegal 2009; Jarosz et al. 2010). When this occurs, over time a population may accumulate genetic variants with muted or 'cryptic' phenotypic effects in the ancestral or normal environmental range (Figure 4.2a) (Gibson and Dworkin 2004; Le Rouzic and Carlborg 2008; Paaby and Rockman 2014).

In a phenomenon referred to as 'phenotypic capacitance,' disruption of robustness by an environmental perturbation can increase the heritable phenotypic variation within a population by impairing the buffering of genetic variation (Rutherford and Lindquist 1998; Queitsch et al. 2002; Bergman and Siegal 2003; Jarosz and Lindquist 2010; Taylor and Ehrenreich 2015). Such environmental perturbations may also modify the effects of loci that were not previously buffered (Figure 4.2). In contrast to phenotypic capacitance, 'potentiation' can occur if an environmental perturbation causes genetic variants to lose their effects or exhibit reduced phenotypic impacts (Figure 4.2) (Jarosz and Lindquist 2010). These phenomena—phenotypic capacitance and potentiation—can substantially modify the relationship between genotype and phenotype, in turn impacting the levels and patterns of phenotypic diversity in a population (Figure 4.2) (Jarosz et al. 2010; Levy and Siegal 2012; Geiler-Samerotte et al. 2013; Schell et al. 2016).

To this juncture, we have discussed robustness as a generic feature of species and populations. However, genetically distinct individuals within a population can in fact vary in their levels of robustness (Queitsch et al. 2012; Felix and Barkoulas 2015; Ehrenreich and Pfennig 2016; Lee et al. 2016, 2019; Mestek Boukhibar and Barkoulas 2016). Differences in robustness among genotypes are caused by loci that individually and jointly render particular genotypes more or less sensitive to environmental change. Variation in robustness among genetically distinct individuals can have significant effects on the manifestation of plasticity across genotypes (Figure 4.2a). Importantly, through a phenomenon known as 'genetic accommodation,' natural selection can act on the genetic variation underlying plasticity to increase or decrease an organism's environmental responsiveness (West-Eberhard 2003). In some instances, this may result in 'genetic assimilation'—the transformation of an environmentally induced trait into a robust phenotype (Waddington 1953; see also Scheiner and Levis 2021 in this volume). Notably, the gain and subsequent loss of plasticity are thought to have a role in the emergence of phenotypic novelty (Baldwin 1902; Schmalhausen 1949; Waddington 1953; West-Eberhard 2003; see also Levis and Pfennig 2021; Pfennig 2021 in this volume), and such changes are likely mediated by loci influencing genotype-environment interaction, plasticity, and robustness.

As a concluding point, it bears mentioning that the concepts and terms presented here are often used differently depending upon the biological context and the perspective of the observer. Such confusion highlights the value of conceptualizing phenotypic plasticity as genotype-environment interaction; a perspective which is

agnostic to whether or not heritable differences in plasticity fit the variable definitions of any given phenomenological case.

4.4 METHODS FOR GENETICALLY DISSECTING PHENOTYPIC PLASTICITY

A large body of theoretical research exists regarding the genetic basis of genotype-environment interaction and phenotypic plasticity (e.g., Via and Lande 1985; Scheiner 1993). In addition, there has also been a substantial effort to empirically address these topics. Often, such work requires 'mapping' the specific loci involved (Figure 4.3) (Fry et al. 1998; Ungerer et al. 2003; Juenger et al. 2005; Gutteling et al. 2007; El-Soda et al. 2014; Lowry et al. 2019), and ideally resolving these loci to the exact genes and genetic variants underlying them (Gerke et al. 2010; Lee et al. 2016, 2019; Matsui and Ehrenreich 2016). After causal loci have been comprehensively identified at high resolution, their phenotypic effects, as well as their interactions with each other and the environment, can be precisely measured (Gerke et al. 2010; Lee et al. 2016, 2019; Matsui and Ehrenreich 2016). These loci can also then be subjected to an additional study aimed at determining the mechanisms by which they affect plasticity.

The first steps in genetic mapping are 'genotyping' and 'phenotyping' (Mackay et al. 2009). Genotyping is the determination of the alleles that a number of genetically distinct individuals possess throughout their genomes. Phenotyping involves measuring traits of interest among genotyped individuals. For work focused on plasticity and genotype-environment interaction, traits must be measured across a number of distinct environments. Once genotypes and phenotypes have been obtained, statistical models can then be employed to identify contributing loci. Historically, the two main approaches for genetic mapping have been 'linkage mapping' and 'association mapping.' Linkage mapping relies on individuals from a known pedigree, which are usually progeny produced by crossing two or more genetically distinct individuals in a controlled manner (Figure 4.3a). In contrast, association mapping employs individuals that have been sampled from nature and have unknown pedigrees.

Linkage and association mapping each have advantages and disadvantages (Mackay et al. 2009). Because linkage mapping is limited to the genetic diversity present in cross parents, association mapping will generally capture more genetic diversity. Also, because natural populations experience many more meioses than occur during the construction of crosses, association mapping will often provide more precise resolution (e.g., Atwell et al. 2010; Brachi et al. 2010; Mackay et al. 2012). Yet, association mapping is confounded by non-causal correlations between genetic variants due to population structure (Pritchard et al. 2000; Zhao et al. 2007), whereas this is a more limited concern in linkage mapping. Furthermore, due to the balanced allele frequencies typically found in laboratory crosses, linkage mapping often affords a superior ability to detect loci (Ehrenreich et al. 2010; Bloom et al. 2013). This advantage is especially pronounced when it comes to detecting epistatic loci, which requires testing on allele combinations (Ehrenreich 2017). Often, the choice of approach will depend on features of the organism under study, such as generation time and amenability to controlled breeding.

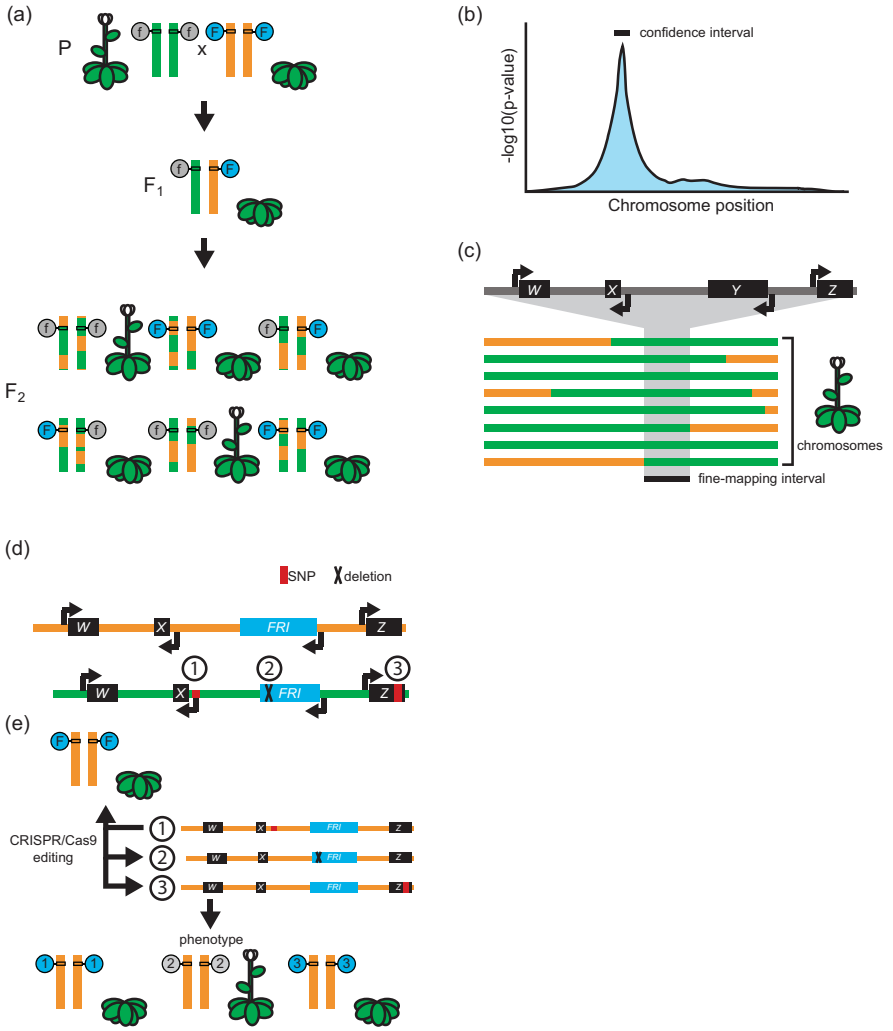

FIGURE 4.3 Mapping loci to the resolution of genes and genetic variants. (a) A typical experimental design for linkage mapping. Two genotyped strains are crossed to produce F_1 progeny, which are subsequently selfed or crossed to each other to produce an F_2 generation. The recombinant F_2 progeny can then be phenotyped for traits of interest, in this case flowering in the absence of overwintering. Often, researchers will inbreed F_2 strains to increase homozygosity and eliminate issues associated with allelic dominance. (b) A linkage scan identifies a highly significant locus on the examined chromosome. (c) Recombination breakpoints present among flowering offspring delimit the locus to four candidate genes. (d) Multiple candidate genetic variants are present in the delimited window of the chromosome. The two parents differ at two SNPs and a deletion. Note that this is an abstraction and real loci often harbor significantly more genetic variation. (e) Genome editing can be used to determine the exact causal genetic variant. Here, each of the candidate genetic variants (1, 2, and 3) is individually introduced into an otherwise isogenic genetic background using genome editing.

Genetic mapping usually detects loci at the resolution of multiple genes and many genetic variants, limiting mechanistic inference (Figure 4.3b–d) (Mackay et al. 2009). Fortunately, there are strategies that can be employed to improve mapping resolution, such as using cross progeny from more than two parents (Kover et al. 2009; Aylor et al. 2011; King et al. 2012) or more than one generation of meiosis (Rockman and Kruglyak 2008; Linder et al. 2016), or by combining data from linkage and association mapping (Yu et al. 2008; Myles et al. 2009). Another option is to use 'site-directed mutagenesis,' a body of techniques for introducing targeted genetic changes into a genome, to resolve a locus spanning multiple genes down to a single gene (Figure 4.3d and e) (e.g., Steinmetz et al. 2002; Taylor and Ehrenreich 2014, 2015; Taylor et al. 2016). However, this approach often depends on the number of genes within a locus being small and the study organism allowing genetic manipulation.

Emerging tools for high-throughput site-directed and random mutagenesis are opening new doors for the genetic dissection of heritable traits, including environmentally responsive phenotypes. For example, improvements in CRISPR/Cas9-mediated site-directed mutagenesis have made it possible to simultaneously explore the effects of thousands of single nucleotide changes in parallel (Roy et al. 2018; Sadhu et al. 2018; Sharon et al. 2018). Edited pools of cells can be grown in different environments to identify exact nucleotide variants that interact with the environment. Another approach with similar potential is 'reciprocal hemizygosity analysis,' a technique that utilizes gene disruption to enable comparison of individuals that carry only one copy of a gene of interest in an otherwise isogenic, diploid genome (Steinmetz et al. 2002). Recently, it was shown that transposon mutagenesis can be used to perform reciprocal hemizygosity analysis on a genome-wide scale (Weiss et al. 2018). Presently, these technologies work best in organisms that are predominantly unicellular, such as the budding yeast *Saccharomyces cerevisiae*, or in cell lines derived from particular tissues.

4.5 EMPIRICAL INSIGHTS INTO THE GENETICS OF PHENOTYPIC PLASTICITY

A number of high resolution studies of natural populations have characterized how segregating loci contribute to phenotypic plasticity and genotype-environment interaction (e.g., Aukerman et al. 1997; Johanson et al. 2000; El-Din El-Assal et al. 2001; Maloof et al. 2001; Werner et al. 2005; Balasubramanian et al. 2006; Filiault et al. 2008; Gerke et al. 2010; Lee et al. 2016, 2019; Matsui and Ehrenreich 2016). Such studies have mainly been conducted in model organisms, such as the thale cress, *Arabidopsis thaliana* and budding yeast, *S. cerevisiae*. These, and other, model organisms possess features that facilitate studying genotype-environment interaction, and are accompanied by deep knowledge bases and powerful toolkits for molecular genetics and functional genomics (Koornneef et al. 2004; Shindo et al. 2007; Ehrenreich et al. 2009a; Liti and Louis 2012; Weigel 2012; Ehrenreich and Magwene 2017). Critically, these foundations make it possible to not only identify causal genes and genetic variants but also situate newly identified genetic factors within biological networks.

Arabidopsis thaliana, the main plant model system, offers numerous advantages for studying the genetic basis of plasticity. This species is predominantly self-fertilizing; thus, strains isolated from nature are typically highly inbred and homozygous throughout their genomes (Koornneef et al. 2004; Shindo et al. 2007; Weigel 2012). Because these strains are genetically stable, they can be grown in parallel in a number of distinct environments, facilitating the study of plasticity using association mapping (Ehrenreich et al. 2009b; Atwell et al. 2010). Linkage disequilibrium decays rapidly in *A. thaliana*, meaning that loci are often detected at high resolution by association mapping (Nordborg et al. 2005; Weigel and Nordborg 2005). In addition, *A. thaliana* has retained the ability to outcross, and strains can easily be mated to each other to facilitate linkage mapping (Ungerer et al. 2003; Juenger et al. 2005). Furthermore, because *A. thaliana* is highly amenable to genetic engineering (Miki et al. 2018), detected loci can also be resolved and validated using mutagenesis techniques.

Linkage and association studies in *A. thaliana* have found an abundance of environmentally responsive genetic variation in nearly all traits, including flowering time (Aukerman et al. 1997; Johanson et al. 2000; El-Din El-Assal et al. 2001; Maloof et al. 2001; Werner et al. 2005; Balasubramanian et al. 2006; Filiault et al. 2008), root architecture (Rosas et al. 2013), pathogen response (Corwin and Kliebenstein 2017), and nutrient utilization (Baxter et al. 2012). Flowering time, in particular, has served as a model phenotype for understanding the genetic architecture of plasticity (Mouradov et al. 2002). Evidence suggests plasticity in flowering time in *A. thaliana* (Zhao et al. 2007; Ehrenreich et al. 2009b; Kover et al. 2009; Atwell et al. 2010; Salome et al. 2011), as well as in other plant species (Gage et al. 2017; Lowry et al. 2019), involves a large number of distinct loci.

Many genes and genetic variants that contribute to heritable variation in flowering time across environments have been identified in *A. thaliana* (Aukerman et al. 1997; El-Din El-Assal et al. 2001; Maloof et al. 2001; Werner et al. 2005; Balasubramanian et al. 2006; Filiault et al. 2008; Ehrenreich et al. 2009b; Atwell et al. 2010). In many cases, these genes are the same genes identified in classic genetic screens focused on flowering time. However, in some instances, the study of plasticity and genotype-environment interaction has led to the discovery of novel, environmentally responsive genes. Arguably, the best example of this is *FRIGIDA (FRI)*, which encodes a component of a regulatory complex that helps suppress flowering until after winter (Figure 4.4a) (Johanson et al. 2000). Many strains carry non-functional *FRI* alleles, meaning they can flower without experiencing overwintering (or vernalization), resulting in a striking example of gene-environment interaction (Figure 4.4a) (Gazzani et al. 2003).

Although *A. thaliana* has provided valuable insights into mechanisms underlying plasticity and genotype-environment interaction in natural environments, this system is more limited in its ability to shed light on the role of genetic interactions. In this regard, *S. cerevisiae* has provided insights that would have been difficult to obtain using other systems (Ehrenreich et al. 2009a; Liti and Louis 2012; Ehrenreich and Magwene 2017; Yadav and Sinha 2018). Yeast grows rapidly under controlled laboratory conditions that are easily manipulated. Additionally, it is possible to culture hundreds, if not thousands, of genetically distinct yeast strains at the same time.

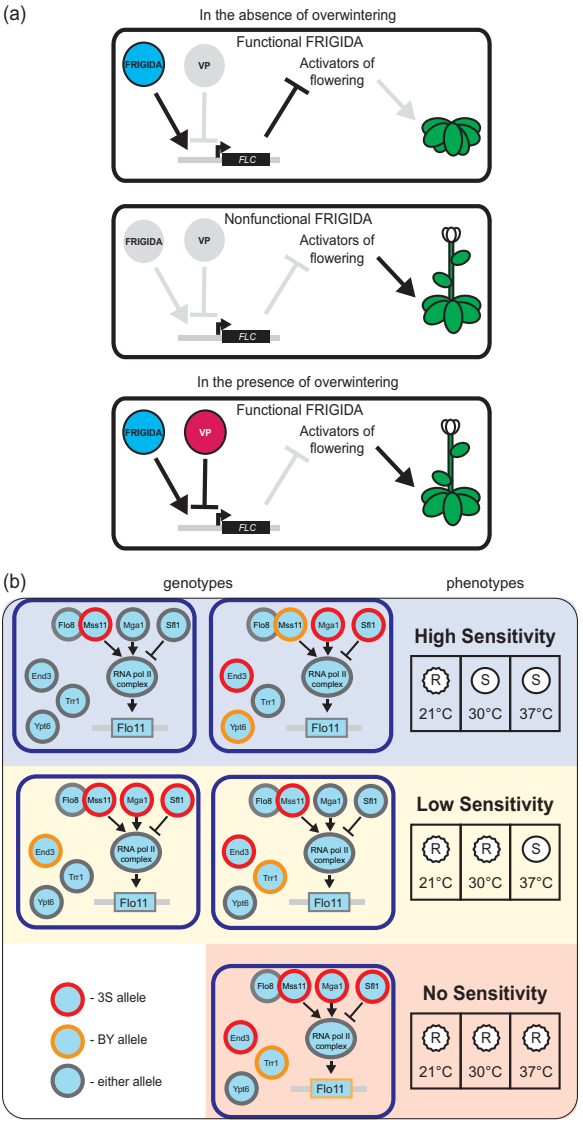

FIGURE 4.4 Genetic mechanisms underlying phenotypic plasticity. (a) A simplified mechanism for plasticity in flowering in *Arabidopsis*. A functional *FRIGIDA* (*FRI*) gene in the absence of overwintering induces the expression of *FLOWERING LOCUS C* (*FLC*), which is a strong repressor of flowering. Without a functional *FRI*, *FLC* is not expressed and flowering proceeds. Overwintering induces the expression of genes in the vernalization pathway. These environmentally sensitive genes counteract *FRI* by inducing repressive chromatin at the *FLC* promoter. Vernalization enables plants to flower by nullifying the effect of *FRI*. (b) Complex genetic architectures of an environmentally responsive rough colony phenotype in a cross of the *S. cerevisiae* strains BY and 3S. Linkage mapping in a yeast cross identified five distinct genotypes composed of seven environmentally sensitive loci that produce rough colonies with varying levels of temperature sensitivity. Most of the involved genes play roles in the same pathway, which regulates the transcription of *FLO11*.

Although association mapping is of limited utility in yeast due to its extensive population structure (Peter et al. 2018), easy cross generation, genotyping, and phenotyping enable statistically powerful genetic mapping via linkage studies (Ehrenreich et al. 2010; Bloom et al. 2013). Thus, with yeast, the genetic architectures of plasticity, including epistatic effects, between a small number of strains can be determined to near completion.

In yeast, evidence supports a model in which environmentally responsive genetic variants in different genes genetically interact to impact the activity of particular pathways that control the transcription of master regulators of cell fate. At least two examples supporting such a model have been comprehensively dissected: one in sporulation (Gerke et al. 2009, 2010) and the other in colony morphology (Figure 4.4b) (Taylor and Ehrenreich 2014, 2015; Lee et al. 2016, 2019; Taylor et al. 2016). In both examples, involved genetic variants interacted with each other and the environment to regulate the transcription of a key master regulator—*IME1* and *FLO11* in the cases of sporulation and colony morphology, respectively. Interestingly, in the case of colony morphology, environmentally responsive genetic interactions modulated sensitivity of the trait to temperature, with some genotypes even showing robustness to temperature (Figure 4.4b) (Lee et al. 2016, 2019).

However, plasticity need not arise through highly epistatic genetic architectures mediated by a single master regulator. Genotype-environment interactions can also involve large numbers of environmentally sensitive loci that exhibit additive effects (Matsui and Ehrenreich 2016; Wei and Zhang 2017) or a mixture of additive and epistatic effects (Bhatia et al. 2014; Yadav et al. 2016). For example, one study in yeast found that genetic variants in unrelated components of the stress response machinery showed additive effects on genotype-environment interaction (Matsui and Ehrenreich 2016). Undoubtedly, other studies that achieve comparable resolution will provide additional examples of distinct molecular mechanisms.

Collectively, studies like the ones described here suggest a number of features regarding the genetic basis of variation in plasticity in natural populations. Plasticity can be controlled by many loci that may interact with not only the environment but also each other. Genetic variants that impact the transcription of key genes may be a common, although not necessarily universal, source of gene- and genotype-environment interaction. This makes sense given that most species harbor an abundance of gene- and genotype-environment interaction in gene regulation (Smith and Kruglyak 2008; Maranville et al. 2012; Grishkevich and Yanai 2013). Yet, it is also clear that there is no singular genetic architecture or mechanism that entirely explains plasticity and genotype-environment interaction in populations. These insights indicate that the genetic basis of plasticity will likely depend on the species, traits, and environments under study.

4.6 THE FUTURE OF THE GENETICS OF PHENOTYPIC PLASTICITY

To this point, we have discussed how genetic variation can interact with the environment to alter phenotype. We have also described how these genotype-environment interactions, visible at the phenotypic level, result from genetic variants altering the molecular function of genes in response to the environment. In this section, we

propose some next steps that have the potential to transform our understanding of the genetic basis of plasticity.

First, non-model species should increasingly be used as focal systems for studying the genetic basis of plasticity. Although the majority of high-resolution research on genetic variation in plasticity has occurred in model systems with well-developed molecular genetics and functional genomics toolkits, important examples exist where researchers have studied the genetic basis of plasticity in non-model organisms (e.g., Sieriebriennikov et al. 2018). Technological advances are reducing the barriers to genetic and genomic research in non-model species and should make it easier to study genetic variation in plasticity in non-model species. For example, progress in sequencing technologies and genome assembly have now made it possible to produce high quality, telomere-to-telomere genomes for almost any organism, as discussed in (Ekblom and Wolf 2014; Simpson and Pop 2015; Jung et al. 2019) and elsewhere. In addition, CRISPR/Cas systems should enable genetic manipulation in nearly any species (Cong et al. 2013; Mali et al. 2013). These technological advances will increasingly facilitate studying the genetic basis of plasticity in non-model species that possess compelling environmentally responsive phenotypes (e.g., Ragsdale and Ivers 2016).

Second, we need a better understanding of how genetic variants that interact with the environment impact entire networks of genes and proteins. Here, it is important to recognize that a genetic variant affecting one gene is likely, in turn, to have downstream consequences for the functions of many other genes. Understanding these downstream consequences becomes especially important when one considers the potential for natural selection to act on and modify plasticity. For example, whether genetic assimilation can occur depends on a system allowing for, and tolerating, genetic variants that promote robustness. Accessing the systems biology of plasticity will likely require combining the genetic mapping approaches described earlier with transcriptomic, proteomic, and other high-dimensionality datasets produced from the same individuals in multiple environments (Sieberts and Schadt 2007; Ayroles et al. 2009; Nadeau and Dudley 2011; Civelek and Lusis 2014).

Third, for multicellular organisms in particular, insight is needed into how genotype-environment interactions impact development. New technologies have made it possible to approach this difficult problem. For example, high-throughput single-cell RNA sequencing enables the generation of transcriptomes from thousands of distinct cells from a given tissue (Macosko et al. 2015; Satija et al. 2015; Briggs et al. 2018; Farrell et al. 2018; Wagner et al. 2018). In addition, CRISPR/Cas9-mediated developmental barcoding provides ontogenetic histories of organs, tissues, and even individual cells (McKenna et al. 2016; Alemany et al. 2018; Kalhor et al. 2018). It may be possible to use these approaches individually or in combination (Kester and van Oudenaarden 2018; Raj et al. 2018; Baron and van Oudenaarden 2019; Wagner and Klein 2020) to learn how genotype-environment interactions modify the developmental process itself. Such research might also provide insights into the relationship between heritable differences in phenotypic plasticity and epigenetic contributions to plasticity.

These ideas illustrate how the next step in understanding the genetics of plasticity is to move beyond standard genetics in model organisms and to begin to better

determine the mechanisms by which genetic variation modifies the relationship between genotype, environment, and phenotype across a broader array of species. Doing this will require characterizing how genetic variants impact the networks underpinning environmentally responsive phenotypes and examining how plasticity manifests during development.

4.7 CONCLUSIONS

Genetically distinct individuals commonly exhibit heritable differences in their phenotypic responses to the environment. This variation in plasticity is a direct result of loci that harbor genetic variants that individually show gene-environment interactions and jointly give rise to genotype-environment interactions. These genetic variants impact the relationship between genotype, environment, and phenotype by modifying how genes function at the molecular level in response to the environment. Because many such loci often segregate within populations, environmental changes can significantly alter the phenotypic variation exhibited by a population, thereby providing a potential substrate for evolutionary change.

Understanding how genetic variation gives rise to these differences in plasticity is important within the contexts of genetics and evolutionary biology. Indeed, such research can shed light on the mechanisms underlying genetic accommodation and genetic assimilation, which, in turn, has the potential to improve knowledge of the environment's role in facilitating the evolution of novel traits. Because genetic variation in plasticity may also impact how populations and species respond to global climate change, research on this topic may also be generally informative regarding the future evolutionary trajectories of life on our planet. Additionally, it is important to recognize that genetic variation in plasticity has bearing on all other areas of biology in which the relationship between genotype, environment, and phenotype matters, including agriculture, medicine, and synthetic biology.

In this paper, we have broadly discussed heritable variation in plasticity from both conceptual and empirical perspectives, emphasizing what is known and what still needs to be learned. Central to this discussion is the importance of understanding mechanism: How do environmentally responsive genetic variants individually and jointly impact the functions of individual genes? How do they affect the activities of pathways, complexes, and networks? And, how do they impact developmental processes? Answering these questions will produce valuable, detailed, descriptive insights into plasticity that can inform all areas of biology and ideally facilitate a future in which our knowledge of plasticity enables the prediction of how organisms will respond to the environment based on their genotypes. We provide some suggestions for future research in Box 4.1.

ACKNOWLEDGMENTS

We thank members of the Ehrenreich lab, Andrew Isdaner, David Pfennig, and two anonymous reviewers for their input on a draft of this manuscript. This work was supported by grant R35GM130381 from the National Institutes of Health to I.M.E., as well as funds from the University of Southern California to I.M.E.

BOX 4.1 SUGGESTIONS FOR FUTURE RESEARCH

- What are the genetic architectures and molecular mechanisms underlying phenotypic plasticity across a broader range of species? Such work can determine the generality of findings to date, such as the observation of high genetic complexity. Application of this work to a diversity of continuous and discrete traits that have experienced varying degrees of natural selection could connect empirical findings about genetic architecture to ecological and evolutionary theory.
- What is the nature of the gene- and genotype-environment interactions that underlie variation in plasticity? It is unclear to what extent environmentally induced changes in heritable phenotypic variation are mediated by cryptic genetic variation as opposed to genetic variation that is already visible. Further work is needed to not only identify at high-resolution loci that contribute to plasticity, but also examine how the effects of these loci change and genetically interact with each other across environments.
- Can we obtain a clearer understanding of molecular, cellular, and developmental mechanisms underlying genetic differences in plasticity? This involves developing a more general understanding of how individual genetic variants contribute to plastic phenotypes across environments. However, it is also necessary to assess how these variants combine to impact the function of pathways and networks. These consequences should be examined within and across cells, tissues, and sexes. The latter work can potentially shed light on how gene- and genotype-environment interactions modify developmental processes in multicellular organisms.
- What are the genetic and molecular bases of genetic accommodation and assimilation? A major reason why genetic variation in plasticity is interesting is because it can potentially help us understand how evolution works. Gene- and genotype-environment interactions contributing to variation in plasticity provide a crucial substrate for selection to act upon to modify the relationship between genotype, environment, and phenotype. Thus, it may be that empirically studying genetic variation in plasticity can provide valuable insights into the mechanisms of genetic accommodation and assimilation.

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5 Physiological Mechanisms and the Evolution of Plasticity

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CONTENTS

5.1 INTRODUCTION AND SCOPE

Phenotypic plasticity is fundamentally a physiological phenomenon. Whether the phenotypic outcome of plasticity is the preservation of form ('homeostasis'), fleeting change, more gradual but reversible change ('acclimation'), or largely irreversible change ('developmental plasticity'), physiology provides the proximate link between the environment and an individual's phenotypic development and expression. A view into the physiological mechanisms underlying plastic traits, particularly plastic traits that have undergone evolutionary divergence, can inform how these traits evolve and the constraints that prevent them from evolving in certain ways.

We begin this chapter by introducing why and how physiology is intimately connected to phenotypic plasticity. We then present examples of how the divergence of physiological mechanisms may have promoted phenotypic divergence in plasticity

DOI: 10.1201/9780429343001-5

among populations and species, a phenomenon that has been studied under the names of 'genetic accommodation' and 'genetic assimilation' (see Scheiner and Levis 2021 and Levis and Pfennig 2021 in this volume). We rely heavily on examples involving organisms derived from natural populations to highlight the ubiquity of physiological evolution and its impacts on phenotypic diversity. In the interest of keeping the chapter focused, we will not be addressing physiological mechanisms in plants, which warrant a chapter entirely of their own (for reviews see Sultan 2000; Gilroy and Trewavas 2001; Herman and Sultan 2011). We then discuss *how* physiological mechanisms and their affected traits evolve (or fail to evolve), sometimes even promoting reproductive isolation. Finally, we argue that characterizing the physiological mechanisms underlying plastic phenotypes can offer us a more nuanced understanding of the evolutionary potential and constraints of phenotypic plasticity.

5.2 HOW IS PHYSIOLOGY INVOLVED IN PHENOTYPIC PLASTICITY?

Most plastic phenotypes are influenced by genetic factors (Pigliucci 2001; see also Goldstein and Ehrenreich 2021 in this volume). At the same time, the ability of organisms to be plastic can itself be modified by the environment (Beaman et al. 2016). Thus, one can argue that most cases of phenotypic plasticity have a genetic and environmental basis. Regardless, there must be some connection between these causal factors and the phenotypes on which natural selection acts. Physiology is likely, in most cases, to be the black box linking these proximal factors to their resulting phenotypes. Further, physiological mechanisms themselves have genetic underpinnings, can harbor genetic variation, and can therefore evolve, producing divergence in plastic phenotypes.

Physiological changes are often at the core of phenotypic responses to environmental variation because physiology is sensitive to environmental change and often systemic. Such changes can thus coordinate responses among traits so that they work together. In particular, endocrine systems are environmentally responsive and can influence several traits simultaneously, sometimes in contrasting ways, through differences in hormone transport, metabolism, and the sensitivity of tissues to hormones. Importantly, many hormone receptors are ligand-gated transcription factors with DNA-binding abilities, such that intercellular signals directly influence gene expression and the corresponding responses in phenotypes (Aranda and Pascual 2001). Further, the modularity of endocrine systems provides multiple ways in which they can evolve to provide evolutionary divergence in plastic traits. Finally, hormones function not only as homeostatic regulators but as signals during development, giving them a special role in developmental plasticity (Nijhout and Wheeler 1982; Dufty et al. 2002). For these reasons, we primarily focus on hormonal mechanisms of plasticity, although they are but one of several physiological systems to consider.

With respect to developmental plasticity, physiological mechanisms can link environmental signals to both continuous and discrete plasticity outcomes. For instance, the butterfly *Bicyclus anynana* is known to display large, bright ventral hindwing eyespots during the wet season, whereas the same eyespots are small and drab during

the dry season. Although these alternate forms are more common than intermediate forms in nature since different broods coincide with different seasons, it is clear that wing patterns change continuously with temperature (Brakefield and Reitsma 1991; Holloway and Brakefield 1995). Differences in temperature, in turn, affect the amounts of ecdysteroid hormones present at a critical larval period during which eyespot formation is determined (Koch et al. 1996; Brakefield et al. 1998). Here, a continuous level of an environmentally sensitive physiological mediator results in continuous phenotypic variation, often referred to as a reaction norm (Figure 5.1).

Alternatively, physiological mediators can evolve such that their relationship with a phenotypic output occurs as a threshold between multiple alternatives, or 'polyphenism' (Figure 5.1). Other species of butterflies that are closely related to *B. anynana*, for example, share ecdysteroid signaling as a physiological mechanism underlying ventral hindwing eyespot formation, yet individuals respond to

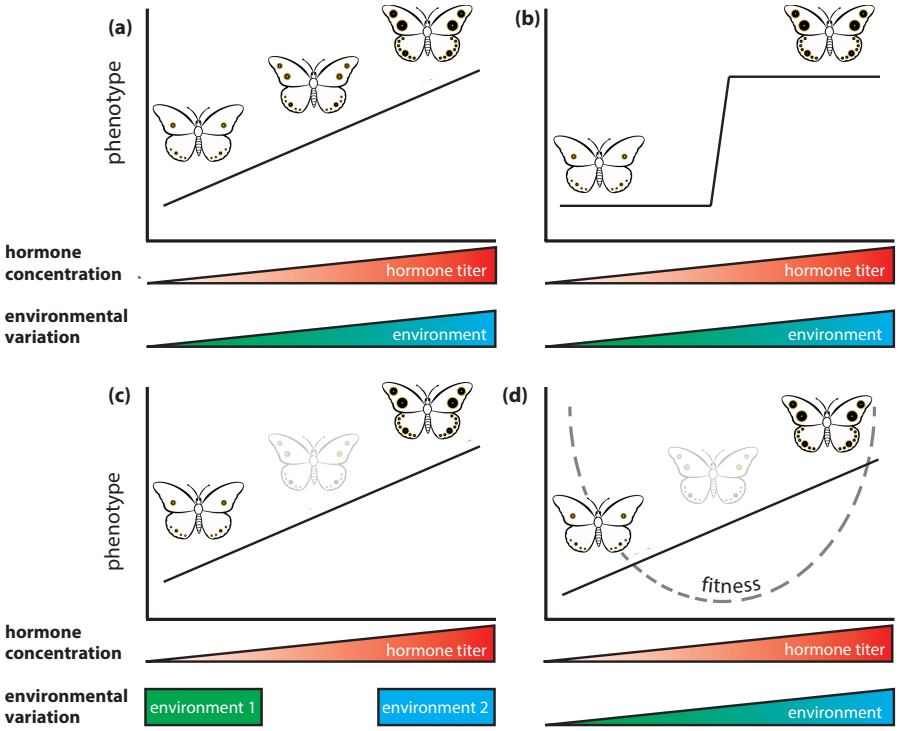

FIGURE 5.1 Continuous and threshold traits and how they relate to physiological mediators. Continuous plasticity, often referred to as a 'reaction norm,' can be generated by a continuous level of an environmentally sensitive hormone (a). Alternatively, the relationship between phenotypic outcomes and as environmentally sensitive hormone can occur in a threshold fashion, often referred to as 'polyphenism' (b). Sometimes polyphenisms are only apparent because organisms encounter discrete environmental variation, as in seasonal polyphenisms (c), or because the fitness of intermediate phenotypes is very low and they are quickly removed from populations (d).

temperature in a threshold pattern where intermediate eyespots are rare even when they are exposed to intermediate temperatures as larvae (van Bergen et al. 2017). Likewise, males of the bull-headed dung beetle *Onthophagus taurus* are either hornless or display fantastic horns if below or above a critical body size, respectively, even though the physiological mechanism informing this decision is a continuous titer of JH (Emlen and Nijhout 1999). Thus, polyphenism does not just require physiological mechanisms in order to convert externally triggered signals into phenotypic outcomes, as does continuous plasticity: it also requires physiological mechanisms that canalize the expression of alternative phenotypes (Sieriebriennikov et al. 2017).

In the following section, we highlight different systems that have been used to understand how the evolution of physiological – often, hormonal – mechanisms have led to divergence in plastic traits. One approach used to understand how this occurs is to compare the physiological plasticity of lineages (populations or species) with a derived trait with closely related lineages lacking that trait, i.e., having the ancestral state. If manipulations of the underlying mechanism in an ancestral-type lineage produce the derived trait, or vice versa, this provides evidence that the physiological mechanism was a crucial mediator of that trait's evolutionary change.

5.3 PHYSIOLOGICAL MECHANISMS PROMOTING THE EVOLUTION OF PLASTIC PHENOTYPES

5.3.1 Mechanisms Promoting Plasticity in Developmental Transitions

The evolution of developmental transitions is a widespread mechanism of phenotypic change in groups ranging from plants to primates (Gould 1977; Raff and Wray 1989). One of the most compelling examples of where tweaks to developmental timing have potentially led to diversification is in salamanders. Ancestrally, the salamander life-cycle begins with an aquatic egg that hatches into a gilled, aquatic larva, which then undergoes metamorphosis to become an air-breathing and terrestrial adult (Duellman and Trueb 1986). From this state, several lineages have independently evolved to become paedomorphic, whereby larvae do not undergo metamorphosis but become sexually mature while retaining their larval form (Alberch et al. 1979; Wiens et al. 2005). It has long been assumed that evolutionary shifts in thyroid hormone (TH) signaling – the key pathway mediating metamorphic transitions in amphibians (Denver 1997; Buchholz et al. 2003) – preceded the emergence of these unique life cycles. Matsuda (1982) noted that some salamander species display facultative paedomorphism in response to their environmental conditions, and he suggested that genetic assimilation of once environmentally sensitive TH signaling was responsible for the evolution of obligatory paedomorphic forms.

At a mechanistic level, these environmentally mediated physiological changes are best understood in the almost entirely paedomorphic axolotl (*Ambystoma mexicanum*). In this species, a pituitary deficiency in TH secretion is controlled by just a few loci, as confirmed by quantitative trait loci (QTL) analysis, and the penetrance of these loci depends on genetic background (Voss et al. 2012; Johnson and Voss 2013). Although the endocrine mechanisms are unknown in the facultative paedomorph *A. talpoideum*, the expression of paedomorphs can be increased using artificial selection

over just four generations (Semlitsch and Wilbur 1989). It seems plausible that, given the presence of heritable genetic variation in a physiological mediator of TH signaling, a population of organisms that were once facultatively paedomorphic like *A. talpoideum* could evolve to be constitutively paedomorphic like *A. mexicanum*. Indeed, stressful environmental conditions will occasionally cause *A. mexicanum* individuals to metamorphose (Smith 1969), possibly a vestige of the phenotype's once environmental control.

The ability to change developmental course when confronted by or anticipating inhospitable environments is featured across all reaches of multicellular life. In vertebrates, this ability is often regulated by the stress axis (Crespi and Denver 2005; Wada 2008). Specifically, in salamanders and frogs, environment-dependent upregulation of the glucocorticoid corticosterone (hereafter, CORT) interacts intimately with TH signaling to accelerate development (Denver 1997; Johnson and Voss 2013; Bohenek 2019). As an example, divergence in CORT production, secretion, transport, or reception may have led to divergence in the relative timing of metamorphosis in a group of frogs called spadefoot toads (families Pelobatidae and Scaphiopodidae). Spadefoot species vary widely in how sensitive their developmental pace is to environmental conditions. At one extreme, Couch's spadefoot toad (*Scaphiopus couchii*) lays eggs ephemeral ponds and is canalized for a very brief larval period. In contrast, the Western spadefoot toad (*Pelobates cultripes*) breeds in long-lasting ponds and has substantial plasticity in developmental timing, while the Mexican spadefoot toad (*Spea multiplicata*) breeds in ponds of intermediate duration and has intermediate plasticity. Commensurate with their variable levels of developmental plasticity, *P. cultripes* and *Sp. multiplicata* respond to pond drying with higher levels of CORT and TH – which work synergistically to accelerate metamorphosis – but the same hormones in *Sc. couchii* do not respond to this environmental stressor (Kulkarni et al. 2017). Thus, the atypically brief and unresponsive developmental speed of *Sc. couchii* may be the consequence of evolutionary divergences in the stress and thyroid axes.

Another example of a plastic developmental transition associated with diversification of life-histories is in nematodes. In several groups of mostly terrestrial species, adverse environments trigger early larvae to enter developmental arrest as an alternative third stage, the dauer larva. The dauer is a metabolically quiescent stage that awaits the return of a favorable environment, often through dispersal to a new one, upon which the animal resumes development. In the model nematode *Caenorhabditis elegans*, the decision to enter dauer is made through physiological cascades that begin with the sensation of external small molecules (ascaroside pheromones) and internal metabolites, followed by signaling through conserved endocrine pathways such as insulin/insulin-like growth factor (IGF), transforming growth factor (TGF)-β, and the steroid hormone DAF-12-dafachronic acid (Hu 2007). In principle, changes to either the induction cues or downstream switches might be selected in response to local pressures. Indeed, genetic variation in this plastic response has been found among populations of a single species (Viney et al. 2003; Green et al. 2013), as have larger differences between deep evolutionary lineages (Ogawa et al. 2011; Bose et al. 2012; Falcke et al. 2018). How these differences correlate with adaptive evolutionary changes is still unknown, though it seems likely that the dauer stage has played a key role in the diversification of nematode life-histories. This is

because of the dauer's association with the invertebrate vectors often needed for dispersal (e.g., Herrmann et al. 2006; Kiontke and Sudhaus 2006): any physiological change that enables a switch to a new dispersal host may provide the initial step in the colonization of previously inaccessible habitats.

Perhaps the most striking example in which the dauer stage may have facilitated the evolution of divergent life-histories is the infective juvenile (IJ/iL) stage of nematode parasites. Several groups of parasites, each with an alternative third stage used to infect their insect or vertebrate hosts, have independently evolved from free-living lineages (Blaxter et al. 1998). Given the long-recognized physiological similarities of IJs to dauers (Rogers and Sommerville 1963), it has been proposed that dauers are a preadaptation for parasitism ('dauer hypothesis'; Hotez et al. 1993). Molecular details of parasites' entry into the IJ stages have provided increasing support for this scenario. For example, in the intestinal parasites *Strongyloides stercoralis* and *S. papillosus* (Strongyloididae), as well as the hookworm *Ancylostoma caninum* (Strongylidae), a dauer inhibitor in *C. elegans*, the hormone Δ7-dafachronic acid, blocks the transition to the IJ stage (Ogawa et al. 2009; Wang et al. 2009). Similarly, more recent RNAi-knockdown experiments in *Strongyloides ratti* confirmed that the decision is made through the hormone's likely target, the Vitamin D receptor homolog DAF-12 (Dulovic and Streit 2019). Genetic perturbations have also shown that the homolog of FoxO in *S. stercorails* controls IJ entry, also similar to the dauer stage of *C. elegans* and *Pristionchus pacificus* (Castelletto et al. 2009). Increasing evidence thus shows it is likely that repurposing the physiological response in a plastic decision has facilitated a radiation of lifestyles in nematodes, including the evolution of parasitism, independently in distantly related clades.

A final example of developmental transitions potentially linked to ecological diversity is another type of dispersal dimorphism: insect wing polyphenisms. Feeding and dispersal polyphenisms are both widespread in insects and perhaps one of the major reasons for their evolutionary success (Simpson et al. 2011). For example, in aphids, females can develop into wingless or winged morphs in response to local cues, and several molecular details of this response's physiology have been characterized, including roles for JH signaling (Hardie 1980), ecdysone signaling (Vellichirammal et al. 2017), and the modification of insulin signaling by micro-RNAs (Shang et al. 2020). It is interesting to note that these signaling pathways, which have ancient roles in molting and growth, have been co-opted to instruct developmental plasticity in other traits such as dauer formation in nematodes (this section), horn development in dung beetles (Section 5.3.2), and eusociality in all insects that possess it (Section 5.3.3). Thus, an issue to resolve with future research is how, at a macroevolutionary scale, physiological systems diverge to produce entirely new developmental functions.

5.3.2 Mechanisms Promoting Plasticity in Morphology

Evolution has offered us an astounding bounty of morphological variation that can be easily measured, not only in living animals but also through fossils, so it is understandable that most adaptive radiations have been studied through the lens of morphology. Physiology can shape morphology, especially during development, so

what is the role of physiological divergence in the evolution of plastic morphologies? One group of fish, pupfish (Cyprinodontidae) that currently inhabit the Death Valley regions of California, has recently undergone morphological divergence as a consequence of both natural and semi-natural experiments (Lema 2020), allowing researchers to answer this question.

Specifically, one species that is endemic to a single pond, the Devil's Hole pupfish (*Cyprinodon diabolis*), has recently evolved to be morphologically distinct from other species, having a small body size, relatively large head and eye sizes, and no pelvic fins (Wales 1930). It was historically suspected that pelvic fin development in this species was in some way responsive to temperature because some *C. diabolis* individuals developed pelvic fins, albeit rarely, while some individuals of *C. nevadensis* – a closely related pupfish species that generally does develop pelvic fins – lacked them (Wales 1930; Miller 1948). More compelling evidence arose for this hypothesis with the establishment of artificial refuges meant to mitigate the extinction risk of this species. Within four years, *C. diabolis* from populations within the refuges, which contained cooler water than Devil's Hole, were much longer than the largest conspecific *C. diabolis* in Devil's Hole and developed relatively shorter heads (Wilcox and Martin 2006).

More recent studies have aimed to determine whether ancestral temperature- and diet-induced plasticity in endocrine mechanisms led to the unique morphologies of *C. diabolis*. To do this, researchers exposed an ancestral-type lineage, *C. n. amargosae*, to environmental conditions experienced by the derived species, *C. diabolis*. In response to more restricted food and modestly higher water temperatures, *C. n. amargosae* individuals were less likely to develop pelvic fins and also developed other attributes typical of *C. diabolis* (Lema and Nevitt 2006). Importantly, larval *C. n. amargosae* that were food-restricted had lower whole-body levels of T_4 (thyroxine, which is converted to biologically active T_3 in tissues), supporting the hypothesis that the observed morphological changes were brought about by temperature- and diet-dependent TH signaling. Finally, these morphological changes, including the loss of pelvic fins, could be recapitulated by treating larval *C. n. amargosae* with inhibitors of TH (Lema 2014). Thus, a close relative of the Devil's Hole pupfish exhibits morphological plasticity in response to energetically challenging conditions that are at least partially mediated by changes in TH signaling. If variation in such hormonally mediated plasticity was also present in the ancestor of Devil's Hole pupfish, that variation may have been selected to produce the species' defining morphological attributes.

Another example of rapid physiological divergence can be found among populations of *Onthophagus taurus*, a beetle that exhibits a dramatic nutrition-dependent polyphenism in horn development. As mentioned above, the common insect hormone JH regulates horn expression in males, with low and high titers of JH resulting in hornless and horned individuals, respectively. Although native to Mediterranean Europe (Balthasar 1963), *O. taurus* has been distributed among different continents in the last 60 years by human introduction (Silva et al. 2016), allowing a comparison of JH titers and its consequences among multiple very recently diverged populations. These populations differ with respect to the degree and timing of their sensitivity to JH and, correspondingly, in the threshold that determines horn production (Moczek

et al. 2002). Specifically, Western Australian individuals induce expression at only very large body sizes, and these individuals also require substantially higher JH titers later during development when compared to their counterparts in the Eastern United States (Moczek and Nijhout 2002). These differences in threshold induction have been maintained in the laboratory after several generations, suggesting that the divergence of a physiological mechanism between the two populations has been genetic.

We have seen now that morphological evolution between two lineages can happen rapidly, but if we zoom out in evolutionary time, we find that physiological mechanisms may ultimately promote a rich, phylogenetic landscape of morphological diversity. An emerging model for studying this is a family of nematodes, Diplogastridae, which – in addition to responding to environments with dauer development – exhibit polyphenism in their feeding morphologies. Nematodes with the polyphenism typically have one morph that has more or larger teeth that enable a broader diet than does its other morph, usually including the consumption of other nematodes as prey (Serobyan et al. 2014; Wilecki et al. 2015). Comparative analyses of form in these nematodes have demonstrated that developmental plasticity is associated with an evolutionary radiation of feeding forms, including those with structural novelties (Susoy et al. 2015). Consequently, mouthparts have likely taken on a wide array of feeding functions in Diplogastridae relative to non-polyphenic outgroups, as both implied by structure (Fürst von Lieven and Sudhaus 2000) and determined empirically (Susoy et al. 2016; Ledón-Rettig et al. 2018).

Was this adaptive radiation spurred by underlying physiological change? Phylogenetic and functional genetic studies have together outlined a program for testing this idea. First, multiple molecular factors making up a developmental 'switch' between two environmentally sensitive morphologies have been identified in one particular diplogastrid model, *Pristionchus pacificus*. Importantly for the study of trait divergence, these factors include genes whose copy number and expression vary among populations and species with different plasticity phenotypes (Ragsdale et al. 2013; Bui et al. 2018). In a study linking polyphenism regulation to morphological effects, Sieriebriennikov and colleagues (2020) discovered a nuclear receptor (NHR-1) that influences both the switch decision and plastic morphology itself. Interrogation of this and other polyphenism regulators (e.g., NHR-40; Kieninger et al. 2016) may thus identify the inter- or intracellular processes the switch controls to instruct alternative forms. Second, comparative studies have begun to determine macroevolutionary changes in both (1) the molecules controlling the switch (Ragsdale and Ivers 2016; Sieriebriennikov et al. 2018; Biddle and Ragsdale 2020) and (2) the molecular targets of the switch (Bui and Ragsdale 2019; Sieriebriennikov et al. 2020; Casasa et al. 2021). Together, these identified changes will enable us to track, in macroevolutionary time, how the physiological mediators of development have influenced morphogenesis in a group with diverse, plastic forms.

5.3.3 Mechanisms Promoting Plasticity in Behavior

To examine how environmentally sensitive physiological changes can result in population or species differences in behavior, we return to the pupfish system. We will recall that an ancestral-type cyprinodont species, *C. n. amargosa*, was used

to demonstrate temperature- and diet-dependent plasticity in morphology (Lema and Nevitt 2006). This subspecies also alters the degree of its aggressive behaviors in response to seasonal variation in population density and water temperature. By comparison, a population of its closely related subspecies that inhabits Big Spring (*C. n. mionectes*), an environmentally stable springhead, is relatively aggressive all year round. A good candidate for studying aggression is arginine vasotocin (AVT), a peptide hormone produced in the preoptic area of the hypothalamus. Among its other roles, this hormone acts with the central nervous system to regulate social behavior (Goodson and Bass 2001). Therefore, researchers set out to determine whether these pupfish subspecies, which have been separated for less than 4000 years, had diverged in AVT signaling to enable differences in territoriality. They found was that pupfish of both sexes from the Amargosa River possessed larger AVT neurons relative to same-sex individuals from Big Spring (Lema and Nevitt 2004b). Further, the researchers functionally determined whether AVT had a role in territoriality by administering AVT to pupfish from both populations; indeed, this treatment caused pupfish to become less aggressive (Lema and Nevitt 2004a).

One of the most striking examples of behavioral plasticity, and a hallmark of eusocial insects, is a division of reproductive behavior (West-Eberhard 1989). In particular, fecund individuals ('queens') exhibit very little care to their offspring while other, less fecund (or often sterile) 'worker' individuals provide sibling care, and the individual development of these alternate forms is largely dictated by environmental conditions. Although eusociality has independently evolved in several insect lineages (bees, wasps, ants, and termites), this extreme developmental plasticity in reproductive behavior is governed by similar physiological mechanisms in all of them (Corona et al. 2016). And although there are important differences in how these mechanisms are regulated among different lineages, in all cases they channel nutritional information into behavioral phenotypes through insulin signaling. Among eusocial lineages, the caste system is a highly evolved developmental system, with a large evolutionary distance from lineages that are not eusocial. This makes comparative studies with ancestral-type populations difficult to conduct. Nonetheless, some research groups have taken creative approaches to shed light on how environmentally sensitive physiological responses have been evolutionarily modified to yield such elaborate societies.

For instance, Chandra and colleagues (2018) studied an ant species, the clonal raider ant *Ooceraea biroi*, which has secondarily lost queens but in which workers display cooperative brood care, such that reproductive asymmetry still exists within colonies. Thus, while this species is derived with respect to reproductive biology, it is similar to the presumed ancestral subsocial state of ants. These researchers focused on the functional role of *insulin-like peptide 2* (*ilp2*) in promoting environmentally dependent reproductive behavior in the ancestral-type species *O. biroi*. The study found that the removal and addition of larval ants up- and down-regulated *ilp2* levels, respectively, and they did so independently of condition, suggesting that social signals *per se* can mediate insulin signaling. Further, by injecting the *O. biroi* ILP2 peptide into workers, the researchers were able to initiate reproduction in these workers, even in the presence of larvae, which are otherwise an inhibitive cue. The findings of this study suggest a plausible scenario in which, during the transition

from solitary to subsocial life, insulin signaling – which is canonically sensitive to nutrition – in adults became responsive to larval signals. Adults with low nutritional stores and ILP2 levels may have been more sensitive to larval signals, suppressing their own reproduction and initiating sibling care. Conversely, adults with high nutritional stores and ILP2 levels would have been less sensitive to larval signals and more likely to reproduce, despite the presence of larvae. In eusocial lineages, a similar type of asymmetry could have been modified by natural selection to ultimately produce sterile workers and obligately reproductive queens.

Another approach to understanding the environmentally sensitive origins of eusociality has been to utilize a species of bee that is facultatively social. For example, the sweat bee *Megalopta genalis* expresses both solitary and social strategies within the same populations, and this facultative expression of sociality is induced by the social and nutritional environment. Specifically, nest-founding females actively adjust the quality and quantity of resources provided to their daughters, which generates physiological differences – again through insulin signaling – that, in turn, govern reproductive behavior (Kapheim et al. 2012; Kapheim 2017). Further, researchers have found that genes involved with the facultative expression of sociality in sweat bees were derived from a set of genes anciently involved in development, supporting the hypothesis that developmental plasticity is intrinsic to the evolution of eusociality (Kapheim et al. 2020). More generally, eusocial insects possess a variety of attributes that make them a promising group in which to determine how environmentally dependent physiological mechanisms contribute to behavioral diversification (Jones and Robinson 2018).

5.4 PHYSIOLOGICAL MECHANISMS, PLEIOTROPY, AND EVOLUTION

Physiological mechanisms are intrinsically pleiotropic in nature: hormones, as an example, typically influence whole suites of traits (Ketterson and Nolan 1999; Nijhout 2003). As a consequence, the phenotypic responses we have described, such as developmental timing and morphology, are not mutually exclusive. For example, selection that has caused divergence in developmental timing among spadefoot toads has gone hand in hand with changes in limb and snout lengths (Gomez-Mestre and Buchholz 2006). Conversely, selection on threshold morphologies in beetles results in a corresponding change in developmental timing because the higher JH levels needed for horn induction also influence molting (Moczek and Nijhout 2002). We begin this section by examining how such physiological pleiotropy influences the evolution of plastic phenotypes; we conclude by exploring how such divergence in physiology can then spur the subsequent evolution of other traits or even reproductive isolation.

5.4.1 Physiological Pleiotropy as a Constraint

The pleiotropic nature of physiological systems means that correlated traits can be at odds with each other. This antagonism would happen if an increase in the expression of a beneficial trait is linked to a corresponding decrease in a different favorable trait or, alternatively, to an increase in the expression of a maladaptive trait. For instance, a hormonal mechanism often implicated in tradeoffs between different male traits is

testosterone, which can augment male reproductive success by helping males secure territories but impedes reproductive success by lowering offspring survival due to its detrimental effects on parental care (Hau 2007). Likewise, increased insulin signaling is thought to increase growth and reproductive output at the cost of a reduced lifespan (Figure 5.2; Dantzer and Swanson 2012).

How might we detect physiological pleiotropy? If selection is acting on variation in the overall levels (i.e., production or release) of a systemic hormone, we might expect that hormone titers vary consistently across species to match their environmental conditions (Vitousek et al. 2019). Further, we would expect that traits influenced by that hormone will respond together. If these traits cannot be easily dissociated from each other, one prediction is that we should see a consortium of traits covarying with hormone titers across species. Such covariance has been studied in a suite of life-history

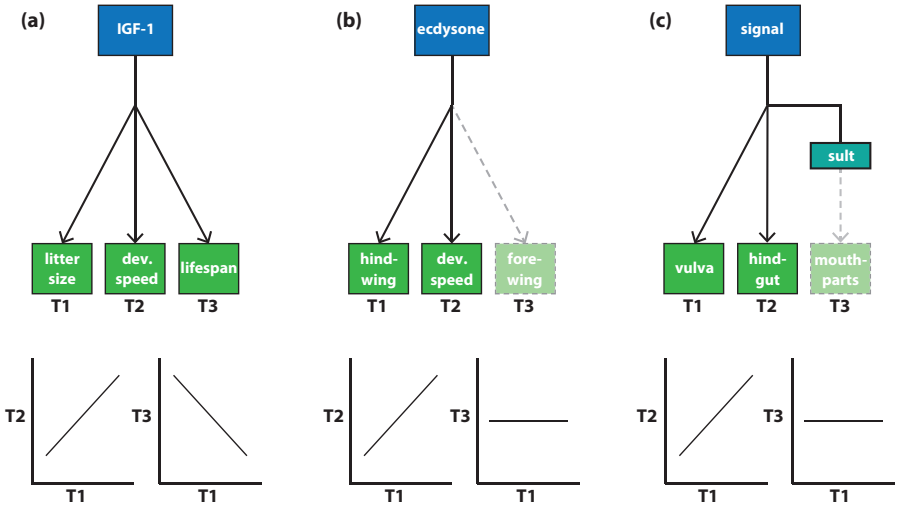

FIGURE 5.2 Breaking physiological pleiotropy. Physiological pleiotropy occurs when a physiological mechanism influences multiple traits and can potentially constrain the evolution of plastic phenotypes. To illustrate, we show systemically acting mechanisms ('signals') in blue, and the hypothetical traits they influence ('T1,' 'T2,' and 'T3') in green. Arrows indicate the influence of a signal on the receiving trait. When traits are linked by a common signal, they will covary as the signal strength changes due to plasticity within species, or selection across species (a). In mammals, for example, if selection favors an increase in IGF-1 because it causes adaptive increases in litter size and developmental speed, it can cause a correlated maladaptive reduction in lifespan. Such pleiotropy can be mitigated via a trait-specific reduction in sensitivity to the signal (b). For instance, ecdysone receptor expression is absent in the forewing spots of *Bicyclus anynana*, rendering them unresponsive to the same environmentally sensitive levels of ecdysone that govern plasticity in hindwing spots. Additionally, pleiotropy can be alleviated by introducing an intermediate modifier (depicted in teal) that dials signal intensity in a trait-specific fashion (c). In polyphenic nematodes (*Pristionchus pacificus*), the receptors NHR-40 and NHR-1, which regulate the expression of alternate mouthparts, are expressed elsewhere in the organism; an intermediate enzyme (sulfotransferase, 'sult') specific to mouthpart-producing cells locally mediates the influence of a hypothetical signal without disrupting other traits.

traits that are influenced by the hormone insulin-like growth factor-1 (IGF-1) (Swanson and Dantzer 2014). This study found that, across mammals, higher levels of IGF-1 were associated with fast life histories, suggesting that the evolution of IGF-1 titers can occur more rapidly than the sensitivities of tissues to it. One interpretation of these studies is that physiological mediators evolve to match environmental conditions, but trait correlations are conserved, potentially limiting adaptive evolution.

5.4.2 Physiological Pleiotropy as an Integrator

An alternative interpretation of the IGF-1 survey in mammals is that the suite of traits linked to this hormone, which operate well together, can be rapidly changed in response to changing selective pressures via tweaks to their common physiological mechanism, thus spurring rapid multivariate adaptation (Ketterson et al. 2009). Genetically correlated traits can synergistically accelerate evolution when selection favors a simultaneous increase or decrease in both (Lande and Arnold 1983; Falconer and Mackay 1996; Agrawal and Stinchcombe 2009). In the same way, a physiological mechanism that influences a suite of traits all performing a common function can promote the evolution of a coordinated response to environmental change (Adkins-Regan 2008; McGlothlin and Ketterson 2008).

That suites of correlated and favorable traits might arise suddenly through a physiological modification and promote adaptation may seem unlikely. However, if past selection pressures have already produced an adaptive and integrated suite of traits, they may still work well together even when they are placed in a new context, whether through plasticity or an evolutionary shift. At one extreme end of this scenario, physiologically mediated suites of traits can be transplanted between life stages or sexes (Raff and Wray 1989; West-Eberhard 2003). For example, sibling care in eusocial systems (described above in Section 5.3.3) may have evolved from the precocious display of maternal care in worker females – which have not yet initiated reproduction – towards siblings instead of their own offspring (West-Eberhard 1987; Linksvayer and Wade 2005). Indeed, a suite of common genes have been implicated in both sibling and maternal care behavior in *Polistes* wasps and carpenter bees (Toth et al. 2007; Rehan et al. 2014). These changes in reproductive behavior have come, not by themselves, but hand in hand with a suite of traits such as sensory perception and foraging (West-Eberhard 1987). Likewise, selection for increased aggression in female hyenas now causes their embryos to be bathed in high levels of testosterone, which promotes a suite of male traits that are now also adaptive (Licht et al. 1998). A rapid, multivariate response to selection may thus be achieved through changes in the strength of a physiological mediator (e.g., a hormone concentration) or by changing the context in which a physiological mediator is deployed.

5.4.3 Breaking Physiological Pleiotropy

Trait correlations are not a necessary upshot of physiological systems. Alternatively, evolutionary adjustments can occur when the responsiveness of traits to a common physiological mediator is easily broken. In this case, the evolution of otherwise linked traits can proceed independently, eschewing maladaptive side effects for the

organism. The ways in which responsiveness can be 'broken' are many, from the way a signal is secreted, broken down, transported, metabolized, or sensed (Nijhout 2003). For instance, hormones are sensed by receptors that can have tissue- and life stage-dependent expression, and without the presence of such receptors a hormone signal will not be seen. Thus, variation in receptor expression among traits can alleviate pleiotropy imposed by a system hormone (Figure 5.2).

Let us return to the butterfly *Bicyclus anynana*, which has highly plastic, ventral hindwing eyespots. As mentioned, ventral hindwing spots in this species differ between two seasonal morphs due to different levels of environmentally dependent ecdysone experienced during their wandering stage. In contrast, ventral *forewing* spots *lack* plasticity, even though they are exposed to the same seasonally dependent levels of ecdysone as ventral hindwing spots. These differences in plasticity between forewing and hindwing spots are enabled by differential expression of ecdysone receptor (EcR) in eyespot centers: at the critical stage of development, EcR is present in the central cells of the hindwing spots of both seasonal forms but is absent in the central cells of the forewing spots (Monteiro et al. 2015). Further, whether this plasticity in eyespots is continuous or discrete, and in what direction it responds to temperature, varies among closely related species (van Bergen et al. 2017), indicating that the evolution of eyespot expression is not physiologically constrained.

Additionally, signaling mechanisms may be modified without requiring changes to signals or receivers, but instead through intermediate modulators. As we have seen in the nematode *Pristionchus pacificus*, mouthpart plasticity is controlled by two receptors, NHR-40 and NHR-1, which directly instruct developmental outputs (Kieninger et al. 2016; Sieriebriennikov et al. 2020). Both receptors are expressed throughout the body of the nematodes and are thus likely regulate several organismal processes besides mouth polyphenism. Furthermore, the DNA-binding activity of at least NHR-40 is highly conserved with other species, ruling out that feature of the receptor as a likely target for selection on plastic phenotypes (Bui and Ragsdale 2019). However, additional enzymes, which have more localized expression and no observed pleiotropy, completely toggle the influence of NHR-40 and NHR-1 (Ragsdale et al. 2013; Bui et al. 2018; Namdeo et al. 2018; Sieriebriennikov et al. 2018). Further, the genes encoding two of these enzymes vary dramatically in their copy number across nematode lineages with the polyphenism (Biddle and Ragsdale 2020). Therefore, it is possible to amplify the effects of tissue-specific signaling modifiers through dosage, whether in copy number or expression level. In this case, selection could act on specific traits without disturbing conserved, system-wide processes.

5.4.4 Variability in Physiological Pleiotropy as Substrate for Evolution

Now that we have addressed both maladaptive pleiotropy that constrains evolution and beneficial pleiotropy that promotes evolution, we will consider what happens when pleiotropy is variable. The effects that physiological responses can have on correlated traits can vary by genetic background, and therefore pleiotropy itself can evolve. Genetic variation in pleiotropy is particularly interesting when considering populations experiencing a novel environmental context. Such a context might cause a hormone to be expressed at a higher maximum, for a longer duration, during a

different life stage, or even in a different sex than it typically is. Because the physiological response has not yet been tested in this new context, selection has not had the opportunity to filter variation in pleiotropic relationships with that physiological response. Thus, when populations encounter novel conditions, they may unleash a reservoir of variation – which previously existed as 'cryptic genetic variation' – via phenotypic plasticity, including variation that is neutral, adaptive, and maladaptive (Ledón-Rettig et al. 2014; Paaby and Rockman 2014) (Figure 5.3). In cases where at least some of the variation is adaptive, this variation can promote trait evolution.

The expression of cryptic genetic variation is often viewed through the lens of buffering mechanisms, which are themselves physiological systems. The classic experiments of Waddington (Waddington 1953; see also Pfennig 2021 this volume), which implied a mechanism buffering standing genetic variation from its expression, would later be realized by more mechanistic studies (Rutherford and Lindquist 1998; Queitsch et al. 2002). When these buffering mechanisms fail due to environmental or genetic stress, the underlying causal genetic variation – in quantitative genetics terms, 'liability' (Falconer and Mackay 1996; Gibson and Dworkin 2004) – becomes phenotypically expressed. Physiological factors such as hormones, which are environmentally sensitive and systemic, are a perfect candidate link between an environmental stimulus and the expression of such underlying genetic variation. For instance, Suzuki and Nijhout (2006) found that, in a population of tobacco hornworm (*Manduca sexta*), mechanisms controlling the regulation of the developmental hormone JH had likely acted as a capacitor for genetic variation in coloration until those mechanisms were disrupted – by genetic and environmental stress – at which point standing genetic variation in the regulation of and sensitivity to JH was revealed in the population.

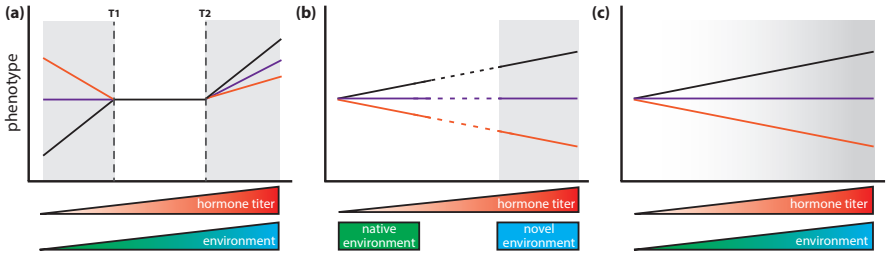

FIGURE 5.3 Physiological mediators as they relate to the expression of cryptic genetic variation. Under native environmental conditions, the effects of a physiological mediator, such as a hormone titer, have uniform or nearly uniform effects on phenotypes across genotypes in a population. If a population experiences extreme environmental conditions (depicted in gray), hormonal titers might be pushed beyond their buffering thresholds (T1 and T2), and variable relationships between genotypes (black, purple, and orange), the hormone, and the resulting phenotypes might be revealed (a). Cryptic genetic variation might also be uncovered when a population experiences a novel and discrete environment, even if the underlying reaction norms are continuous (b). Although it is easiest to model the evolutionary consequences of cryptic genetic variation under the assumption of discrete environments (panels a and b), conditionally expressed genetic variation may, in principle, also be continuous (c). However, the implications of this more biologically realistic variation await better description, both theoretically and empirically.

To explain this scenario in a natural population, we return to spadefoot toads, but this time with respect to a novel larval feeding strategy: predaceous carnivory. In the *Spea* clade, tadpoles have the ability to develop as ancestral-type omnivore morphs, which are passive and feed on decaying plant and animal material; alternatively, they develop as carnivorous and often cannibalistic morphs that are aggressive and specialize on shrimp and tadpole prey (Pfennig 1990; Ledón-Rettig and Pfennig 2011). Experiments have shown that feeding a closely related ancestral-type species the novel diet (shrimp) exposes genetic variation in traits associated with the novel larval feeding strategy (Ledón-Rettig et al. 2010a; Levis et al. 2018). Additionally, consuming the novel diet increases their levels of endogenous CORT in this ancestral-type species (Ledón-Rettig et al. 2010b), and treating them with exogenous CORT while feeding them their native diet recapitulates the increases in genetic variance (Ledón-Rettig et al. 2010a). Taken together, these findings implicate diet-induced changes in CORT as a mechanism mediating the expression of previously cryptic genetic variation, specifically variation that could be selected to allow the evolution of a novel feeding strategy.

5.4.5 How Does Physiological Divergence Promote Diversification?

In this section, we have so far addressed the question of how free or constrained physiological responses are to evolve. A different question is how the evolution of physiological mechanisms promotes subsequent evolution. Here we highlight two processes that can result from physiological divergence – range expansion and differences in reproductive timing – using a North American songbird, the dark-eyed junco.

The evolution of physiological mechanisms can help populations expand into new habitats where they were not previously able to exist, allowing new or relaxed selection pressures to act on traits (Price et al. 2003; Ghalambor et al. 2007). Although dark-eyed juncos (*Junco hyemalis*) inhabit most of North America, they have also colonized a novel, urban habitat within San Diego, California (ca. 1983). This new yet stable population experiences an environment dramatically different from the montane environment: increased noise and light levels, constant human disturbances, and novel predators, all of which are ways that would likely favor a bolder, more fearless personality (Sloan Wilson et al. 1994; Sih et al. 2004). A candidate mechanism that might govern variation in such personality traits is the stress axis: more robust stress responses (e.g., higher levels of CORT) are associated with shy personalities while muted stress responses are associated with bolder, fearless personalities (Koolhaas et al. 1999). Indeed, researchers found that juncos from the San Diego population exhibit bolder exploratory behavior than a nearby (70 km) montane population and, likewise, lower maximum CORT levels (Atwell et al. 2012). Importantly, by detecting persistent differences between the two populations under common garden conditions, they found compelling evidence that the variation in both physiology and associated behaviors is due to genetic divergence, and that rapid evolution of CORT responses and boldness can occur.

Interestingly, the contemporary evolution of physiological mechanisms in juncos that allowed them to expand into a novel environment also indirectly promoted

genetic evolution in other traits. The change in habitat (a milder climate and more abundant food) resulted in colonists experiencing a much longer breeding season. This extended breeding period effectively reduces the social challenges imposed by an annual competition for mates, and males correspondingly produce less testosterone (Atwell et al. 2014). However, while the differences in testosterone between urban and mountain juncos are entirely plastic, differences in plumage patterns – which are typically mediated by testosterone – have genetically diverged between the two populations. In other words, once urban juncos produced atypically low titers of testosterone in response to their novel environment, selection on (or drift in) genetic variation in the sensitivity of plumage to this hormone caused them to diverge genetically from montane populations. Thus, a muted HPA response may have allowed juncos to expand into a new habitat, and novel or relaxed selective pressures introduced by that habitat thereafter resulted in an evolutionary divergence of an unrelated trait.

In addition to geographic isolation, the divergence of physiological mechanisms underlying plasticity in the reproductive timing of conspecific individuals can lead to reproductive isolation (Taylor and Friesen 2017). These differences in reproductive timing (also called 'allochrony') can occur even within a population inhabiting the same geographic area. Although the importance of sympatric speciation is debated, there are compelling examples where such divergence at a physiological level is taking place. For example, two subspecies of juncos seasonally coexist in the Eastern United States during their non-breeding season, foraging together in mixed flocks (Cristol et al. 2003). However, their underlying physiologies begin to change as the breeding season approaches (Fudickar et al. 2016). Even when kept under common garden conditions that mimic changes in photoperiod that occur in late winter, sedentary dark-eyed juncos (*J. h. carolinensis*) produce higher levels of testosterone and larger testes to prepare them for breeding, while migratory juncos (*J. h. hyemalis*) possess larger fat stores to help them prepare for migration (Fudickar et al. 2016). The physiological differences between the subspecies' response to photoperiod likely inhibit gene flow between migrants and residents, thus creating the potential for reproductive isolation.

5.5 CONCLUSIONS

In this chapter, we have highlighted examples where environmentally sensitive physiological responses generate variation within populations and may have even spurred trait diversification among lineages. Given that variation in physiology can be selected upon to produce evolutionary change in laboratory experiments (Suzuki and Nijhout 2006) and that the same process is often invoked in adaptive radiations (Pfennig et al. 2010; Susoy et al. 2015), a major goal now is to connect our laboratory observations with the rich diversity we see in nature (see Box 5.1). Towards this goal, as seen in our examples, physiological divergence is being studied in populations undergoing dramatic environmental change – and commensurate changes in selective regimes – due to the hand of human intervention, whether that be climate change (see Diamond and Martin 2021 in this volume), transportation, urbanization, or even restoration efforts. Under these accelerated conditions, it may be possible to characterize the processes that occur over longer evolutionary timescales.

Because evolutionary diversification through plasticity-led evolution is still a controversial topic (Laland et al. 2014; see also Futuyma 2021; Levis and Pfennig 2021; and Schlichting 2021 in this volume), we propose that a better view into the physiological mechanisms governing plasticity, both within and across lineages, will offer a more nuanced understanding of the potential and the limits of this process. In Box 5.1, we offer some suggestions for future research.

ACKNOWLEDGMENTS

We thank two anonymous reviewers for their comments on the manuscript. We also acknowledge support from the National Science Foundation (grants DEB-1754136 to C.C.L.-R. and IOS-1911688 to E.J.R.).

BOX 5.1 SUGGESTIONS FOR FUTURE RESEARCH

- What are the molecular mechanisms underpinning physiological plasticity and how do they evolve? Understanding where heritable variation in physiological responses underlying plasticity resides in a developmental pathway can tell us:
  - How pleiotropic a physiological response is likely to be: is it high in the chain of regulatory logic and likely to influence many other traits, or is it terminal and likely dissociable from its ancestral pathway?
  - Whether there is bias in how physiological plasticity evolves: does plasticity preferably evolve at a certain node in a physiological pathway? There are at least two ways to approach this question. First, given a clade of organisms that vary in their physiological responses and corresponding plastic phenotypes, one could comparatively quantify, at a molecular level, how the components of a physiological pathway have been modified to produce diversity (e.g., via gene amplification, the addition or subtraction of modifier loci, protein evolution, or novel genes). Second, given a tractable laboratory system, one could use artificial selection to determine whether the evolution of plasticity occurs repeatedly by the same or different modifications.
- Does physiological pleiotropy generally constrain or facilitate the evolution of plastic phenotypes? It can be difficult to delineate when a common physiological mechanism covaries with traits because it is constraining phenotypes or because it has helped promote the diversification those phenotypes. One approach suggests measuring the fitness of individuals alongside physiological parameters and their correlated traits over time (Dantzer and Swanson 2017). This would require extensive effort in many systems but would potentially reveal the evolutionary consequences of physiological pleiotropy.

- How do physiological responses that affect multiple life stages evolve? The pleiotropy of physiological responses extends not only across traits but also across life stages: when the environment elicits a plastic response in early life, it can persist as a 'carry-over' effect even after individuals have transitioned to a new life stage. Even when a physiological response is adaptive early during life, this response may have a different effect on fitness later during life. Yet, there is little empirical evidence to demonstrate whether carry-over effects can evolve to become more or less sensitive to early life conditions. Quantifying how selection pressures jointly act on physiological plasticity during early and late life stages will be difficult given that (1) environmental conditions may fluctuate across generations and (2) populations may be in intermediate stages of evolution such that adaptation may not be complete (Moore and Martin 2019). Nonetheless, understanding the relationships between the mechanisms underlying carry-over effects and their fitness repercussions can reveal how they evolve and diversify.
- How do physiological responses to novel environments interact with standing genetic variation? Understanding where the raw phenotypic variation for phenotypic change – and even evolutionary innovations – comes from is an outstanding question. However, there is mounting evidence that, in many cases, this variation is preexisting, heritable, and environmentally sensitive (i.e., is cryptic genetic variation). Understanding the physiological systems gating this reservoir of phenotypic variation in natural populations can yield a mechanistic insight into the origins of novel trait expression, and potentially allow us to *predict* in which other systems and situations this process might be important.
- In situations where lineages have recently split, demonstrating that the same physiological mechanism mediating intraspecific plasticity is responsible for interspecific trait variation could offer an additional method to test hypotheses of genetic accommodation (West-Eberhard 2003). This may not work for long-separated lineages, as underlying mechanisms for trait expression can diverge over evolutionary time. However, demonstrating a common underlying physiological basis to plasticity between lineages may be particularly useful for instances of contemporary evolution.

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6 Ecology and Evolution of Plasticity

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CONTENTS

6.1 INTRODUCTION

Adaptive phenotypic plasticity allows organisms to cope with a range of environments through adjustments in a range of traits, from gene expression and physiology, to behavior and morphological development (West-Eberhard 2003; Sultan 2015;

DOI: 10.1201/9780429343001-6

Gilbert and Epel 2015; see also Pfennig 2021; Sultan 2021 in this volume). The degree of adaptive plasticity varies both within and across species, and such variation in plasticity has implications for understanding why some organisms survive and diversify in novel environments (Sol et al. 2005; Pfennig et al. 2010; Diamond and Martin 2021; Levis and Pfennig 2021). Thus, biologists have long been interested in the ecological and evolutionary drivers of plasticity. Why are some organisms more phenotypically flexible, while others are relatively more fixed in trait expression? The parallel literature on variation in niche breadth has considered a similar question: Why do some organisms use a range of environments and resources while others are more specialized (Futuyma and Moreno 1988; Richards et al. 2006; Sexton et al. 2017)?

In this chapter, we focus on the ecological and evolutionary causes of variation in adaptive phenotypic plasticity versus a more specialized strategy (Figure 6.1). We first review the evolutionary drivers of plasticity, evaluating the classic role of environmental variation relative to other factors such as novel, extreme environments. Second, we review the costs and limits associated with phenotypic plasticity and review how

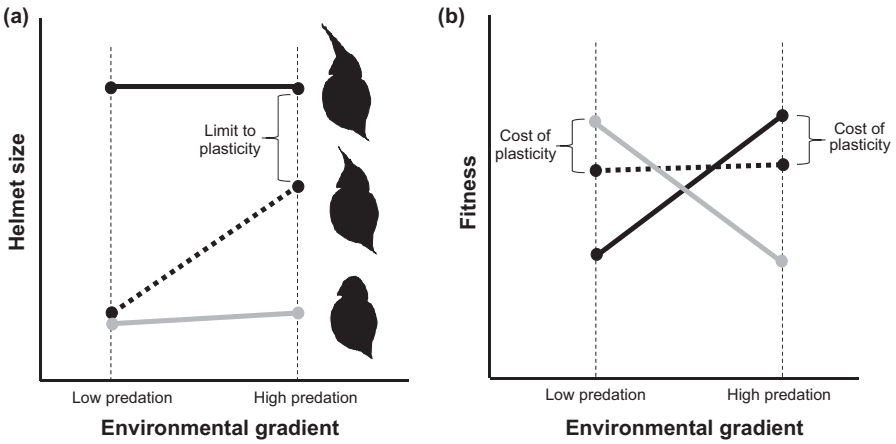

FIGURE 6.1 Variation in adaptive phenotypic plasticity. To illustrate the costs and benefits of plasticity, consider the zooplankton *Daphnia*, which induces a protective spine in response to predator cues. Panel (a) uses reaction norms to illustrate variation in plasticity – The plastic genotype (indicated by the dotted line) induces spines in the predator environment, in contrast to the fixed genotypes that are specialized to low or high predation environments (solid gray or black lines, respectively). The difference in spine length between the plastic genotype and the fixed genotype represents a limit to plasticity. Panel (b) shows the fitness of each genotype (e.g., offspring number, lifespan, adult body size). Adaptive plasticity should translate into maintenance of relatively high fitness across an environmental gradient (as shown here). A cost of the ability to be plastic is represented by lower fitness of the plastic genotype (dotted line) relative to the specialist genotype in the environment where the plastic trait is not expressed (solid lines). While there is a cost of the induced phenotype itself, the benefits of plasticity result in a net fitness benefit relative to the spine-less specialist in the high predation environment. However, any developmental or genetic limits to plastic spine production should result in relatively lower fitness of the plastic genotype when compared to the specialist spine producer. While this example shows reaction norms as genotypes, the same approach can be used for individuals, populations, or species.

empirical findings have challenged our conceptual understanding. Finally, we discuss exciting areas of future research. Throughout this chapter, we also consider the parallel literature on variation in niche breadth, as in many cases, variation in the degree of generalization is due at least in part to underlying plasticity.

6.2 SELECTION FOR PLASTICITY: ENVIRONMENTAL VARIATION AND ENVIRONMENTAL EXTREMES

We first review the conditions expected to favor the evolution of plasticity: variable or novel environments. Experiments in both the lab and field provide support linking the degree of environmental variability to plasticity, but also show that plasticity does not always evolve when expected, pointing to the importance of costs and limits. We often treat characteristics of the environment, such as variability and cue reliability, as external to the organism. However, organismal traits such a dispersal and diapause influence these selective drivers of plasticity, resulting in opportunities for complex feedbacks in the evolution of plasticity.

6.2.1 Theory Emphasizes the Importance of Environmental Variation

Phenotypic plasticity is generally favored when the environment varies and different phenotypes are optimal in different environments, especially when costs of phenotype adjustments are low and predictive environmental cues are present (Scheiner 1993; Schlichting and Pigliucci 1998; Berrigan and Scheiner 2004; Scheiner 2020). However, the time scale, or temporal graininess of environmental variation, matters (Figure 6.2). Developmental plasticity is particularly favored when the environment changes across generations, but is relatively consistent within generations ('coarse-grained variation,' Levins 1968; Van Tienderen 1991; Moran 1992). When the environment changes within a generation, more continuous phenotypic adjustments are favored, such as context-dependent expression of behaviors or enzymes ('fine-grained variation,' Bradshaw 1965; Piersma and Drent 2003; Snell-Rood 2013). When conditions change over longer time frames but are consistent across successive generations, transgenerational plasticity can be adaptive (Figure 6.2; Day and Bonduriansky 2011; Bell and Hellmann 2019; see also Bonduriansky 2021 in this volume). While variation tends to favor plasticity, extreme variation can disfavor such strategies. Highly variable environments, especially when reliable cues are lacking, result in the evolution of bet-hedging (Tufto 2015) such as stochastic gene expression in microbes (Veening et al. 2008) or dormancy periods in desert annuals (Gremer and Venable 2014).

Theoretical models show that both temporal and spatial environmental variation can play a role in the evolution of plasticity (Berrigan and Scheiner 2004). Plasticity is usually favored in the face of temporal variation with reliable cues, but specialization may emerge in spatially variable conditions if one environment is more common or if patterns of selection across environments are very different (Van Tienderen 1991; Scheiner 1993). As dispersal between spatially variable environments increases, population-level selection on plasticity increases; when migration rates are lower, spatial variation results in a relative increase in the strength of local selection (Scheiner 1998). Models that integrate both spatial and temporal variation suggest that spatial variation can be a

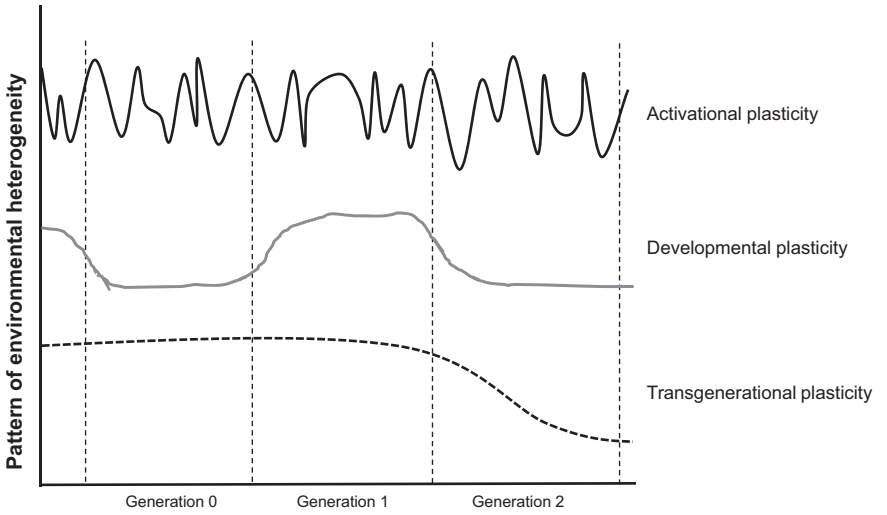

FIGURE 6.2 Patterns of environmental variability and forms of plasticity. The environment can vary over space or time. Fine-grained environmental variation (top) refers to environmental variation within a generation and favors the evolution of 'activational' plasticity (the activation of underlying pre-existing networks, such as behavior or physiology) or forms of developmental plasticity that are reversible. Fine-grained variation can also select for fixed average traits or bet-hedging if the rate of change is rapid, unpredictable, or 'cue-less.' Coarse-grained variation refers to environmental change where environments are relatively constant within generations. Such variation can be due to relatively slower environmental change, or organismal traits such as habitat choice that increase environmental constancy within generations. This form of variation tends to favor developmental plasticity, where early cues on the state of the environment trigger different developmental trajectories, which tend to have broader, more integrated phenotypic effects that are often less reversible. Finally, when rates of environmental variation are very slow across generations, there may be selection for transgenerational plasticity, where parental cues or conditions shape the development of offspring.

more potent driver of plasticity, but how they combine depends on the life history of the organism (Scheiner 2013). Across these models, theory shows that environmental variation tends to favor the evolution of plasticity, but also combines with other factors, such as costs of plasticity and cue reliability, as we discuss more below.

6.2.2 Empirical Data Suggests Environmental Variation Is Important, but Not Entirely Predictive

Empirical studies support the prediction that plasticity should be higher in genotypes that experience greater environmental variation. Genetic variation in plasticity has been linked to the degree of environmental variation in both abiotic and biotic factors (e.g., Van Kleunen and Fischer 2001; Baythavong 2011). Species with higher dispersal rates, which tend to experience greater spatial variation also tend to show greater developmental plasticity (Hollander 2008). Similarly, species with greater

diet breadth often exhibit greater behavioral plasticity (Hoedjes et al. 2011). While environmental variation often correlates with variation in plasticity across species and populations, in some cases it explains little to no variation in plasticity (Karban and Nagasaka 2004), reinforcing the idea that environmental variation is only a piece of the equation in the evolution of plasticity.

Experimental evolution studies also provide empirical support for the expectation that environmental variation favors the evolution of plastic generalists (Kassen 2002). Some experimental evolution approaches suggest that the evolution of plasticity may be more likely in the face of temporal, rather than spatial, variation (Reboud and Bell 1997; Condon et al. 2014), and the time scale of that variation (fine- or coarse-grained) may matter less than expected (Kassen and Bell 1998). While environmental variation tends to favor the evolution of plasticity in the lab, there are just as many cases where predicted patterns of plasticity and wider niche breadth do not evolve in variable environments (Riddle et al. 1986; Bell 1997; Jasmin and Kassen 2007), suggesting the importance of costs and limits in the evolution of plasticity.

6.2.3 Evolution of Plasticity as a Byproduct of Strong Directional Selection

Much of the literature has stressed the importance of environmental variation as a driver of the evolution of plasticity. However, selection in novel or extreme environments, regardless of current levels of environmental variation, can also result in the evolution of plasticity as a byproduct (but see Scheiner and Levis 2021 in this volume). Theory suggests that in extreme environments, outside of the range of recent environmental variation, there may be selection on plasticity as it shifts populations to the new fitness peak (Lande 2009; Chevin and Lande 2010; Chevin et al. 2013). This is similar to other models suggesting that selection away from the population mean is more likely to result in the evolution of plasticity (Gavrilets and Scheiner 1993). Such theory has been offered as an explanation for why experimental evolution sometimes results in an increase in plasticity or phenotypic variance (e.g., Falconer 1990; Czesak et al. 2006; Garland and Kelly 2006). Similarly, artificial selection close to ancestral mean phenotypes results in the evolution of specialists, while selection in more extreme novel environments results in plastic generalists (Hughes et al. 2007). Outside of the lab, extreme novel environments (such as cities) select for behavioral plasticity through both species sorting (Sol et al. 2008; Maklakov et al. 2011) and population differentiation within species (Snell-Rood and Wick 2013). Across these cases, plasticity emerges in more extreme environments but not necessarily in more variable environments. Thus, understanding the evolution of plasticity requires considering not only current patterns of environmental variation but also the difference between ancestral and current conditions.

6.2.4 Organismal Traits Structure Environmental Variation

Predictions about the evolution of plasticity often treat environmental variation as a force external to an individual organism. Some of the disconnect between theoretical expectations and diverse empirical findings likely stems from the fact

that organisms choose and modify their environment such that our measures of environmental variation are poor measures of the variation organisms are actually experiencing (Sultan 2015, Snell-Rood and Steck 2019). Behavioral and physiological traits that influence how organisms experience environmental variation can themselves evolve, setting the stage for complex evolutionary feedbacks (Kylafis and Loreau 2008; Scheiner 2016). In some cases, individual behavior may increase environmental variation, such as neophilic or exploratory behaviors (Greenberg 1990; Tebbich et al. 2009), which could result in positive feedback in the evolution of plasticity, until associated costs take effect. More commonly, individual behavior tends to decrease experienced environmental variation such that an otherwise complex or variable environment is more stable or predictable. For instance, dispersal behavior, coupled with habitat preferences, reduces habitat heterogeneity experienced by an organism (Kotliar and Wiens 1990), and diapause or microhabitat selection can buffer temperature variation (Huey et al. 2003). In many cases, individuals choose habitats based on their own phenotypes or previous experiences (Davis 2008; Edelaar and Bolnick 2012). Such habitat selection or modification tends to reduce environmental variation and drive the evolution of specialization (Ravigne et al. 2009). Combining behaviors that influence environmental variation with important details of life histories, dispersal, and natural history offer more informed predictions for the evolution of plasticity (Scheiner et al. 2012; Scheiner 2016).

6.2.5 Selection on Plasticity Is Limited by Cue Reliability

Environmental variation tends to set the stage for the evolution of plasticity. However, plasticity is favored in variable environments only when there are reliable cues about the state of the environment (Moran 1992; DeWitt et al. 1998). In other words, plasticity should evolve when future environmental states vary *and* cues received in the present provide some reliable information about environmental states in the future (McLinn and Stephens 2006; Botero et al. 2015). If this condition is not satisfied, plastic organisms risk expressing phenotypes that are poorly matched to their environments. Thus, a major limit to the evolution of phenotypic plasticity is the degree to which available cues are reliably predictive of future environmental states.

However, just as organismal traits structure environmental variation, they also influence cue reliability. Organisms are bombarded with a wealth of information on the state of the environment, but, in an evolutionary sense, they have some degree of 'control' in the specific cues they 'use' in developmental decisions. Organisms may increase their certainty in evaluating the current state of the environment by paying close attention to individual cues (Szpiro and Carrasco 2015; Kurtz et al. 2017) or using a combination of different cues (Munoz and Blumstein 2012). Cue use itself is also evolving, and organisms focus their attention on more reliable cues (Dunlap and Stephens 2014) and attend to these particularly relevant cues during certain developmental windows (Bateson 1979; Fawcett and Frankenhuis 2015). Thus, cue reliability can itself evolve and likely carry associated costs, setting the stage for complex feedbacks in the evolution of plasticity.

6.3 COSTS AND LIMITS SELECT AGAINST PLASTICITY, BUT ARE OFTEN LOWER THAN EXPECTED

Variable and extreme environments can drive the evolution of plasticity, especially if reliable and predictive cues of environmental states are available. We next review the costs and limits that select against plasticity (DeWitt et al. 1998; Callahan et al. 2008; Murren et al. 2015). Interestingly, literature searches suggest that existing research focuses on the costs and limits of plasticity more than twice as often as the drivers of plasticity. 'Costs' represent fitness tradeoffs associated with plasticity, while 'limits' on plasticity stem from situations where the plastic genotypes cannot achieve the same phenotype as fixed genotypes (DeWitt et al. 1998; Auld et al. 2010). For example, in *Daphnia*, a cost of the ability to induce a spine in response to a predator would be detected as a lower fitness of the plastic genotype in the predator-free environment relative to a specialist that does not express a spine (Figure 6.1). However, a limit to plasticity would be seen as the plastic genotype inducing a less pronounced spine relative to a specialist that produces a spine across all environments. Depending on the traits measured, an experiment may detect fitness costs that stem from a phenotypic limit (Figure 6.1); considering the underlying mechanism of costs and limits can provide insights into the processes influencing the evolution of plasticity.

6.3.1 Conflict between Theoretical Expectations of Costs and Empirical Observations

Models of the evolution of plasticity show that costs of plasticity reduce the likelihood of plasticity evolving, often favoring the evolution of a fixed average phenotype or specialization (Scheiner 1993; Schlichting and Pigliucci 1998; Sultan and Spencer 2002; Berrigan and Scheiner 2004). In considering the evolution of plasticity, we must distinguish between costs of an induced plastic phenotype and costs of the ability to be plastic (DeWitt et al 1998; Callahan et al. 2008; Murren et al. 2015). Many traits induced through plasticity are themselves costly, for instance the fitness tradeoffs associated with growing a defensive spine in *Daphnia* (Black and Dodson 1990), or the costs of upregulating chemical defenses in plants (Cipollini et al. 2014). The trait- or environment-specific costs of plastic or induced phenotypes are rampant. However, costs of the *ability* to be plastic are thought to be of primary importance in explaining genetic variation in plasticity because: (1) trait-specific costs should also be experienced by fixed genotypes expressing that trait, and (2) trait-specific costs are often offset by the benefits of an induced trait in a particular environment. We focus our discussion on the costs of the ability to be plastic, noting that trait-specific costs can be important limits in the evolution of plasticity when developmental time lags create phenotypic mismatches (Padilla and Adolph 1996).

A large body of empirical work has sought to measure the costs of the ability to be plastic. In some cases, studies have found these expected costs. For instance, in radish, the ability to mount an induced defense in response to predation is costly in terms of lifetime fruit mass (Agrawal et al. 2002). In frogs, the ability to alter developmental timing in response to pool drying is costly in terms of size at metamorphosis,

at least in some populations (Merila et al. 2004). However, empirical studies often do not find the expected costs of plasticity. For example, there are few to no costs of the ability to induce a defensive spine in *Daphnia*, despite costs of the spine itself (Scheiner and Berrigan 1998). Similarly, there are weak to absent costs of predator-induced plasticity in frogs (Steiner and Van Buskirk 2008). Overall, meta-analyses across 27 studies show that while costs of plasticity are detected more often than expected by chance, overall, they are infrequent (<30% of tests for costs) and their effects are relatively small ($|\text{selection coefficients}| < 0.1$, Van Buskirk and Steiner 2009). In addition, costs of plasticity tend to be higher when traits are measured in stressful conditions (Van Buskirk and Steiner 2009). For instance, in mustards, a cost of the ability to adaptively adjust leaf area with light is only seen under low light conditions (Steinger et al. 2003).

The literature on costs and tradeoffs of niche breadth (Futuyma and Moreno 1988; Sexton et al. 2017) provides similarly varied evidence for costs. There is ample evidence for the often-assumed generalist-specialist tradeoff (e.g., Kelly and Bowers 2016; Thuy et al. 2016), but there are often cases where costs are lower than expected, or completely absent (Dutilleul et al. 2017; Fukano and Nakayama 2018). Experimental evolution studies of adaptation to alternate environments overwhelmingly show tradeoffs in fitness between environments, but detecting costs of simultaneous adaptation to both environments is less likely (Kassen 2002). We next explore the mechanisms that may generate costs, and explanations for why costs may be less common than expected.

6.3.2 When We See Costs, Where Are They Coming From?

Fitness tradeoffs of plasticity and wide niche breadth can come from a number of underlying mechanisms (DeWitt et al. 1998; Auld et al. 2010). Costs of plasticity refer to fitness tradeoffs of the ability to be plastic (Figure 6.1), and some of the best examples stem from developmental costs of plasticity itself. Plastic genotypes, relative to specialist genotypes, must sense cues about the state of the environment, process those cues, and develop the appropriate matched phenotype. Regardless of the actual phenotype, this process can be costly, especially for forms of plasticity that develop through learning-like processes ('developmental selection,' Snell-Rood et al 2018). For instance, generalist insects that use a range of host plants must attend to a wider range of cues than specialists, which takes time and divides attention thereby potentially increasing the risk of predation (Bernays 2001; Dukas 2002). Organisms that adjust their behavior through learning must spend time and energy sampling the environment and consolidating information (Dukas 1998; Dukas 2019). The associated neural machinery and process of forming long-term memories are metabolically costly (Laughlin et al. 1998; Mery and Kawecki 2005), and large brains result in exponential increases in developmental time (Workman et al. 2013). Thus, the evolution of learning results in tradeoffs in juvenile competitive ability (Mery and Kawecki 2003) and adult reproduction, often delaying reproduction and resulting in greater investment in fewer offspring (Barrickman et al. 2008; Snell-Rood et al. 2011; Kotrschal et al. 2013). In many

cases, there are even direct tissue tradeoffs between plasticity machinery (such as brain size) and other costly tissues, such as gut or flight muscle (Isler and van Schaik 2006; Liao et al. 2016). Similar costs of the process of plasticity are seen for other types of plasticity that develop through variation and selection within individuals such as acquired immunity or plant morphological architecture (Snell-Rood et al. 2018).

Limits to plasticity stem from constraints on plastic genotypes expressing optimal phenotypes relative to a specialist. These limits may be measured in terms of fitness costs, but these costs of 'phenotypic inferiority' originate from development and genetic constraints. These limits more broadly refer to the idea that 'jack-of-all-trades is master of none.' First, let us review limits that stem from developmental processes. The 'developmental range' limit states that by specializing on a particular fixed trait, non-plastic genotypes may be able to achieve more extreme trait values or those that are better matched to the environment in question (DeWitt et al. 1998). This idea is supported by the observation that the range of plasticity seen within species is often dwarfed by trait divergence across species. For instance, developmental plasticity in relative limb length induced by rearing substrate within *Anolis* species is much smaller than differences across species of twig- and trunk specialists (Losos et al. 2000). Similarly, activity-induced variation in mouse morphology is much less than genetic variation across lines artificially selected for running activity (Kelly et al. 2006). Another developmental constraint is time lags. The development of well-matched phenotypes often takes time, which may be an issue when the environment itself is changing at rate that is faster than an organism can develop an appropriate phenotype, resulting in a suboptimal phenotype in the current environment (Padilla and Adolph 1996; Gabriel et al. 2005). A final limit to the developmental process is the 'epiphenotype problem,' which posits that the earlier in development that salient cues are received, the greater range of possible resultant phenotypic outcomes (DeWitt et al. 1998). Thus, plastic genotypes may have less integrated and more poorly performing phenotypes than specialists due to limits on the timing of developmental processes. Some empirical support exists for the epiphenotype hypothesis: for instance, in snails that alter shell shape in response to predator cues, those that received cues earlier in development are able to achieve a greater range of shell phenotypes (Hoverman and Relyea 2007). Species of shorebirds and ducks that hatch relatively earlier show greater phenotypic variation within species in relative bill and wing dimensions (Snell-Rood et al. 2015). Limits to developmental processes can result in poorer performance of plastic phenotypes compared to when those phenotypes are relatively more fixed in development.

Limits to plasticity can also arise from evolutionary constraints at the level of the underlying genetic architecture of plasticity. Antagonistic pleiotropy refers to situations where the action of a gene in one situation is negatively correlated with its action in another environment. Experimental evolution studies commonly show evidence of antagonistic pleiotropy when organisms are adapting to heterogeneous environments (Kassen 2002). For instance, flies selected in variable temperature environments are plastic generalists but perform poorly

in specific temperature conditions relative to evolved specialists (Berger et al. 2014). Tradeoffs resulting from adaption to a particular environment can be more pronounced when considering environments that are relatively more dissimilar (Travisano and Lenski 1996). This observation is consistent with plasticity theory showing that highly divergent environments, with different optimal trait values, favor the evolution of distinct specialists (Levins 1968; Scheiner 1993), resulting in phenotypic divergence across species that dwarfs within-species plasticity (e.g., Losos et al. 2000). Limits to the evolution of plasticity and niche breadth may also arise from the efficacy of selection across environments. Because specialists are adapting to one environment, and selection is spread across multiple environments in generalist species, specialists will fix beneficial mutations and purge deleterious mutations faster for traits specific to individual environments (Kawecki 1994; Whitlock 1996; Van Dyken and Wade 2010, Snell-Rood et al. 2010). Empirical support for this idea comes from experimental evolution studies where specialists adapt more rapidly due to increased efficacy of selection (Bennett et al. 1992; Kassen and Bell 1998) and genes biased in their expression between alternate morphs being more genetically variable, presumably due to relaxed purifying selection (Kijimoto et al. 2014). However, other experiments fail to find support for this idea (Cooper and Lenski 2000), and still others question the theoretical assumption of plastic gene expression being entirely specific to alternate environments (Snell-Rood et al. 2010).

6.3.3 Why Don't We See Costs of Plasticity More Consistently Across Studies?

As this discussion shows, a diversity of mechanisms can generate fitness tradeoffs associated with plasticity. These explanations are not mutually exclusive, and the mixed evidence for each suggests that they are all important to varying degrees. Indeed, this diversity of mechanisms is one explanation for why the evidence for costs of plasticity and generalization is so varied (Kassen 2002; Van Buskirk and Steiner 2009). It is likely that different types of costs apply differently to plastic traits depending on how they develop and function. Indeed, it has been argued that developmental switch mechanisms of plasticity are more likely to be limited by developmental and genetic limits acting at the population level, while learning-like mechanisms of plasticity are more likely to be limited by costs to the individual of the developmental process itself (Snell-Rood 2012; Snell-Rood et al. 2018). Here we review several additional explanations for why the costs of plasticity tend to be lower and less frequent than often expected. These explanations fall into two general categories: patterns of past selection and how the complexity of organisms both affect our ability to detect costs. This discussion recalls broader discussions around the challenges of detecting costs and tradeoffs (Reznick et al. 2000; Agrawal 2020).

First, patterns of past selection affect our ability to detect costs. There should be strong selection against the costs of plasticity, and over time, mechanisms should evolve to reduce the costs of plasticity (Murren et al. 2015). For example,

predator-induced spines in *Daphnia* are themselves costly, resulting in lower fecundity (Riessen and Sprules 1990). However, some *Daphnia* also reduce their metabolic rate in the face of predation, which can sometimes offset the costs of an induced spine (Scheiner and Berrigan 1998). The idea that the costs of plasticity are evolutionarily transient is similar to the argument that costs of elaborated sexual traits are fleeting as there is strong selection on mechanisms to reduce these costs (Badyaev 2004). For instance, changes in underlying metabolic pathways can affect the costs of acquiring carotenoids and the development of colorful ornaments (McGraw 2005; Higginson et al. 2016). Evolutionary changes that reduce costs can also explain why we sometimes detect costs within species, but such tradeoffs are not seen across species (Agrawal 2020). Evolutionary innovations can explain instances of synergistic pleiotropy, instead of the commonly assumed antagonistic pleiotropy (e.g., Sackman and Rokyta 2019; Ruark-Seward et al. 2020): as genotypes adapt to multiple environments, some mutations are just better than others. From a methodological standpoint, how do we deal with selection altering the relative costs and benefits of plasticity over time? Experimental evolution studies offer one approach to tease apart the effects of time: for instance, costs of specialization take time to emerge in flies adapting to different environments (Olazcuaga et al. 2019). Similarly, one could account for evolutionary time in comparisons of costs across populations or species. Another approach is the use of recombinant inbred lines in the lab; because these lines have not experienced selection in the field, they may better reveal costs of the ability to be plastic (Weinig et al. 2006). Experimentally detecting costs of plasticity also depends on the presence of variation in plasticity—either variation in the degree of plasticity or the presence of specialists and generalists. In many cases, selection on plasticity may be so strong or historically ancient that there is little current variation in the degree of plasticity. For instance, there is no standing genetic variation in adaptive developmental plasticity in some populations of spadefoot toads (Newman 1988). While we may detect such 'genotype-by-environment' interactions (i.e., *GxE*) in our analyses, in some cases it could be a function of noise, instead of functional standing variation in a population. Negative correlations between the number of lines in a study and the probability of detecting costs of plasticity support the idea that meaningful variation in *GxE* may limit the ability to detect costs (Van Buskirk and Steiner 2009).

Second, the complexity of organisms means that it is often difficult to determine the best traits to measure with respect to adaptive plasticity. Many interacting traits, at levels from proteins to behavior, affect the fitness of individual organisms. How does one choose the best traits to measure when studying costs of adaptive plasticity? Meta-analyses show that we are just as likely to detect costs of plasticity as to detect costs of 'canalization;' i.e., keeping a phenotype stable in the face of environmental variation (Van Buskirk and Steiner 2009). Traits closely related to fitness, such as body size and development time, are often under selection to be maintained across an environmental gradient (Van Tienderen 1991). Hence, a cost of canalization may indeed reflect a cost of plasticity, but for a trait not measured. In support of this interpretation, meta-analyses show that studies lack evidence for

adaptive plasticity in 71% of cases testing for costs of plasticity (Van Buskirk and Steiner 2009), suggesting in many cases we may be choosing the 'wrong' traits as proxies of plasticity. What does this mean for future studies? There have been a number of calls to quantify biological complexity more thoroughly, from more complete measures of fitness (Shaw et al. 2008) to measures of the diversity of cues triggering plasticity (Westneat et al. 2019). However, we caution that more data does not necessarily give more clarity without informed expectations: given the natural history of a system, which phenotypic traits does one expect to vary to maintain constant fitness-related traits across environments? In expanding the range of traits measured, we may also want to consider what aspects of performance we tend to miss in laboratory assays that could be relevant to costs of plasticity in the field. For instance, in the lab, plastic generalists are sometimes more fecund overall (e.g., *Drosophila* thermal generalists; Condon et al. 2014), opposite to expectations. It is possible that such genotypes are tolerant of novel environments with few competitors, but intolerant to competition (sensu 'Grimes Triangle'), but we would miss such tradeoffs in lab assays with abundant resources (Agrawal et al. 2010; Cipollini et al. 2014).

6.4 FUTURE DIRECTIONS IN ECOLOGY AND EVOLUTION OF PLASTICITY

Given the extensive work to date on the ecological and evolutionary drivers and constraints on plasticity, what do we have left to explore? Increasing attention to environmental change and developmental mechanisms of plasticity are opening new doors for theoretical and empirical advances in our understanding of why plasticity varies.

6.4.1 Plasticity and Cryptic Genetic Variation in Novel Environments

The role of plasticity in novel environments has received increased attention in the last two decades as we gain a greater understanding of rapid, human-induced environmental change (Sih et al. 2011; Merila and Hendry 2014; see also Diamond and Martin 2021; Levis and Pfennig 2021; and Scheiner and Levis 2021 in this volume). Theory has established that plasticity may allow organisms to achieve greater fitness in novel environments, allowing for population persistence and further adaptation (Price et al. 2003; Lande 2009; Chevin and Lande 2010). However, we know less about when plasticity in novel environments will fuel subsequent adaptive evolution. Cryptic genetic variation can be seen in laboratory experiments (Gibson and Dworkin 2004), but how does it affect evolution in nature (Ledon-Rettig et al. 2014)?

Microevolutionary models that incorporate development—such as simple regulatory network models—are well-suited to address this issue since they model mechanisms through which hidden variation can accumulate (Hoke et al. 2019). Asymmetries in the degree of variation released in novel environments may be a result of underlying developmental systems that channel genetic and environmental variation along phenotypic dimensions that have been favored in the past (Watson and Szathmary 2016). In an important simulation model, for example, genotypes with plastic gene regulatory networks that were allowed to evolve in variable

environments showed greater alignment of genetic and mutational variance along the dominant axis of environmental variation compared to fixed genotypes (Draghi and Whitlock 2012). These same plastic genotypes, when challenged with a more extreme novel environment along the same dominant axis, were better phenotypically matched to novel environments. This theoretic result demonstrates that selection for plasticity may result in developmental or regulatory systems that constrain the dominant axes of genetic variation, with important implications for further evolution in novel environments. Indeed, a recent meta-analysis found support for this idea that the 'direction of plasticity' was aligned with the dominant axis of genetic variation in novel environments (Noble et al. 2019). Such approaches pave the way for future research addressing whether certain patterns of past selection harbor greater underlying cryptic genetic variation and how such variation interacts with environmental change.

6.4.2 Costs of Plasticity and Genetic Assimilation in Novel Environments

As highlighted in this chapter, decades of effort have been spent searching for the costs of plasticity, and while costs have been found, they are incredibly variable in frequency and magnitude. Are there exciting future research directions on the costs of plasticity, or are they an act of futility in an over-studied area? We argue above that one way forward in studies of costs are studies that consider multiple dimensions of fitness in realistic conditions. A particularly interesting context to study costs is with respect to the importance of plasticity in novel environments. Plasticity is more likely to lead to genetic assimilation in novel environments when those new conditions are constant and plasticity is costly (reviewed in Scheiner and Levis 2021 in this volume). We can test such ideas using extreme but predictable anthropogenic environments (e.g., cities, agricultural monocultures; see also Diamond and Martin 2021 in this volume) and forms of plasticity that are known to be costly (e.g., trial-and-error learning). We can also extrapolate to understudied forms of plasticity that should have important costs and benefits in new conditions (e.g., acquired immunity).

6.4.3 Feedbacks in the Evolution of Plasticity: When the Environment Evolves

We know organism physiology and behavior can influence how a genotype experiences environmental variability and the reliability of cues, important drivers of plasticity. When these traits are evolving alongside plasticity, complex dynamics can emerge. To what extent could such negative feedbacks explain the evolution of specialists across lineages, or, conversely, positive feedbacks explain the evolution of plastic generalists and situations where generalists fuel evolutionary diversification (see Levis and Pfennig 2021 in this volume)? Organismal traits such as resource use, diapause, and movement affect the ecological conditions that favor or constrain the evolution of plasticity. Thus, a future direction in understanding plasticity lies at an intersection with the emerging literature on eco-evolutionary feedbacks (Govaert et al. 2019).

6.4.4 Evaluating Null Models in the Evolution of Plasticity

Theory recognizes that neutral processes may explain patterns of variation in plasticity and niche breadth (Via 1993; Hardy et al. 2016; Forister and Jenkins 2017), but most empirical studies focus on adaptive explanations. To what extent does genetic drift shape the distribution of specialists and plastic generalists across the tree of life? Does this matter when making predictions about environmental change (Ghalambor et al. 2007; Snell-Rood et al. 2018)? We should move past our adaptationist biases in our explanations of the causes and consequences of plasticity (see also Futuyma 2021).

6.4.5 Developmental Realism in Evolutionary Models: Predicting Diversification and the Emergence of Specialists and Generalists

Incorporating more developmental realism into models changes the dynamics of evolutionary theory (Draghi and Whitlock 2012; Kriegman et al. 2018). As we incorporate more features of development into our conceptual links between genotype and phenotype (e.g., Salazar-Ciudad and Jernvall 2010), we believe the field will have more power to develop theory related to macroevolutionary questions, such as speciation and the diversification of body shapes. To what extent does plasticity play a role in these processes? Reviewing the costs and limits of plasticity suggests that antagonistic pleiotropy could explain why, over long periods of evolutionary time, species with highly divergent traits are favored over 'infinitely plastic generalists' – relative to individual specialists, it is not possible for one plastic genotype to develop alternate phenotypes that are high-performing over their lifetime. Models of this process that incorporate more developmental realism could integrate underlying costs and limits and ask questions about the emergence of depressed hybrid fitness and speciation, as selection in divergent environments generates antagonistic pleiotropy.

6.5 CONCLUSIONS

Adaptive phenotypic plasticity is favored in novel and variable environments and can underlie broad niche breadth. Plastic generalists can colonize new environments, paving the way for diversification. Costs and limits of plasticity play an important role in maintaining variation in plasticity and promoting the evolution of specialists, for instance in constant conditions or when alternate environments favor drastically different phenotypes. Despite decades of research on the ecology and evolution of plasticity, there are still many exciting and new directions of future research (Box 6.1), especially around developing new theory and testing theoretical predictions in the field. Anthropogenic environments such as cities offer a promising context where we can test plasticity theory while also generating insights with conservation implications. Finally, as we expand our conceptualization of the genotype to phenotype link from a simple view of a gene to trait mappings, to more complex network-based interactions, we generate novel insights especially with respect to broader macroevolutionary questions.

BOX 6.1 SUGGESTIONS FOR FUTURE RESEARCH: QUESTIONS AND HYPOTHESES

- When does plasticity result in adaptive traits in novel environments? Developmental selection mechanisms of plasticity are more likely to result in adaptive plasticity in novel environments (relative to evolved switches).
- How does plasticity interact with cryptic genetic variation in novel environments? Patterns of past selection bias underlying patterns of genetic variation (e.g., along the same axes of environmental variation).
- Why do studies vary so much in the likelihood of detecting costs of plasticity? Costs of plasticity play out across multiple dimensions of fitness and are often obscured in laboratory conditions.
- When do costs of plasticity shape the ecology and evolution of plasticity? Costs of plasticity may play a prominent but transient role in genetic assimilation in novel environments.
- Why does plasticity vary within and between species? Neutral theories can explain patterns of plasticity, in some cases, better than adaptive explanations.
- How does plasticity bridge the divide between micro- and macroevolutionary questions? Developmental realism in evolutionary models could generate predictions around the emergence of speciation and specialization as developmental systems adapt to different environments.

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7 The Loss of Phenotypic Plasticity Via Natural Selection: Genetic Assimilation

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CONTENTS

7.1 INTRODUCTION

Given that all species are confronted with a heterogeneous environment, why isn't every species highly plastic? Why are all or most species not adapted to a wide range of environments? Despite the breadth of plasticity across traits and species (see Chenard and Duckworth 2021; Lister 2021; Snell-Rood and Ehlman 2021; Sultan 2021 in this volume), adaptive phenotypic plasticity is limited. Two recent surveys (Palacio-López et al. 2015; Acasuso-Rivero et al. 2019) found that adaptive plasticity was found in less than half of measured traits. The absence of trait plasticity may be because that plasticity never existed. Or it may be because trait plasticity used to be present in a lineage and the trait evolved to be non-plastic. This latter possibility is the focus of this chapter. We will examine a specific pattern of evolution, where

DOI: 10.1201/9780429343001-7

plasticity is initially favored or able to enhance adaptation but then disappears due to natural selection, a process often referred to as 'genetic assimilation'. Plasticity can also be lost through non-selective processes, including genetic drift or mutational degradation (Masel et al. 2007), as might occur when alternative phenotypes are seldom expressed and thereby experience relaxed selection (Kawecki 1994; Whitlock 1996; Van Dyken and Wade 2010). Such non-selective causes are the alternative hypotheses that must always be considered when assessing evolution in natural populations (see Section 7.5).

Understanding why and how plasticity is lost can provide insights into two classes of processes. The first class is adaptation in a changing environment; that process has been the focus of studies of genetic assimilation. The second class is divergence and speciation. New phenotypes and novel structures might originate through phenotypic plasticity and then become fixed in a population (West-Eberhard 2003). Such trait differentiation could then lead to reproductive isolation if hybrid or intermediate phenotypes have a lower fitness than either the ancestral or derived lineages (Pfennig et al. 2010; see also Levis and Pfennig 2021 in this volume).

To understand the loss of plasticity, we must also consider the conditions that maintain or promote plasticity, which requires distinguishing between adaptive and non-adaptive plasticity (Doughty and Reznick 2004). Phenotypic plasticity is a change in the phenotype of an organism in response to a change in the environment, for labile traits, or different phenotypes expressed by a single genotype, for fixed traits. For example, if an organism takes in fewer nutrients it grows less; this is a plastic response to differences in nutrient availability. In addition to being labile, this type of response to the environment is sometimes referred to as passive plasticity. Most likely passive plasticity is not adaptive, although the potential exists that the response was molded by selection. In contrast, some plastic changes, such as the shift in spadefoot toad tadpoles from a detritus diet to a meat diet, requires an active switch in the developmental program (Pfennig 1992). Active plasticity can involve both continual (e.g., metabolic rate) and categorical (e.g., winged or wingless) responses to the environment (see Pfennig 2021 in this volume). It is more likely that active plasticity is, or at one point was, adaptive. Both types of plasticity can be cryptic. Cryptic plasticity (i.e., 'hidden reaction norms') occurs when trait plasticity is not expressed in the ancestral environment, but then a shift in the environment reveals the potential for a plastic response. We contrast that with a trait which shows a limited range of plasticity in the ancestral environment because the range of environmental variation is limited. In this latter case, the new environment might expand the phenotypic range, but this is a case of extending the reaction norm rather than revealing its existence. To understand the loss of plasticity, we have to consider whether the plasticity is labile or fixed, active or passive, continual or categorical, adaptive or not, and cryptic or merely limited (Table 7.1).

Our goal in this chapter is to cut through that complexity of possibilities and indicate when and how the loss of plasticity via natural selection is likely to occur. We first review the various concepts and processes that are often grouped under the heading of 'genetic assimilation' and examine the few theoretical models of this process. Then we explore the empirical evidence for the process in both laboratory and natural systems.

TABLE 7.1
Five Characteristics of Trait Plasticity

Can the trait take on multiple phenotypes during an individual's lifespan?	Labile: The trait value can change in response to environmental inputs (e.g., physiological processes)	Fixed: The trait value cannot change (e.g., adult body size in holometabolous insects)
How does the trait respond to the environment?	Active: A switch occurs in a metabolic or developmental system in response to an environmental signal	Passive: The trait responds by a general shift in the phenotype, but not by an active switch
Is the plastic response continual or categorical?	Categorical: The phenotype exists as just two or more discrete forms	Continuous: The phenotypic response to the environment can be described as a smooth mathematical function (a reaction norm)
Is the plasticity adaptive?	Adaptive: The plastic response of the trait changes the phenotype so as to increase the fitness of the individual	Not adaptive: The plastic response of the trait does not increase the fitness of the individual
Is the plasticity cryptic or limited?	Cryptic: The trait plasticity is not expressed in the range of conditions in the ancestral environment	Limited: The range of trait plasticity expressed in the ancestral environment is much less than the new plasticity expressed by the novel environment

7.2 GENETIC ASSIMILATION CONCEPTS

Consider a situation in which there is an abrupt change in the environment that alters the fitness optimum of a trait of a population. That abrupt change could occur due to a temporal shift in the environment (e.g., a change in the climate or the arrival of a new competitor), or due to a spatial displacement (e.g., long-distance dispersal). Following that shift there will be selection for the mean trait value in the population to move to the new optimum. In the absence of phenotypic plasticity, that change would occur through typical Darwinian evolution. However, if the trait is phenotypically plastic, and that plastic response can be triggered by either the altered environmental factor or some correlated signal, other evolutionary dynamics are possible.

Now we have to track the evolution of two components of the phenotype: (1) those components that are due to genes whose phenotypic expression are not responsive to the novel environment (let's call them 'non-plastic genes'), and (2) those components that are due to genes whose expression is responsive to the novel environment (let's call them 'plasticity genes'). In the absence of plasticity (as in the previous scenario), only non-plastic genes were evolving; now both types could evolve. Of course, categorizing any gene as plastic or non-plastic is somewhat simplistic. A gene that is responsive to one type of environmental signal (e.g., food type), might be unresponsive to a different environmental signal (e.g., day length). Also, the responsiveness of a gene to a given

signal can evolve, thereby converting a plasticity gene into a non-plastic gene. However, this categorization is useful conceptually and leads to consideration of various combinations of evolutionary responses depending on two properties of the plasticity genes: (1) the phenotypic range of the plastic response, and (2) whether that plasticity has a fitness cost (Table 7.2). We follow the convention of DeWitt et al. (1998), who defined a 'cost of plasticity' as any factor that decreases the fitness of an individual even if the plastic phenotype matches the optimum; in contrast, a 'limit of plasticity' is any factor that prevents an individual from expressing that optimal phenotype.

If the plasticity is sufficient to alter trait expression to the new fitness optimum (i.e., no limits), and if there are no fitness costs of plasticity associated with the new phenotype, there would be no change in the genetics of the population. The phenotypes of the individuals would simply shift. If there is a cost and that cost is due to a developmental pathway that allows for trait plasticity, there would be selection to reduce that trait plasticity while retaining the optimal trait value through evolution of the non-plastic genes. This dynamic—a plastic change in the trait value followed by the loss of plasticity—was termed 'genetic assimilation' by Conrad Hal Waddington (1942; see reviews in Pigliucci and Murren 2003; Crispo 2007).

It may be that because of developmental limits on trait plasticity, the shift is insufficient to alter the trait value completely so as to match the new fitness optimum. If so, the non-plastic genes could then evolve to move the trait the rest of the way to the new optimum. Plasticity might remain depending on whether or not it was costly, with costs resulting in selection to eliminate the plasticity. It is also possible that the plasticity genes could evolve to overcome the developmental limits with no evolution of the non-plastic genes. Of course, it could be that both the plastic and non-plastic genes could evolve synergistically to shift the phenotype to the optimal value. Which of the two types of genes would evolve would depend on the amount

TABLE 7.2
Possible Evolutionary Responses Resulting from a Shift in the Optimal Trait Value Depending on Whether Plasticity Has Developmental Limits or Is Costly

	Fitness Costs of Plasticity	
Developmental Limits to Plasticity	**No**	**Yes**
No	A plastic shift in the phenotype with no evolution	A plastic shift followed by the evolution of the non-plastic genes to the fitness optimum and the loss of plasticity
Yes	A partial plastic shift followed by the evolution of the non-plastic genes to the fitness optimum or The plasticity genes evolve to eliminate the developmental limits	A partial plastic shift followed by the evolution of the non-plastic genes to the fitness optimum and the loss of plasticity

of standing genetic variation in each type and/or the amount of mutational variation generated in each. To make this even more complicated, both types of genes could evolve synergistically and then later costs could act to decrease plasticity. Depending on when during that process the system was measured, plasticity might be increasing or stable, even if the eventual outcome would be the loss of plasticity.

The term genetic assimilation has a somewhat fraught history, as does the concept itself. (For a review of the history of the conceptual controversy, see Futuyma 2021 and Pfennig 2021 in this volume.) It has often been used as an umbrella term for a variety of related processes (Table 7.3), most likely because it is the most well-known of those terms. Even Waddington, who coined the term, used it in different ways. In his initial, concept-only paper, he used the term to refer to instances in which the plastic response completely shifts the phenotype in a particular direction, with subsequent selection eliminating the environmental-dependency for that phenotypic expression (Waddington 1942). However, his experimental work involved traits that were only partially expressed in the new environment, and so the phenotypic expression (i.e., the form of the trait) was evolving along with the plasticity (e.g., Waddington 1956). Making this more complicated, the traits he was manipulating were bimodal in their phenotypes, and what he reported on was the change in the percentage of individuals expressing each form, treating them as if they were strictly categorical. So, it is unclear to what extent he considered changes in the mean trait value within the scope of his concept.

A half-century earlier, James Mark Baldwin (1896) had described a pattern of evolution that included the potential for the evolution of both the plasticity of a trait and its non-plastic component. Because his papers were published prior to the rediscovery of Mendel, its terminology is a bit hard to parse. His paper was almost entirely ignored by evolutionary biologists, possibly because Baldwin was primarily interested in the evolution of learning and framed his argument in those terms. It has only been in the last 20 years that Baldwin is receiving his due as the originator of ideas about the evolution of plasticity (Scheiner 2014a). Because Baldwin was mostly thinking in terms of the evolution of learning, for him the optimum would move continuously. This contrasts with the ideas of Waddington who conceived of a single, step change in the environment. Baldwin's ideas could apply to a continual directional change in the abiotic environment (e.g., climate change) or to a competitive or

TABLE 7.3
Three Processes of Trait Evolution Modified by Phenotypic Plasticity

Term	Trait Induction Basis	Change in Plasticity	Evolution of Mean Trait Value	Citation
Baldwin effect	Environmental	None or increase	Changes	Baldwin (1896)
Genetic assimilation	Environmental	Decrease	Stays the same	Waddington (1942)
Genetic accommodation	Environmental or genetic	None, decrease or increase	Changes or stays the same	West-Eberhard (2003)

Source: Modified from Crispo (2007).

predator-prey Red Queen type arms race (Whitlock 1996; Rice and Holland 1997). His ideas were termed the 'Baldwin effect' by Simpson (1953), although being somewhat mischaracterized in that paper as a process by which nonheritable, plastic traits would be replaced by fixed genetic factors, rather than recognizing that plasticity itself was heritable (Scheiner 2014a).

The most recent version of these ideas comes from Mary Jane West-Eberhard (2003). She expanded on Baldwin's and Waddington's ideas by considering how changes in one trait might be driven by a genetic mutation as well as by a shift in the external environment. Hers is the most expansive view as it includes the potential for plasticity to both increase (ala Baldwin) and decrease (ala Waddington). She termed this process 'genetic accommodation.'

We are torn as to what terminology to use in this chapter. As you will see, the process of plasticity loss due to natural selection does not necessarily occur as described by Waddington. Yet, genetic assimilation is the term generally used to describe this process. So, we will use that term, but emphasize that it is referring to a concept and process that goes well beyond its original definition.

7.3 MODELS OF THE EVOLUTION OF GENETIC ASSIMILATION

Despite Waddington's original paper appearing over 70 years ago and being very widely cited, there have been only four publications that present formal models of the process of genetic assimilation (Lande 2009; Chevin and Lande 2010; Scheiner et al. 2017, 2020). What distinguishes these models is that in all cases the environment goes through a step change and plasticity can potentially evolve. Although any model of the evolution of phenotypic plasticity has a bearing on this process (see reviews by Berrigan and Scheiner 2004; Scheiner 2019), we focus on these four. The key distinction between the models of Lande and Chevin on the one hand and Scheiner, Barfield, and Holt on the other is that the former are analytic, quantitative genetic models while the latter are individual-based simulations that directly model genes. Those distinctions mean that each has different assumptions and can answer somewhat different questions. That the general results are similar gives confidence that the overall conclusions are robust. Both, however, do not cover the potential complexity of conditions outlined in Table 7.1, so there is clearly more work to be done in this area.

The model by Lande (2009) considered the case of a fixed trait with a continuous, linear reaction norm that was maintained by a limited amount of temporal variation in a single population. That population then experiences a step change in the mean of the environment, after which the temporal variation continues, but now around the new mean. [NB: In all of these descriptions, 'environment' can be considered synonymous with 'the optimal trait value.'] Initially plasticity increases, followed by evolution of the non-plastic component of the phenotype with plasticity evolving back to its initial value. This decrease in plasticity is not because there is a cost or limitation associated with the plasticity; rather, it is returning to the optimal amount determined by the temporal variation. Notably, this return takes on the order of 10,000 generations. The model of Chevin and Lande (2010) differed from the previous model in two ways: there was no temporal variation, just the step change, and plasticity had a cost. The dynamics of trait evolution are similar with two exceptions:

First, plasticity rises and falls as before, but now it eventually disappears entirely because of the cost. Again, this process takes on the order of 10,000 generations. Second, the non-plastic component initially has a small rise, and then a much greater increase as plasticity disappears.

The initial model by Scheiner and colleagues (2017) had no temporal variation within environments, just the step change. It contrasted three cases: (1) no cost or limitation to plasticity, (2) a cost of plasticity, and (3) plasticity linked to developmental noise so that greater plasticity resulted in a higher chance of developmental errors. This last case was closest to the initial verbal model of Waddington (1942), where he argued that such errors of development would lead to the replacement of plasticity by a fixed phenotype. The second case, with a cost of plasticity, was similar to that of Chevin and Lande (2010). In a second paper, Scheiner and colleagues (2020) mimicked the model of Lande (2009) by including temporal variation, along with the step change, although they considered this without and with a cost of plasticity. From these models, they found that in the absence of any process acting on plasticity in the novel environment, plasticity did not disappear. In the presence of either a cost of plasticity or linkage with developmental noise, plasticity decreased substantially (Figure 7.1). In the presence of temporal variation, plasticity evolved in a fashion very similar to that seen by Lande, although it was an order-of-magnitude slower (~100,000 generations) for the parameters used in that model (Figure 7.2).

These models lead to two general conclusions. First, genetic assimilation requires a process that will select for reduced plasticity. Those processes can be internal, such as a cost of plasticity or a developmental limit to plasticity, or they can be external, such as a pattern of environmental variation that results in a lower optimal amount of trait plasticity. In the absence of such a process, there is no selection to eliminate

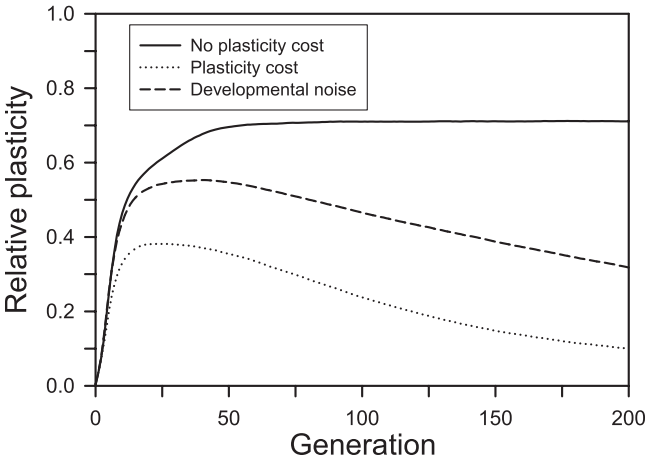

FIGURE 7.1 Relative plasticity as a function of the time following a step change in the environment for three scenarios: no cost of plasticity, a cost of plasticity, and developmental noise linked to plasticity. A value of 1.0 indicates adaptation is due only to plasticity; a value <1.0 indicates a mixed plastic and non-plastic response. See Scheiner et al. (2017) for details of the simulations.

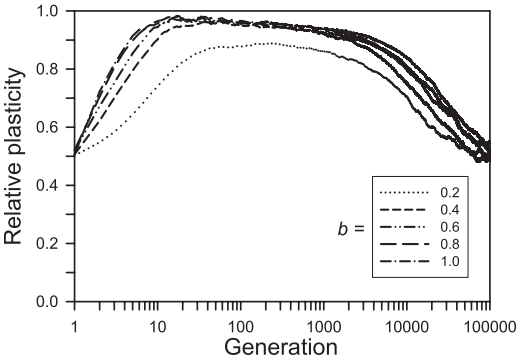

FIGURE 7.2 Relative plasticity as a function of the time following a step change in the environment in the presence of temporal variation before and after the step change. A value of 1.0 indicates adaptation is due only to plasticity; a value <1.0 indicates a mixed plastic and non-plastic response. The plasticity parameter (*b*) determines the magnitude of the plastic response by the phenotype for a given plastic genetic value; higher values of (*b*) indicate greater phenotypic plasticity for the same genetic value of plasticity. See Scheiner et al. (2020) for details of the simulations.

plasticity. Second, the time it takes for plasticity to be reduced can be very long. Meanwhile, in the short term, the amount of plasticity can actually increase. In natural populations, for genetic assimilation to happen, after the initial environmental change, the environment must have little or no variation (i.e., the change must remain relatively stable), otherwise there is the potential for continued selection for plasticity, rather than selection to eliminate plasticity. The qualitative change in competitor presence driving the loss of plasticity in spadefoot toad tadpoles (see Section 7.5.1) is a good example of a change followed by little variation. In contrast, environmental changes in something like temperature, which is continually changing in complex ways, is unlikely to lead to genetic assimilation, as there will be constant selection in favor of plasticity.

Despite environmental stability being an important determinant of whether or not plasticity will be lost, the most favorable conditions for genetic assimilation to happen are the presence of either costs or limitations of plasticity. However, all of the empirical evidence says that costs of plasticity are rare or small (Murren et al. 2015) except in single-celled organisms (Callahan et al. 2008) or under very stressful conditions (Van Buskirk and Steiner 2009). The most likely causes for the loss of plasticity are, thus, developmental limits because a plastic developmental pathway either is unable to achieve the optimal phenotype or is linked to greater developmental noise.

Our focus in this chapter is on models of genetic assimilation. There is an extensive, related literature on the evolution of phenotypic plasticity that indicate the conditions that favor or disfavor plasticity more generally (see reviews by Berrigan and Scheiner [2004] and Scheiner [2019]). Another class of models explores the evolution of 'canalization,' which refers to the intrinsic robustness that developmental processes can display in response to external (environmental) or internal (genetic) perturbation (Waddington 1942). In the absence of evolving plasticity, developmental

systems may be selected to be canalized (Gavrilets and Hastings 1994; Kawecki 2000; Meiklejohn and Hartl 2002; Siegal and Bergman 2002; Masel 2004; Scheiner 2014b). While those models are relevant to the issues explored here, for the most part, they are not models of the genetic assimilation process *per se* because they do not involve a potentially adaptive reaction norm.

Having examined the theory of genetic assimilation, we now turn to the empirical evidence that this process can and does occur. First, we look at laboratory experiments that tested the potential for genetic assimilation to occur, and then consider natural systems and ask: has it happened?

7.4 LABORATORY EXPERIMENTS OF GENETIC ASSIMILATION

Before moving into the experimental evidence for genetic assimilation, we first want to make a quick note about how the evolution of plasticity is typically studied in the lab. We only provide a short overview here; see Scheiner (2002) for more in-depth coverage of the topic. There have been three main approaches for artificial selection experiments used to study plasticity's evolution. In the first approach, plasticity itself is the target of artificial selection. In the second, a trait (often induced by plasticity) is the target of artificial selection and plasticity evolves as a correlated response. Most laboratory studies using these first two approaches also use some form of group-level (e.g., sibship-level) selection. They do so because individuals might exhibit 'fixed' plasticity (Table 7.1) wherein they only express a single phenotype despite harboring the developmental capacity to express others in alternative environments. Such fixed plastic responses make it impossible to assess plasticity at the level of a single individual. The best case for such studies is using clonal replicates, but more often full-sib or half-sib designs are used. While such group-level selection has been very useful, it is limited by the fact that natural selection most often acts at the level of the individual. Thus, a third experimental design—simultaneous or alternating individual selection in multiple environments—has also been employed. Together with quasi-natural selection experiments (i.e., experimental evolution *without* artificial selection) these three approaches form the bedrock for laboratory studies of the evolution of plasticity generally and genetic assimilation specifically.

As with models of genetic assimilation, explicit efforts to experimentally evaluate this process are limited. The greatest number of studies were by Waddington (1953, 1956, 1959, 1960, 1961), Waddington and Robertson (1966), and his student Bateman (1959a,b), where they used both artificial selection and experimental evolution to test for genetic assimilation in various traits of fruit flies (e.g., wing venation, thoracic segments, eye facet number, anal papillae). In general, the experimental protocols involved exposing laboratory populations of flies to a sudden environmental stress (heat shock was used most often, but so was exposure to ether and high levels of sodium chloride) that induced a morphological response in some proportion of individuals. Individuals that did and did not respond were then used to establish separate populations in the next generation whose progeny were also exposed to the environmental stress. This process was repeated for several generations. Almost invariably, they found that after relatively few generations some flies would express the induced form of the phenotype without ever experiencing the inducing cue themselves.

This early work on fruit flies revealed two key points. First, it established that a change from a plastic to a non-plastic developmental pathway could happen, it could happen quickly, and it could happen in a variety of traits (some of which—anal papillae—had known adaptive value). However, these experiments did not demonstrate genetic assimilation as defined by Waddington, because they did not involve a step change in the environment; rather they involved a short-term stress. Second, these experiments suggested that pre-existing (i.e., standing) genetic variation formed the heritable basis of genetic assimilation, in contrast with *de novo* or environmentally induced mutations, which at the time was a point of major contention for all evolution. Indeed, their work spurred other researchers (e.g., Milkman 1960, 1961; Mohler 1965a,b; reviewed in Dworkin 2005) to explore the genetic basis of these assimilated traits.

In recent decades, additional laboratory studies have not only broadened the range of taxa used to evaluate genetic assimilation but also emphasized the importance of the evolution of a response threshold. For example, Suzuki and Nijhout (2006) experimentally evolved tobacco hornworm (*Manduca sexta*) coloration using a similar approach to Waddington. By using caterpillars that develop black coloration under normal rearing conditions, but that have variation in coloration ranging from black to green following heat shock treatment, they were able to establish three lines. In the first, they selected caterpillars that showed a strong response to heat shock by developing green coloration. This line was essentially selecting for greater plasticity and the evolution of a 'polyphenism' (i.e., expression of discrete phenotypes associated with different environments). In their second experimental line, they were selecting for reduced heat shock sensitivity and the maintenance of black coloration. This was their genetic assimilation line. Finally, they had a control line that was not selected upon. After only 13 generations, they were able to evolve a highly plastic line (via genetic accommodation) and a canalized line (via genetic assimilation). By following up on these phenotypic observations with hormonal manipulation, these authors found that genetic assimilation of color production was driven by an evolutionary reduction in sensitivity to juvenile hormone.

Shifting thresholds of induction seems to be common mechanism by which polyphenic traits evolve. An artificial selection study exposing nematodes (*Caenorhabditis remanei*) to heat shock found that genetic assimilation in response to the heat shock treatment was actually the result of a shifted reaction norm (Sikkink et al. 2014). In this case, the researchers assayed the phenotypic response across a broader range of temperatures than the heat shock temperature and found the induced plasticity was not fixed across a wider range of temperatures. Instead, the threshold for the response was shifted to higher temperatures over evolutionary time (~10 generations). Their results further demonstrate that what appears to be genetic assimilation can result from a shift in the threshold of induction across environments, i.e., an evolution of plasticity rather than its elimination. A similar example of selection for a shifting threshold is the nutrition-induced horns of exotic populations of dung beetles (*Onthophagus taurus*), which shifted their threshold for induction of male horns both upward and downward compared to ancestral populations in roughly 40 generations (Moczek and Nijhout 2003). These studies demonstrate that developmental threshold shifts in response to selection seem to be a

common route by which plasticity rapidly evolves. Because the population no longer expresses a shift in phenotype with the environment, it appears as if the plasticity has been lost, while that plastic capacity might simply be unexpressed. This distinction is important because a further shift in the environment could re-express the plasticity only in the latter case.

The loss of plasticity requires the appearance of non-plastic genotypes in a population. For example, the buckeye butterfly (*Precis coenia*) normally develops pale beige or reddish-brown wing coloration depending on the time of year (or temperature and light cycle during rearing). In a laboratory colony maintained under long-day conditions that normally induce the beige phenotype, a single individual spontaneously arose possessing the reddish-brown phenotype (Rountree and Nijhout 1995). Subsequent work determined that this constitutive expression of the reddish-brown phenotype resulted from a single recessive gene. Whether such a *de novo* mutation would then come to dominate the population would depend on selection for or against it versus the plastic genotype. Alternatively, it might also come to dominate by drift either in the original population or through a founder event.

Genetic assimilation might occur through an indirect cost of plasticity linked to an increased mutation rate wherein an environmental change (heat shock) induces epigenetic changes which result in phenotypic changes (i.e., plasticity). It has been posited that chromosomal regions with stress-induced epigenetic change are more susceptible to genome instability, including transposon insertions (Piacentini et al. 2014; Fanti et al. 2017; Kasinathan et al. 2017; Pimpinelli and Piacentini 2020). Indeed, transposition rates increase during heat shock because of stress-induced damage to transposition inhibitors (Gangaraju et al. 2011). As a result, heat shock can lead to transposon mobilization in the germline and an increased number of mutant offspring. Under this scenario, repeated rounds of heat shock alter the chromatin state via epigenetic modifications, produce novel phenotypes, and make DNA hypermutable. Transposon insertions at hypermutable regions produce new mutations and enable phenotype production even in the absence of heat shock. The loss of plasticity might be selected for if there is selection against such hypermutability. Additionally, the increase in genetic variation throughout the genome might create the potential for selection for novel non-plastic genotypes. To what extent various selective and non-selective mechanisms (e.g., induced mutation, typical *de novo* mutation, drift) underlie the loss of plasticity is an open question (Box 7.1).

One study that sought to evaluate genetic assimilation failed to find it (Smolinsky et al. 2019). In that study, mice were selected for locomotor activity (time running on a mouse wheel), and limb morphology was evaluated after 82 generations. Bone morphology responses to exercise differed between the high running line and the control line. However, the skeletal morphology of the high running line did not resemble the running-induced plastic response of the control line. Thus, the authors concluded that genetic assimilation had not occurred. Yet, genetic assimilation could have occurred in earlier generations (as plasticity did differ between lines), but was then followed by subsequent modifications to the phenotype in the high running lines so that they no longer resembled the ancestral plastic response (see the bottom right outcome in Table 7.2). Nevertheless, this study highlights the problem of semantics around what constitutes ‘true’ genetic assimilation.

7.5 NATURAL EXAMPLES OF GENETIC ASSIMILATION

The above examples establish that genetic assimilation can occur quickly, that it can occur in a diversity of traits and taxa, and that mechanisms underlying the loss of plasticity can be varied. However, just because genetic assimilation can happen does not mean that it has contributed to the evolution of any trait in any natural population. What do the data say? Rather than performing an exhaustive literature review, we highlight three examples (for a more comprehensive list, see Levis and Pfennig 2021 in this volume).

Two approaches have been used to evaluate genetic assimilation in natural populations. The choice of taxa and traits for either approach is important because both require evaluating extant lineages to infer historical events. Since plasticity can evolve quite rapidly, the signature of genetic assimilation might not be detectable in extant lineages (Pigliucci and Murren 2003). Nevertheless, studies are possible in taxa with well-understood ecologies and phylogenies.

One common approach is to use comparative phylogenetic inference to determine ancestral plastic states and identify if and when lineages have transitioned from a plastic to a canalized form. This approach requires knowledge of the extent of plasticity in a diversity of extant lineages and a well-known phylogeny of the species under investigation. If these data are available, one can determine, for example, whether or not plasticity preceded canalization in the trait of interest, whether the loss of plasticity occurred multiple times, and if that loss facilitated subsequent diversification. This methodology has been successful in investigating the loss of plasticity in a myriad of traits and taxa (Schwander and Leimar 2011), for example, resource use in nematodes (Susoy et al. 2015) and sexual expression in *Solanum* (Diggle and Miller 2013).

A second common approach is to leverage ancestor-descendant comparisons. With this approach, plastic trait expression of populations or species that have colonized a new environment is contrasted with that of the putative ancestral/source population by rearing individuals from each population type in both the ancestral and derived conditions (Levis and Pfennig 2016). This method requires knowledge about relationships among populations (or species) so that appropriate directionality can be assigned (i.e., whether plasticity is lost or gained) and about the environmental cue(s) governing plasticity. This approach may be better suited to evaluate subtle changes in plasticity because it directly determines reaction norms rather than inferring them from trait presence or absence. In addition, this approach may be better able to determine what evolutionary mechanisms and/or ecological factors drove fixation of the formerly plastic trait. In many ways, these comparisons are the closest to Waddington's original scenario for genetic assimilation.

We now describe three possible examples of genetic assimilation in nature. We say 'possible examples' because without access to the actual ancestors, as in a laboratory experiment, we can never say with 100% confidence whether a particular trait in nature evolved by genetic assimilation, through a non-selective process, or had not evolved at all.

7.5.1 Spadefoot Toad Ecomorphs

North American spadefoot toads of the genus *Spea* have evolved a unique larval polyphenism in resource acquisition that has undergone genetic assimilation in some

populations (Figure 7.3a). Like most anurans, *Spea* tadpoles normally develop into an 'omnivore' morph, which has small jaw muscles, smooth mouthparts, numerous denticle rows, and a long gut. This form eats detritus, algae, and small crustaceans. However, if *Spea* tadpoles eat fairy shrimp or tadpoles, some individuals facultatively produce an alternative 'carnivore' morph (Pfennig 1990). This complex, coordinated phenotype differs from the default omnivore morph behaviorally, developmentally, and morphologically. In many parts of its range, the plains spadefoot toad (*Spea bombifrons*) more frequently produces omnivores. However, in populations where *S. bombifrons* co-occurs with a congener (*S. multiplicata*), it produces nearly all carnivores (Figure 7.3b). This divergence in morph production results from the competitor species being superior at acquiring the omnivore resources and thereby

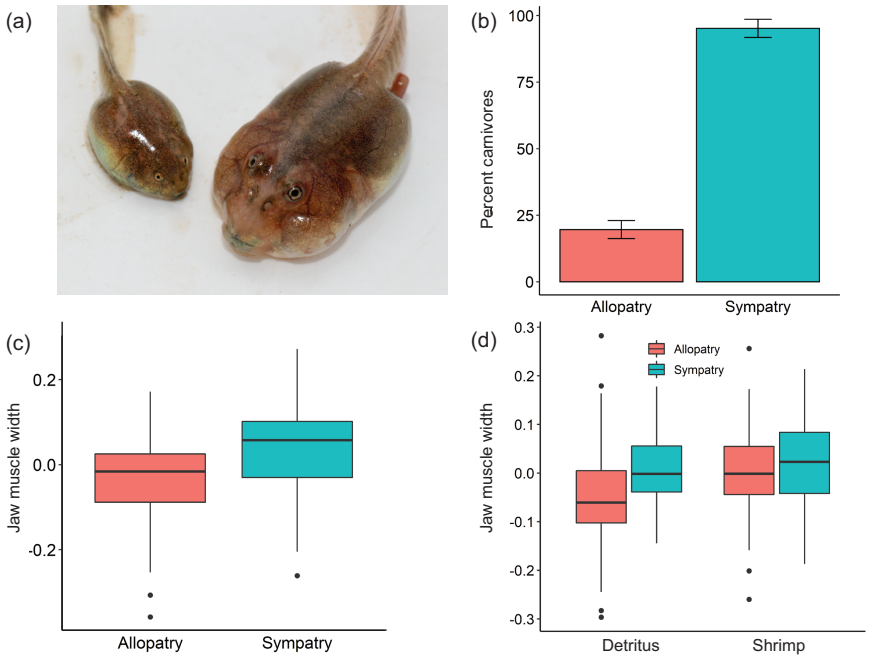

FIGURE 7.3 Spadefoot toads are a possible example of genetic assimilation in nature. (a) Tadpoles in the genus *Spea* can develop as either an 'omnivore' morph (left) or a 'carnivore' morph (right) depending on cues such as diet: carnivores are induced by consumption of shrimp and other tadpoles (photograph courtesy of David Pfennig). (b) In ancestral populations where tadpoles of the Plains spadefoot toad (*Spea bombifrons*) occur by themselves (Allopatry), they produce roughly 20% carnivores. In contrast, derived populations that co-occur with a congener (Sympatry) are nearly fixed for carnivore production (Pfennig and Murphy 2003). (c) In sympatric populations, even without exposure to the inducing dietary cue, tadpoles hatch out as carnivores with larger jaw muscles than tadpoles from allopatric populations (Levis and Pfennig 2019). (d) Whereas tadpoles from allopatric populations have carnivore-like jaw muscles only when reared on a shrimp diet, tadpoles from sympatric populations have carnivore-like jaw muscles even when reared on a detritus diet (Levis and Pfennig 2019).

preventing omnivore formation by *S. bombifrons* (Pfennig and Murphy 2000, 2002, 2003). Because these co-occurring (i.e., sympatric) populations arose as a result of *S. bombifrons* expanding its range into *S. multiplicata*'s range, they can be considered the derived state, whereas populations where *S. bombifrons* is by itself (i.e., allopatric) are ancestral.

On the surface, the observation that morph production has shifted from polyphenic to nearly fixed in sympatric populations of *S. bombifrons* hints that these populations are evolving toward genetic assimilation. However, simply sampling tadpoles in the field does not necessarily indicate if plasticity has been lost; it may only indicate that plasticity is not expressed because the correct environmental trigger may not be present. Additional evidence, however, suggests that selection is favoring the evolutionary reduction of plasticity in sympatry. First, sympatric populations are experiencing directional selection for more exaggerated carnivore phenotypes (Pfennig et al. 2007). Indeed, sympatric carnivores are the most exaggerated form observed in nature (Levis et al. 2018). Second, by rearing tadpoles derived from each environment type (allopatry or sympatry) on each diet (detritus or shrimp), Levis and Pfennig (2019) found that sympatric *S. bombifrons* tadpoles were superior competitors for shrimp (the carnivore resource) compared to allopatric *S. bombifrons* tadpoles. This evolutionary exaggeration of carnivore features and carnivore performance suggests that there are developmental limits to a plastic genotype, and that selection is directly selecting for reduced plasticity in sympatric populations. As a result, sympatric tadpoles: (1) hatch more carnivore-like than allopatric tadpoles (Figure 7.3c), prior to ever experiencing the carnivore-inducing cue; (2) exhibit constitutively carnivore-like jaw muscles on either diet (i.e., large jaws even on a detritus diet; Figure 7.3d); and (3) have expression profiles of genes associated with the alternative morphs that are unaffected by diet (Levis et al. 2017).

As predicted by theory (see Section 7.3), this evolutionary shift toward reduced plasticity (incomplete assimilation) was due, in part, to a transition from a variable environment where both morphs were favored to a stable environment where only carnivores were favored, and from possible developmental limits to a plastically induced carnivore morph. This evolutionary transition has taken considerably less time than that predicted by models (~70 generations, Levis and Pfennig 2019). Although sympatric populations seem to be evolving toward genetic assimilation, the complete loss of plasticity might take hundreds of generations, a state that those populations might never reach. Nevertheless, these results indicate that sympatric *S. bombifrons* are experiencing selection for reduced plasticity and enhancement of the carnivore morph.

7.5.2 *Acacia* Extrafloral Nectar

The ant-*Acacia* association has become a textbook example of coevolution. Generally, in return for food (e.g., nectar and Beltian bodies), *Acacia*-ants defend plants from attack by phytophagous insects. The food is produced in response to leaf damage by an herbivore, and this abundance of food recruits ants to defend the *Acacia* from herbivory. Whereas some *Acacia* species facultatively recruit ant defenders, others are obligate hosts to *Acacia*-ants (Janzen 1966; Heil et al. 2004).

The evolutionary change from induced to constitutive secretion of extrafloral nectar and other characteristics of myrmecophytic (ant-associated) *Acacia* suggests that genetic assimilation has occurred. Facultative overproduction of extrafloral nectar in response to attack is the ancestral condition of the *Acacia* genus; obligate myrmecophytes are derived (Heil et al. 2004). Derived *Acacia* constitutively produce higher levels of extrafloral nectar than non-myrmecophytes, and even their levels of undamaged extrafloral nectar are higher than damaged (i.e., induced) non-myrmecophytes. Several other characteristics of myrmecophtyic *Acacia* are essential to, and emergent from, coevolution with *Acacia* ants, such as enlarged foliar nectaries, enlarged thorns tenanted by the ants, and modified leaflet tips eaten by the ants (Beltian bodies) (Janzen 1966). Thus, selection has converted myrmecophytic *Acacia* from a facultative phenotype into a constitutive one. Moreover, the derived phenotype has been extended and refined (e.g., greater nectar production, Beltian bodies) relative to the ancestral-induced phenotype. The latter changes again suggest that there are limits to a plastic developmental pathway of these traits that resulted in genetic assimilation through selection against plasticity.

7.5.3 *Cyanobacteria* Heterocyst Composition

Fischerella sp. are thermophilic, multicellular cyanobacteria found around hot springs and other high-temperature bodies of water worldwide. When the environment lacks nitrogen covalently bonded to other elements, these bacteria facultatively produce specialized cells—heterocysts—that can convert N_2 into a useable form. A requirement for this nitrogen fixation is the formation of a glycolipid layer on the surface of the heterocyst that acts as the primary barrier to gas diffusion. Many cyanobacteria that form heterocysts harbor temperature-dependent plasticity in the composition of this glycolipid layer, with less permeable glycolipid layer compositions being beneficial at higher temperatures. Such temperature-dependent plasticity in glycolipid composition represents the ancestral state for *Fischerella* (Miller et al. 2020).

Glycolipid composition has undergone genetic assimilation in strains of *Fischerella thermalis* living in Yellowstone National Park (Miller et al. 2020). Some canalized strains produce heterocysts with the high-temperature glycolipid isomers even when reared at lower temperatures. Notably, when grown at low temperatures, the levels of these isomers in canalized strains were greater than the levels of these isomers in plastic strains grown at high temperatures. Overproduction of these key isomers limited diffusion (determined via the rate of nitrogen fixation) and enhanced growth at high temperature. Compared to these canalized strains, plastic strains experienced a performance cost at high temperature. Environmental stability is also important here: canalized strains predominate in parts of the habitat in which temperature exhibits relatively low variability. The transition from a plastic glycolipid composition to a canalized composition was due to a single mutation in a gene responsible for heterocyst development. Plasticity likely allowed *F. thermalis* to survive at high temperatures and bought time for a new mutation to arise that ameliorated the costs of plasticity and improved trait functionality in the new, stable environment. Thus, in this system, there were both developmental limits to the plastic phenotype and costs

of plasticity, and there was a change to a low-varying environment. All of these factors together meet the description and criteria put forth by Waddington in his 1942 paper. Regarding the heritable basis of genetic assimilation, whereas the spadefoot example likely involved selection on standing genetic variation for plasticity, for *F. thermalis* it resulted from a single new mutation. Such mutational origin is more likely to occur in bacteria which are haploid and single celled and so do not have complex development. This difference may hint at broader patterns among taxonomic groups in how genetic assimilation occurs.

BOX 7.1 SUGGESTIONS FOR FUTURE RESEARCH

- Continue evaluating genetic assimilation in diverse traits and taxa. Ideal systems include the ability to make ancestral/derived comparisons and multiple, independent evolutionary transitions. Find systems that variously show plasticity costs and developmental limits and see whether the evolutionary responses are as predicted in Table 7.2. For these tests, it is just as important to examine systems where genetic assimilation has not occurred.
- Identify the types of traits most conducive to genetic assimilation and compare their evolution against that of other traits. Does genetic assimilation occur more often when traits are: labile or fixed, active or passive, categorical or continuous? A powerful test would be to compare different parts of a complex syndromic response because all of the other conditions (i.e., the history of lineages and the processes of selection and drift) are held constant. The potential limitation of this approach is that genetic or developmental correlations may make their evolution non-independent.
- Obtain data on the developmental limits of plasticity and what causes such limits. The most plausible examples of genetic assimilation all seem to include some sort of limitation on development through plastic pathways. Data are needed on developmental limits of plasticity. Are such limits greater or lesser depending on the types of plasticity as listed above, and can that suggest when genetic assimilation is more likely to occur?
- Determine how often genetic assimilation utilizes standing genetic variation, *de novo* mutation, induced mutation, and/or epigenetic variation. These different sources of variation might influence the probability that, or rate by which, genetic assimilation occurs.
- Develop models based on different types of traits, genetic architecture, and costs/limits to plasticity. Current models have considered only continuous traits, linear reaction norms, a lack of developmental limits, and strictly additive genetics. Models are needed for threshold traits (polyphenisms) and more complex genetic architectures.

Models could be developed for specific examples that reflect information about the genetic architecture of the traits and its plasticity and developmental pathways. Such models could be used to test the plausibility of genetic assimilation.

- Reconcile differences between model predictions and empirical data. So far, models of genetic assimilation suggest that this process takes a very long time. However, empirical investigations of this process are often two orders of magnitude (or more) faster. Is this difference simply due to the strength of selection or the types of traits being modeled? Perhaps the empirical examples have been sampled prior to the completion of genetic assimilation. What parameter sets are needed to recapitulate empirical findings and what model assumptions are empirical studies violating?

7.6 CONCLUSIONS

Genetic assimilation is a long-standing idea that is simultaneously both extremely popular and controversial, in large part because it is built on limited theory and data. Only four models have explicitly tried to describe this process and robust empirical data come from only a limited sampling of taxa (mostly insects). Yet, there is a growing body of examples (much of which is from natural populations) where the evolution of traits and their plasticity is compatible with genetic assimilation. Thus, while models, laboratory experiments, and data from natural populations all point to the plausibility of genetic assimilation contributing to evolution and highlight some of the conditions that favor genetic assimilation, more work is needed before we fully understand this interesting process.

ACKNOWLEDGEMENTS

We thank David Pfennig, Carl Schlichting, and an anonymous reviewer for their cogent comments.

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Section III

Consequences of Plasticity

Adaptation, Origination, Diversification

DOI: 10.1201/9780429343001-III

8 Buying Time: Plasticity and Population Persistence

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CONTENTS

8.1 INTRODUCTION

As the environment changes, populations can respond by adapting via evolutionary change. If the rate or magnitude of environmental change is too great for evolution to keep pace, then populations face the risk of extinction (Chevin et al. 2010; Sinervo et al. 2010; Radchuk et al. 2019). While the majority of species that have ever lived have gone extinct, the wealth of extant biodiversity on Earth proves that many lineages have successfully survived and diversified in response to the extensive environmental challenges thrown at them since life began (see Lister 2021 in this volume).

In this chapter, we will evaluate the important role phenotypic plasticity—the ability of a single genotype to produce different phenotypes in response to environmental variation—may play in 'buying time' for populations to persist and potentially then evolve when confronted with rapidly changing or novel environments. We briefly describe the history of this concept and review the theoretical predictions

DOI: 10.1201/9780429343001-8

regarding when phenotypic plasticity will or will not be able to buffer populations in changing and novel environments. We also review the empirical evidence for plasticity buying time and lay out a framework for future tests focusing on organismal responses to novel urban environments. Finally, we discuss the open questions and future directions for further research.

8.2 A BRIEF HISTORY OF BUYING TIME

Populations can evolve at a remarkably fast pace. Although for a long time, the action of natural selection was thought too slow to be readily observed over the course of a human life, in fact, evolution often occurs at the same timescale as ecological change (Reznick et al. 2019). Even so, adaptive evolutionary change can still be constrained for many reasons (e.g., through a lack of heritable variation, environmental stochasticity, and costs of selection). And, evidence suggests that many species are not evolving fast enough to keep pace with the current rate and magnitude of environmental change caused by a warming climate, habitat alteration, species invasions, and other anthropogenic forces (e.g., Radchuk et al. 2019). For some populations, plastic responses to these environmental challenges might be able to buffer populations from extinction, allowing time for adaptive evolution (Merilä and Hendry 2014; Diamond and Martin 2016; Fox et al. 2019).

The idea that phenotypic plasticity might buy populations the time they need to evolve (and perhaps subsequently shape evolutionary responses) is surprisingly old. Following Darwin's publication of *The Origin of Species*, one of the primary criticisms with the theory of natural selection was that the small intraspecific variations viewed as critical by Darwin were too slight for natural selection to effectively work upon (see Costa 2021 in this volume). This criticism led to the promotion of neo-Lamarckian theories of evolution, where in place of natural selection, the acquisition and inheritance of environmentally induced traits was instead the major mechanism of adaptive evolution (Simpson 1953; Crispo et al. 2010; see Bonduriansky 2021 in this volume). As a counterweight, several scientists independently proposed theories incorporating the role of environmentally sensitive traits into the framework of natural selection and Darwinian evolution (Baldwin 1896; Morgan 1896; Osborn 1896). These ideas were most thoroughly developed by James Baldwin and now are collectively known as the 'Baldwin effect' (Simpson 1953; see also Futuyma 2021 and Pfennig 2021 in this volume). Baldwin proposed that through the process of 'organic selection,' plasticity allows individuals to survive in novel and changing environments. Natural selection can then act either on standing genetic variation or on novel mutations with phenotypic effects along the same direction as the plastic effects, promoting adaptation to the novel environment by the further evolution of plastic or canalized responses. While evolutionary biologists during the modern synthesis, such as G.G. Simpson, considered the Baldwin effect plausible, they also thought it to be of little general importance and often misconstrued aspects of Baldwin's theory (West-Eberhard 2003; Crispo 2007; Scheiner 2014).

After Baldwin, Gause, Schmalhausen, and Waddington developed their own distinct theories incorporating a role for plasticity in evolution (Gause 1942; Schmalhausen 1949; Waddington 1961). Both Schmalhausen and Waddington were

key for initiating the developmental and genetic framework for understanding phenotypic plasticity. Importantly, all of these scientists saw an important role for plasticity preceding and influencing evolutionary change. For a detailed review of the overlap and distinctions among these ideas see West-Eberhard (2003), Pigliucci (2001), Crispo (2007), as well as Futuyma (2021) and Pfennig (2021) in this volume. Nevertheless, for most of their contemporaries, phenotypic plasticity was thought to play little role in evolution overall (Pigliucci 2001; West-Eberhard 2003). While Bradshaw (1965) mentioned that plasticity could help populations with limited genetic variation adapt to strong directional selection, there was otherwise little further research on plasticity's role in adaptation to novel environments until the 1980s when West-Eberhard (1989) and Wcislo (1989) reviewed and discussed behavioral plasticity's potential roles in the evolution of novel traits and in adaptation to novel environments. West-Eberhard went on to develop the concepts of phenotypic accommodation (adaptive adjustment to mutational or environmental change among integrated traits via development) and genetic accommodation (adaptive evolution of novel traits induced by mutational or environmental change), building on the theories proposed by Baldwin, Waddington, and others (West-Eberhard 2003; Crispo 2007). Around the same time as these early reviews and verbal models, formal modeling approaches started to explore the evolution of adaptive plasticity (Via and Lande 1985) and the effects of plasticity on the speed of adaptive evolution (Hinton and Nowlan 1987).

8.3 THE THEORY AND MODELING BEHIND BUYING TIME

From modeling and theory, how does plasticity buy time for evolution, and what are the conditions under which this will occur? Imagine a population exposed to a novel environment. If the population's mean phenotype has low fitness in this novel environment, and is not plastic, then the population faces the risk of extinction. However, if fitness-determining traits are plastic in response to the environmental change, then this plasticity could: (a) further push the population away from the new fitness optimum; (b) move the population closer to the new fitness optimum; or (c) place the population directly upon the peak (Figure 8.1). In cases (b) and (c), plasticity in the novel environment can promote population persistence and reduce the intensity of selection (Ghalambor et al. 2007). But what other factors influence the fitness effects of plasticity in novel environments and its impact on future evolutionary change? Mathematical and simulation models provide answers and testable predictions to these questions. The first model to explore this question asked if learning (a common form of plasticity in animals) could accelerate evolution. In this model, only a single phenotype conferred adaptation, while all other phenotypes were equally maladaptive, meaning that there was no slope of increasing fitness approaching the peak in the absence of plasticity. From this model, Hinton and Nowlan (1987) found that learning greatly accelerated the adaptive evolution of the population. Simply put, in this and other models of non-evolving plasticity, phenotypic plasticity smoothed out the fitness landscape by allowing individual genotypes to explore more of the phenotype space. As a consequence, less fit genotypes are able to survive and, in the presence of genetic variation, evolve towards the fitness optimum (Figure 8.2; reviewed in Frank 2011).

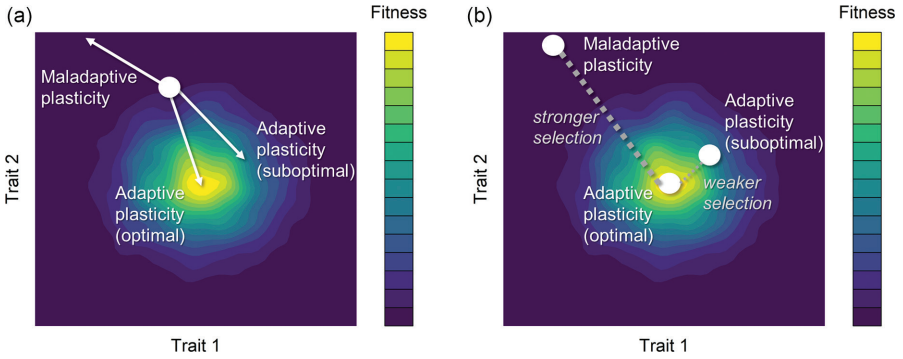

FIGURE 8.1 Variation in plastic responses to environmental change in a novel environment. In both panels, a fitness landscape for two traits is depicted, with the fitness peak in the center of the heatmap plot (and in warmer colors). (a) The population's mean phenotype (represented by the point) begins off its fitness optimum in the novel environment. Three different scenarios for plasticity are shown, including optimal adaptive plasticity that can return the population to its fitness peak; suboptimal adaptive plasticity that increases fitness but does not get the population to its fitness peak; and maladaptive plasticity that moves the population farther away from its fitness peak. (b) Consequences of the evolutionary response for different types of plasticity. Populations begin at their respective points following different forms of plasticity, i.e., the arrowheads from panel (a). Selection is weakest for optimal adaptive plasticity as there is no variation to act upon; selection is weak, but present for suboptimal adaptive plasticity; and selection is strongest in the case of maladaptive plasticity. Modified from Ghalambor et al. (2007).

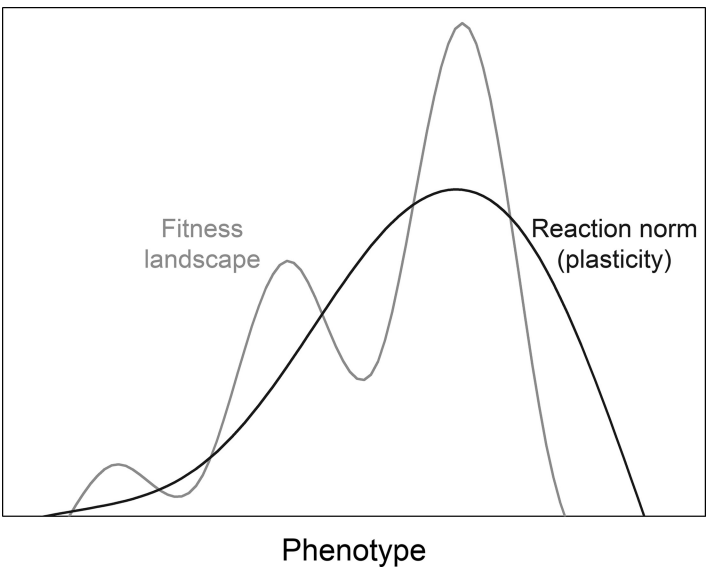

FIGURE 8.2 A high degree of plasticity results in smoothing of a bumpy, multi-peaked fitness landscape. Redrawn from Frank (2011).

However, plasticity can also slow the rate of evolution and negatively impact persistence (Ancel 2000; Ghalambor et al. 2007; Paenke et al. 2007). For plasticity to facilitate evolution and persistence, plasticity must be somewhat adaptive in the new environment, although perfect plasticity that matches the fitness optimum will increase persistence but prevent further evolution (Figure 8.1; Price et al. 2003; Ghalambor et al. 2007; Paenke et al. 2007). Moreover, the effects of plasticity on evolution depend on which genotypes benefit. Plasticity positively affecting relatively more-fit genotypes will generally speed evolution but evolution will be impeded if instead less-fit genotypes are more plastic (Paenke et al. 2007).

In general, the models discussed above assume an unchanging environment, do not model population persistence directly, and omit potential costs of plasticity. Using a model incorporating a continuously changing environment and costs to plasticity, Chevin et al. (2010) found that adaptive plasticity did enable population persistence, although it also reduced the strength of directional selection. Moreover, they found that costs of plasticity constrained the population's ability to track environmental change and that persistence was greatest with intermediate levels of plasticity (Figure 8.3; also see Nunney 2016). Building further upon these models, Reed and colleagues (2010) modeled plasticity's ability to buffer populations in response to a temporally fluctuating environment. Here, they found that when environmental stochasticity was moderate and predictable (i.e., by an environmental cue), even weakly adaptive plasticity could buffer populations and allow them to persist. However, when environmental stochasticity was high and unpredictable, plasticity resulted in a greater phenotype-to-environment mismatch, and increasing the magnitude of plasticity could even increase the risk of extinction.

The models described above show that, as long as plasticity is somewhat adaptive in the new environment, plasticity can buy time for populations to evolve under some conditions. However, when genetic variation in reaction norms exist, plasticity itself can evolve (Scheiner 1993), creating a dynamic interplay between plasticity and evolution. How does the evolution of plasticity affect its ability to buffer populations from the impact of environmental change?

Verbal models (e.g., Baldwin 1896; Waddington 1961; West-Eberhard 2003) suggest that the evolution of plasticity can speed the rate of adaptation to novel environments (reviewed in Price et al. 2003; Ghalambor et al. 2007), and recent formal modeling approaches have been useful in identifying under what conditions this can occur. There is broad consensus among these models that phenotypic plasticity, when there is little associated cost, significantly increases population persistence in novel and changing environments, and that allowing plasticity to evolve enhances this effect and speeds up the rate of adaptation (Lande 2009; Chevin et al. 2010; Scheiner et al. 2017, 2020). This is true whether environmental change is modeled as an abrupt shift (with small temporal variability before and after) or as a directionally moving optimum. Moreover, models show that plasticity, and the evolution of plasticity, can also facilitate persistence and adaptation to new environments even in the presence of gene flow from source populations (Chevin and Lande 2011; Thibert-Plante and Hendry 2011). Altering the predictability of environmental change however can limit the ability of populations to persist, even with evolving plasticity (Ashander et al. 2016). And when costs of plasticity are

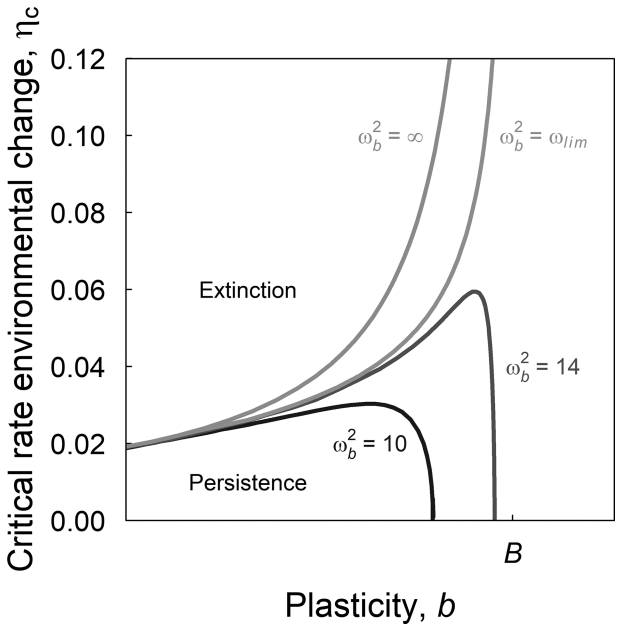

FIGURE 8.3 Results from a model showing that the critical rate of environmental change depends on the cost of phenotypic plasticity. The maximum rate of environmental change that supports long-term persistence of a population, η_c, is expressed as a function of plasticity, b, at four values of the cost of plasticity, ω_b^2. Increasing costs of plasticity are indicated by smaller values of ω_b^2, the width of the fitness function for plasticity. In this example, ω_b^2 includes values of 10 (the highest cost of plasticity considered), 14 (a comparatively lower cost of plasticity), ω_{lim} (the threshold for the cost of plasticity), and infinity (no cost of plasticity). Rates of environmental change higher than each line indicate population extinction, and rates of environmental change below each line indicate population persistence. The populations with relatively high costs of plasticity, that is, when $\omega_b^2 = 10$ and when $\omega_b^2 = 14$, only persist at relatively low values of plasticity, b (shown in the purple and blue lines). However, for these scenarios where $\omega_b^2 < \omega_{lim}$, note there are medium-high values of plasticity that maximize the critical rate of environmental change. In this model, B indicates the environmental sensitivity of selection. Redrawn from Chevin et al. (2010).

high (Scheiner et al. 2020) or when predictability is very low, the evolution of greater plasticity can potentially be maladaptive, increasing the risks of population extinction (also see Lande 2015).

In sum, theory suggests that in novel and changing environments, adaptive plasticity can buy time for populations to evolve, and can even speed this process, especially when plasticity itself can evolve, costs of plasticity are low, and environmental variability is predictable. However, these results also point towards the need for more empirical data to test the models' assumptions and predictions (Box 8.1). For example, when they are measured, costs of plasticity are generally small. However, these are rarely measured in novel environments where additional costs may be imposed (Chevin and Hoffmann 2017; Snell-Rood et al. 2018).

8.4 CRITERIA AND APPROACHES

Several authors have enumerated aspects of the criteria and approaches for buying time (Robinson and Dukas 1999; Pigliucci 2001; Wund 2012; Morris 2014). We synthesize this previous work here to develop a unified set of criteria and review the different ways in which buying time might be assessed. Our main goal in this section is to develop a generalizable summary of criteria and approaches. In the following section, we then apply these criteria and approaches to our review and synthesis of empirical case studies of buying time.

Effectively, there are two criteria for demonstrating buying time via plasticity. First, the population must be exposed to novel environmental conditions beyond those experienced in the ancestral environment. We take a broad view of this criterion to include changes in both the frequency and magnitude of the environmental change. For example, under this definition, it would be sufficient for a population that occasionally experiences extreme high temperatures to experience them more frequently as the environment changed, even if those temperatures were not, in a strict sense, outside the actual range of temperatures experienced by the ancestral population. Of course, populations might also experience novel conditions completely outside the range of ancestral variation, such as in the case of exposure to a novel pesticide or other novel environmental toxin. Second, the population must persist under the novel environmental conditions as a direct consequence of plasticity. Because the causal relationship can be difficult to establish, weaker evidence involves demonstrating that the population has equal or greater fitness due to plasticity expressed in the novel environment. Specifically, the presence of plasticity and the demonstration of population persistence over time would be incomplete evidence of buying time via plasticity. Only through the causal association of variation in plasticity with variation in population persistence can buying time be conclusively shown. Either plasticity or population persistence on its own is insufficient, as populations might persist via other mechanisms (e.g., immigration; rapid evolution) and plasticity, while present, might be unlinked with population persistence. A third aspect of buying time via plasticity—subsequent genetic adaptation of the population, i.e., genetic accommodation—is more a consequence of buying time rather than a necessary criterion for its existence. Although, importantly, the evolution of plasticity itself can enhance its effects on population persistence (see Section 8.3).

Buying time via plasticity can be tested in a number of different ways by using laboratory and field experiments and population- and species-level comparative approaches. The most direct test of this hypothesis would be to choose replicate populations that differ in their plasticity for a single or suite of traits in a novel environment, but are otherwise matched, and assess whether the magnitude of plasticity is positively associated with persistence after being exposed to novel environmental conditions. Such an approach could be carried out in either the field or laboratory setting. However, there is likely to be a limited subset of organisms for which this approach is possible given the constraints on having sufficiently short generation times to be able to track changes in population size over reasonable timeframes. Alternatively, historical versus contemporaneous population samples can be used to test for buying time via plasticity. To establish an association between

plasticity and persistence, at least one historical population size sample, or preferably regular monitoring of the population, must be present. This method could be less able to establish a causal link between plasticity and population persistence, as many such studies lack an experimental manipulative component and records on the nature of the precise environmental change could be sparse. Further, as in many fields of study, historical samples to match against contemporary samples and regular monitoring of populations over time tend to be relatively rare. There are, however, a number of benefits of tests of buying time via plasticity that are performed using matched historical-contemporary samples in field settings. Organisms with longer generation times can be tested, and environmental changes experienced *in situ* are inherently more biologically relevant.

Extant, contemporaneous population and species comparative approaches are yet another way that buying time can be tested. For example, natural variation of the reaction norms among populations of a species exposed to ancestral versus novel environmental conditions can be compared. Similarly, such approaches can be extended to higher levels of biological organization, such as species that differ in their plasticity. As with any comparative approach of this nature, increasing the level of biological organization of analysis tends to increase the potential for confounding variables. Further, like the historical-contemporary comparisons described earlier, with the use of comparative approaches on contemporaneous groups, it can be challenging both to establish a causal link between plasticity and population persistence and to disentangle current plasticity from ancestral plasticity that allowed species to persist under some previous environmental change. By contrast, the major benefit of comparative approaches lies in the large number of species that can be evaluated for the plasticity-persistence association.

In context of these different approaches to testing buying time via plasticity, it is worth considering what types of traits should be examined. As a general rule, examining plasticity in traits that determine fitness should be preferred, since the ability of a population to maintain high mean population fitness while experiencing changing environmental conditions is the core tenet of this hypothesis (Lande 2014). Indeed, while there should be minimal plastic variation in fitness across ancestral and novel environments, plasticity in traits such as morphology, behavior, and physiology will ideally allow populations to maintain high fitness when experiencing environmental change. Depending on the type of trait and nature of the plasticity, greater plasticity can manifest as, for example, a steeper slope in the relationship between the trait value and the environment, or as a broader curve over a range of phenotypic values. Alternatively, trait values might be fixed across ancestral and novel environments via trait canalization to maintain high fitness under changing environmental conditions; however, such fixation of traits across environments can be achieved via plasticity in other traits (Grether 2005).

Beyond demonstrating the ability of plasticity to promote population persistence, studies should also aim to test specific predictions from theory to evaluate the conditions in which buying time will occur. For example, models suggest that costs of plasticity, the predictability of environmental variation, and the covariance of plasticity and fitness affect the likelihood that plasticity will rescue populations (see Section 8.3). These parameters can be measured in natural systems and model

systems of experimental evolution could be well-suited for manipulating these effects. In addition, different mechanisms of phenotypic plasticity have themselves evolved in response to these same conditions, suggesting that some mechanisms of plasticity may be more likely to buy time than others in novel environments.

Recently, Snell-Rood et al. (2018) formalized definitions of three general types of plastic response to changing environmental conditions including: developmental selection, generalized physiological responses, and phenotypic switches. These different plastic mechanisms carry different costs to produce the phenotype and confer different likelihoods that the phenotype will be adaptive in a novel environment (Figure 8.4; see also Lande 2014). While developmental selection carries the highest cost of phenotype, it is also the most likely to be adaptive in the novel environment. Developmental selection mechanisms encompass environment-induced changes in learning, acquired immunity, tissue architecture and the microbiome. As a consequence, developmental selection mechanisms are highly targeted to the novel environment but are costly because they require considerable investment throughout development in response to feedback from the environment. Generalized physiological responses are intermediate with respect to both cost of phenotype and the likelihood of being adaptive in the novel environment. Such generalized physiological responses are not specifically targeted to the new environment, so there can be a waste of resources into broad stress responses that might not cover all possible stressors which increase the cost of phenotype. However, there is often cross-protection from generalized physiological stress responses that can increase the likelihood of being adaptive in the novel environment: for example, increased heat shock proteins can protect against both heat and desiccation stress (Chown and Nicolson 2004). Finally, phenotypic switches, while carrying low costs of phenotype, are the least likely to be adaptive in the novel environment. Phenotypic switches are some of the most commonly described forms of plasticity and encompass polyphenisms and graded developmental responses. Because these responses are highly tailored, the likelihood that phenotypic switches will be adaptive in the novel environment is quite low. However, this expectation does not preclude the fact that there might still be a relatively small subset of environmental changes that allow such phenotypic switches to preadapt organisms for a given novel environment. Although any one of these plastic mechanisms can buy time for populations experiencing environmental change, this framework allows us to develop predictions for their ability to match and be adaptive in the novel environment against the costs required to produce these phenotypes.

In a similar vein, whether plasticity is adaptive in the novel environment will also depend on the nature of the environmental change. Assuming that plasticity evolved in the ancestral environment in response to a specific set of environmental conditions, if the novel environment is an extension of the ancestral environment, such as an increase in environmental temperature, then the plastic response could be somewhat adaptive in the novel environment. However, if the novel environment is either characterized by an extreme shift well beyond the range of the ancestral environment or truly novel (i.e., an environment never experienced before over the evolutionary history of the species, such as a novel environmental toxin), then it is much less likely that plasticity will be adaptive in the new environment. Although importantly, these

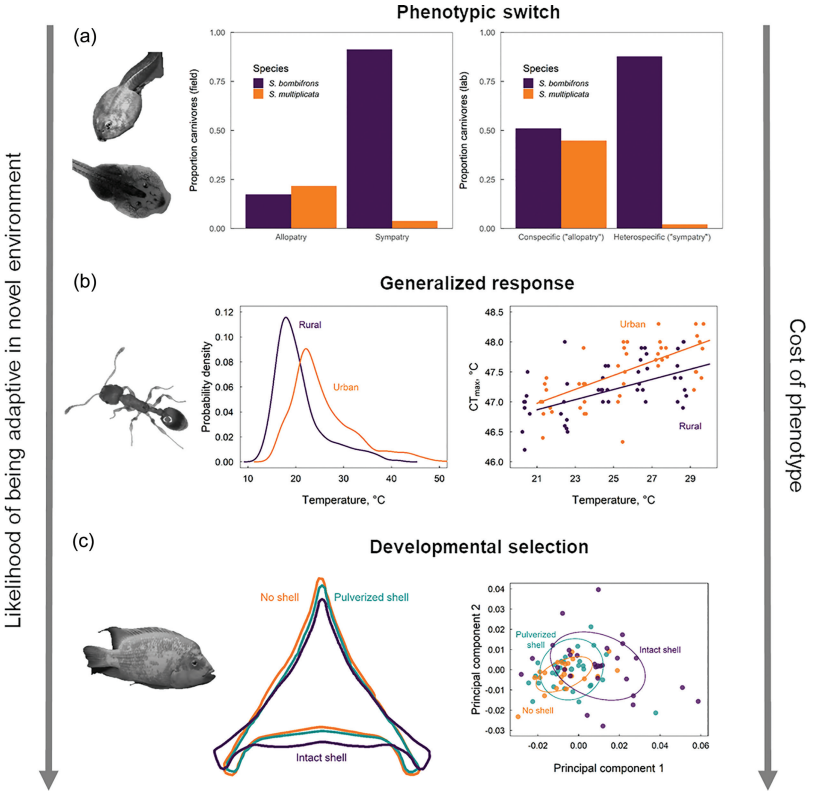

FIGURE 8.4 Case studies of different types of plasticity rescue expressed as functions of their likelihood of being adaptive in the novel environment and the cost of phenotype (*sensu* Snell-Rood et al. 2018). (a) Spadefoot toad tadpoles (*Spea* spp.) exemplify a phenotypic switch, with a low likelihood of being adaptive in a novel environment but a low cost of phenotype. In this system, consumption of shrimp or other tadpoles induces a developmental switch from a typical omnivore morph to a carnivore morph. Under allopatric conditions in the field or reared with conspecifics in the lab, *S. multiplicata* and *S. bombifrons* produce carnivores and omnivores in similar ratios. However, under sympatric conditions in the field or reared with heterospecifics in the lab, *S. bombifrons* adaptively produces a high proportion of carnivores whereas *S. multiplicata* adaptively produces almost none, reducing interspecific competition [data from Pfennig and Pfennig (2012).] (b) Acorn ants exemplify a generalized response, with intermediate likelihood of being adaptive in a novel environment and an intermediate cost of phenotype. In this system, a plastic increase in heat tolerance (the critical thermal maximum, CT_{max}) allows acorn ants to cope with elevated temperatures and might have allowed rural population acorn ants to persist until the evolution of higher heat tolerance could occur [data from Diamond et al. (2018).] (c) The Midas cichlid exemplifies developmental selection, with a high likelihood of being adaptive in a novel environment but a high cost of phenotype. Fish that were fed on snails with their shells intact had shorter, less concave jaws with broader, more outwardly pointing horns (towards the base of the jaw) compared with fish fed on either standard fish food or snails with pulverized shells [data from Muschick et al. (2011)]. Photo credits (grayscale of originals): spadefoot toad tadpoles: David Sanders; acorn ant: Ryan Martin; Midas cichlid: Mahufi/Wikimedia Commons.

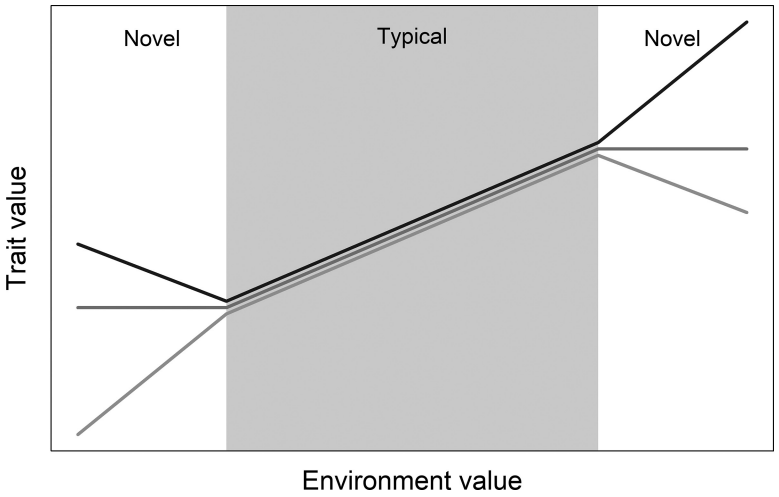

FIGURE 8.5 Reaction norms of three genotypes under typical and novel environmental conditions. Genotypes have similar responses to environmental variation under typical conditions. By contrast, genotypes have divergent responses to novel environmental conditions, under both lower and higher than usual environment values. (Redrawn from Ghalambor et al. [2007].)

novel environmental conditions could also expose cryptic heritable variation in the reaction norm (Figure 8.5), potentially allowing for adaptive plasticity to quickly evolve (Ghalambor et al. 2007).

8.5 EMPIRICAL EVIDENCE OF BUYING TIME

In this section, we discuss the currently available evidence for buying time via plasticity. However, first we aim to direct readers to reviews of buying time via plasticity. Representative studies can be found throughout more general compilations such as West-Eberhard (2003), and within specific sections on the topic of buying time of many additional works (Robinson and Dukas 1999; Pigliucci 2001; Ghalambor et al. 2007; Wund 2012; Morris 2014; Schlichting and Wund 2014; Fox et al. 2019).

We begin by highlighting strong examples of buying time, specifically those that either directly meet both criteria for buying time, or at least, have strongly suggestive data. These studies show evidence of populations experiencing changing and novel environmental conditions and establish a strong link between plasticity and population persistence. We then highlight related fields of study that provide other examples of buying time, including the literature on genetic accommodation, invasion biology, biological responses to global climate change, and recent work on plasticity of the microbiome.

Perhaps the most complete example of buying time via plasticity is that of plastic changes in breeding phenology that allow dark-eyed juncos from a temperate, montane habitat to establish in a lowland, coastal habitat (Yeh and Price 2004). This example of buying time is especially strong, as the authors were able to pinpoint the

actual colonization of the coastal habitat in the early 1980s and were able to monitor population size for six years after the introduction, from 1998 to 2003. Further, the ability to plastically extend the breeding season in the coastal habitat was associated with the maintenance of population size over the study period, strongly suggesting a link between the plasticity and population persistence without evolutionary change.

Alternative phenotypes can also be important types of plasticity that buy time for populations in novel environment conditions. Ecological character displacement in two species of spadefoot toad tadpoles is a prime example. In the American Southwest, *Spea bombifrons* and *S. multiplicata* produce approximately equal ratios of two environmentally induced phenotypes when found alone in allopatry. The two phenotypes represent a developmental switch to a carnivore morph with large jaw muscles, serrated beaks, and shortened intestines that allows them to specialize on larger prey such as fairy shrimp and tadpoles, from an omnivore morph that lacks these features and feeds primarily on algae and detritus. However, in sympatric ponds, there are species-specific shifts in the ratios of these two morphs. *S. bombifrons* produces mostly carnivores whereas *S. multiplicata* produces mostly omnivores (Figure 8.4a; Pfennig and Murphy 2000, 2002). By being able to modulate this developmental switch, the species reduce competition for food which, in turn, facilitates persistence (Pfennig et al. 2006, 2007). Further, in *S. multiplicata*, the differences in the production of carnivore morphs between allopatric and sympatric populations appear to be mediated by plasticity via a condition-dependent maternal effect (Pfennig and Martin 2009; Martin and Pfennig 2010; Levis and Pfennig 2019).

Despite their specificity (Snell-Rood et al. 2018), alternative phenotypes under the control of developmental switches may be an effective means of buying time in situations where preexisting plasticity is immediately adaptive in novel environments because the selective pressure, although novel, is similar in action to a fluctuating selective pressure in the ancestral environment (West-Eberhard 1989, 2003; e.g., gain or loss of predators, competitors or prey). Indeed, such developmental switches based on resource-use traits appear to be a highly repeatable form of buying time via plasticity. Pfennig and McGee (2010) used a series of replicated sister-group comparisons in spadefoot toads as well as centrarchid fish, salmonid fish, neotropical cichlids, and mole salamanders to show that plasticity in resource use and the consequent developmental switch was positively associated with geographic range size and species richness for a given clade, two proxies of persistence.

Comparative approaches of a similar nature, though without relying on sister-group comparisons, have also recently been used to test for buying time via plasticity across a diverse suite of bird species. Ducatez et al. (2020) performed a meta-analysis of over 8000 species of birds and over 3800 novel behaviors and found that those species with a higher propensity to innovate were at a lower risk of global extinction and are more likely to have increasing or stable populations than less innovative birds (see also Sol et al. 2002, 2005). Studies of this nature, while less able to establish direct causation between plasticity and persistence, nonetheless suggest that this mechanism could be fairly widespread.

Experimental transplantation studies also provide evidence for plasticity promoting persistence in novel environments. For example, Volis et al. (2014, 2015) transplanted wild emmer wheat from four natural populations into a low rainfall desert site, outside

the current environmental conditions for the species. Using plasticity measured in a separate greenhouse experiment with drought and normal water conditions, they found that the most plastic population was also the most successful, measured by estimated lifetime fitness, in the novel desert environment (see Donohue et al. 2001 for another example). In a related phenomenon, the 'polyploid plasticity hypothesis' suggests that the widespread success of polyploid plant species (and other taxa) at colonizing novel and diverse habitats, in comparison to closely related diploid species, is due in part to a greater propensity for plasticity in polyploids (Levin 1983; Van de Peer et al. 2017). However, the status of this hypothesis is currently unclear with both some positive (e.g., Hahn et al. 2012) and negative (e.g., Wei et al. 2019) support for the role of plasticity in the success of polyploids across diverse environments.

In addition to these examples, other likely places to look for buying time comes from the genetic accommodation literature. As genetic accommodation describes the process by which newly exposed plasticity (or other novel traits) are subsequently refined by selection, such studies are likely to contain not only the after-effects of buying time, but varying degrees of evidence of the process itself, namely the link between plasticity and population persistence. For example, Badyaev (2009) summarized the Baldwin effect processes operating in the rapid range expansion and colonization of novel habitats across North America by the house finch. Novel high temperatures at the southern range edge and novel cool temperatures at the northern latitude range edge exceed egg-viability limits in the expanding range edge populations. These conditions induce novel behavioral and physiological variation including changes to the traits of environmental assessment, incubation behavior, and oogenesis. Because these traits are all underlain by a common hormonal pathway, they shift in concert in response to environmental change. Female finches can alter offspring size via shifts in hatching order which, in turn, determine offspring growth rate. At the northern range edge, small males and large females are favored and breeding females produce daughters in first-laid eggs and sons in last-laid eggs. However, at the southern range edge, large males are favored, and breeding females produce sons in the first-laid eggs and daughters in the last-laid eggs. As a consequence, the direction of sexual size dimorphism is opposite at the expanding northern and southern range edges. Following induced plasticity in egg laying order, natural selection has acted to refine this variation to maintain reproductive homeostasis under novel environmental conditions. As part of this study, the finch populations were monitored over a several-year period, allowing the researchers to establish a fairly strong association between the egg laying plasticity and the maintenance of population size over time.

Other genetic accommodation papers lack such population persistence data, but nonetheless represent plausible cases where plasticity buys time for evolution to occur. For example, Hua et al. (2015) found that plasticity in the ability to tolerate elevated concentrations of the insecticide, carbaryl, likely facilitated the evolution of insecticide tolerance in wood frog populations. As selection continued to refine the tolerance plasticity, the plastic variation was lost (i.e., the trait was canalized) in populations with high exposure to the insecticide. By contrast, populations with minimal exposure to the insecticide retained plasticity in tolerance. Although population sizes were not directly monitored as part of this study, the fact that initial plasticity was acted upon by natural selection and populations remain extant are suggestive of

plasticity buying time in this system. Similarly, Corl et al. (2018) found evidence of evolutionary divergence in the coloration of side-blotched lizards across light-colored sand habitats and dark-colored lava flow habitats, and that this divergence was likely promoted by plasticity in body coloration in response to the background coloration of the habitat. Shifts in the resource base can also drive the genetic accommodation process. Aubret and Shine (2009) document evolutionary divergence in the head size of tiger snakes across different islands. The islands harbor populations of snakes that differ in their time since colonization of the habitat, and such differences are associated with evolutionary divergence in their responses to prey. The older island populations have become canalized for larger head size. This response appears to be facilitated by a plastic morphological response to variation in prey size, as the newer island population snakes have relatively small head size at birth, but plastically increase their head size in response to large prey items. Finally, tests of the flexible stem hypothesis (West-Eberhard 2003), which predicts that the diverse phenotypes within an adaptive radiation emerge from ancestral developmental plasticity, provide evidence of plasticity's importance in the adaptation to novel environments. Diverse examples, including sticklebacks (Wund et al. 2008), *Anolis* lizards (Losos et al. 2000; Kolbe and Losos 2005) and spadefoot toads (Gomez-Mestre and Buchholz 2006; Kulkarni et al. 2017) match this pattern. Similarly, West-Eberhard (2003) hypothesized that the repeated, independent evolution of crassulacean acid metabolism (CAM) photosynthesis in plants in response to environmental stress was facilitated by widespread ancestral plasticity in photosynthetic pathways.

Invasion biology is another, if less obvious, place to look for evidence of buying time. Greater plasticity of invasive species has been hypothesized as a key mechanism underlying their success (Baker 1965). This idea has been especially well-tested in plants, and while there does seem to be some positive support for a link between persistence of invasive species in novel habitats and plasticity, a number of counter-examples exist (reviewed in Richards et al. 2006). Davidson and colleagues (2011) performed a large comparative study of phenotypic plasticity in 75 invasive versus non-invasive plant species pairs. They found that while invasive species harbored greater plasticity, it was the non-invasive species that maintained greater fitness homeostasis, potentially maintaining their population sizes over time. However, individual studies do provide some evidence of a plasticity-persistence link. For example, Funk (2008) found evidence that plasticity in plant functional traits was higher in invasive plant species compared with non-invasive species pairs among a diverse group of plant taxa in Hawaii. These traits were strongly associated with plant performance and fitness suggesting that such plasticity could aid the persistence of the invasive species in the novel habitat. Similar results were found in native-introduced range population comparative study of an Asian annual plant recently introduced to North America (Matesanz et al. 2012). On the other hand, plasticity may buffer native species from invaders. Trussell and Smith (2000) found that a rapid increase in shell thickness in an intertidal snail during the early 20th century was in fact due to adaptive plasticity that may have quickly evolved in response to an introduced crab. It is unclear if this plasticity was present before the predator invasion, but the response may have evolved as adaptive plasticity rather than a canalized change due to the large cost of shell thickening on body mass (Trussell and Smith 2000).

Arthropod systems provide further support of a plasticity-persistence link in the context of invasion biology. For example, the invasive Mediterranean fruit fly *Ceratitis capitata* has much greater acute thermal tolerance plasticity compared with its native congener *C. rosa*, and this difference in plasticity is associated with a much larger geographic range of *C. capitata* compared with *C. rosa* (Nyamukondiwa et al. 2010). In addition, Chown et al. (2007) examined plasticity in desiccation tolerance in response to chronic and acute temperature treatments among native and invasive springtail species. While they did not find plastic differences in the absolute limits of tolerance between the species, they did find that the shape of the plasticity differed between the species. In particular, the invasive species were consistent with a 'hotter is better' relationship whereas the native species were consistent with a 'colder is better' relationship for desiccation tolerance. Under global climate change, the invasive species were predicted to have higher population persistence.

Indeed, the literature on biological responses to climate change is likely to be another fruitful area for exploring buying time via plasticity. In addition to the springtail system described above, tolerance plasticity appears to be a common feature of many ectothermic species, and while the magnitude of plasticity is, on its own, generally insufficient to cope with global climate change (Gunderson and Stillman 2015; Sørensen et al. 2016), it could still buy time for populations to evolve. Similar scenarios are unfolding for other aspects of global change including alterations to natural habitats for agriculture and human settlement. Recent work in *Anolis* (Campbell-Staton et al. 2020) and *Leiocephalus* (Neel et al. 2020) lizard species suggests that plasticity has been important for the establishment of populations within heavily urbanized areas. Transgenerational plasticity could likewise facilitate persistence, although importantly, such a mechanism relies on the reliable environmental cues (Donelson et al. 2018). For example, a long-term study on blue tits found that the expected temporal trend of earlier laying dates was only detected in populations inhabiting evergreen forests, but not in nearby deciduous forests. Laying date phenology (a maternal effect) is critical in this system for being able to track the timing of peak caterpillar prey abundance. The divergence in the evergreen versus deciduous forest responses was driven by the fact that climatic warming was greatest during the spring and minimal during the winter. The evergreen forest populations have cue windows during a short time in the spring whereas the deciduous forest populations have a very broad cue window spanning the winter and part of the spring (Bonamour et al. 2019).

Finally, some of the newest research areas that could be used to test the buying time hypothesis involve plasticity in the microbiome. The underlying idea is that organisms, by changing the composition or expression of their microbiome, can alter their (extended) phenotype (i.e., plasticity) to respond to novel environments (Alberdi et al. 2016). For example, naive *Drosophila* switched to a novel high-salt diet performed better and were able to persist when given the gut microbiome from flies already experimentally evolved on the resource (Markov and Ivnitsky 2016). Although the microbiome represents a relatively new research frontier and there are few studies to summarize at this point, there is still high potential for the microbiome to serve as an important mechanism mediating the buying time response and allowing populations to cope with environmental novelty (reviewed in Alberdi et al. 2016).

Up to this point, we considered studies that provided positive support for the buying time hypothesis. However, it is worth discussing examples where plasticity failed to buy time. A prominent example is the work of Sinervo et al. (2010) in which, despite having plasticity in thermoregulatory behavior to avoid overheating, many lizard populations in Mexico were found to have gone extinct under recent climate change. In this case, the plasticity was in an adaptive direction to avoid overheating but restricted the available foraging times for lizards. As a consequence, thermoregulatory plasticity was unable to rescue the lizard populations outright, nor to buy time for the populations to adapt. In fact, the genetic architecture of the thermoregulatory plasticity and other thermal traits further limited adaptation. Of course, one could argue that the plasticity still bought some time for the lizards, just not *enough* time. Another example, also in lizards, comes from Telemeco et al. (2017). In this system, *Sceloporus* lizards historically adjusted the depth of their nests based on environmental temperature, such that warmer temperatures would elicit shallower nest depths, and cooler temperatures would elicit deeper nest depths as a protection against cold extremes. However, with climate change the thermal plasticity in nest depth has become maladaptive, as shallower nests under warmer conditions now place the developing lizard eggs at risk of heat stress. Thus, plasticity is potentially hastening vulnerability to environmental change rather than buying time against it.

8.6 BUYING TIME FOR WHAT?

Arguably, one of plasticity's most important roles is to buy time for populations to adapt evolutionarily, i.e., to undergo heritable changes that increase fitness and thus persistence in the new environment. The conditions under which plasticity is able to rescue populations outright appear to be quite limited, either due to an insufficient magnitude of plastic response to fully cope with environmental change or the fact that plasticity will not necessarily be adaptive in novel environments. Such limitations are magnified in light of recent global change processes, characterized by unprecedented shifts in the environments that most organisms inhabit. Thus, the combination of both plastic and evolutionary mechanisms, and in particular, plasticity's role as a means of buying time for evolution to occur, might be expected to be critical for rescue and persistence of many populations experiencing environmental change (Diamond and Martin 2016).

Although our primary focus is on buying time for populations, potentially with the second step that evolutionary change will have sufficient time to take place, we think it is important to also address the fact that plasticity can likewise hinder evolution. In this case, plasticity technically buys time for populations, but rather than allowing sufficient time for evolution to occur, it shields variation from selection and limits evolutionary change. For example, in *Anolis* lizards, behavioral shifts to using microclimates on tops of boulders at high altitude allow thermoregulation via basking in these cool environments; however, the thermoregulatory behavior shields variation in thermal performance from selection (Muñoz and Losos 2017). And as environmental temperatures continue to increase, the buffering effect of such thermoregulation will likely be insufficient to prevent eventual population extinction (*sensu* Sinervo et al. 2010). If there is heritable variation for the plastic trait itself, or if cryptic genetic variation is released in novel environments (Figure 8.5), then the

evolution of plasticity may be able to rescue populations from these types of evolutionary traps (Diamond and Martin 2020). However, evolving increased plasticity in changing environments can carry its own risks as models have found that this itself can lead to extinction under some conditions (Scheiner et al. 2017, 2020).

8.7 NEW VENUES TO TEST FOR BUYING TIME

Although evidence of buying is accumulating, many outstanding questions on the prevalence and mechanisms of buying time remain (Box 8.1). Indeed, while a number of different types of approaches have been used to test for buying time via plasticity, there are still untapped outlets for evaluating this hypothesis. Cities represent an excellent setting in which buying time via plasticity can be tested, as they are often typified by substantial environmental change over a short spatio-temporal range (Rivkin et al. 2019). This property permits the assessment of contemporary population persistence via plasticity. Cities might act as environmental filters which plasticity could overcome (Figure 8.6a). Plasticity could also allow persistence of these newly immigrated individuals or resident urban populations (Figure 8.6b). Both mechanisms would have the same general outcome with respect to the variation of plasticity in the population. In the ancestral environment, a range of plastic genotypes might be supported (Figure 8.6c), whereas in the novel, urbanized environment, only the most (adaptively) plastic genotypes are able to persist (Figure 8.6d). Below, we outline a research program that uses cities as a tool to explore the buying time hypothesis.

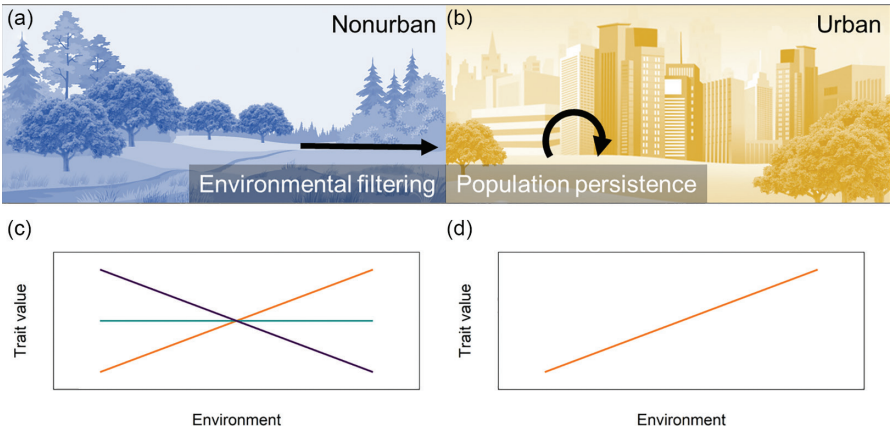

FIGURE 8.6 Patterns and processes of how plasticity might allow organisms to live in human-altered urbanized environments. (a) Plastic phenotypes are those that can traverse the boundary from nonurban environments to urban environments via environmental filtering. (b) Plasticity might allow population persistence of organisms already in urbanized environments. (c) Reaction norms of three genotypes, including one with adaptive plasticity (positive slope, orange line), no plasticity (zero slope, green line), and maladaptive plasticity (negative slope, purple line) in the novel, urban environment. Nonurban environments might be expected to harbor all types of reaction norms. (d) Only the reaction norm exhibiting adaptive plasticity (positive slope, orange line) either enters or persists in the urbanized environment.

8.7.1 Evaluating the Pattern of Buying Time Using Field Observations and Common Garden Experiments

Through already-available and new surveys, species can be identified that either occur only in nonurban habitats or occur in both nonurban and urban habitats. These species can be collected from the nonurban habitat and reared under common garden conditions until a new generation of individuals is produced. Species can be reared under two or more environmental conditions to assess developmental plasticity, or species can be reared under a single environment and assessed for acute plasticity. Common urban stressors include temperature, aridity, environmental toxins, and sound/light pollution, which could serve as the environmental treatments to assess plasticity. Following this list of stressors, relevant traits would then include thermal tolerance, desiccation tolerance, and toxin tolerance. If plasticity buys time for urban populations, then the steepness of the reaction norm or the width of the tolerance/performance curve should be positively associated with the presence across both urban and nonurban habitats. Conversely, less plastic species should be found in only the nonurban habitats. Of course, some aspects of urbanization might in fact alleviate stress on organisms, for example with food supplementation in cities (Rivkin et al. 2019). In this case, a link between plasticity in, for example, resource-acquisition or resource-use traits and persistence would not be expected, and thus could serve as a useful control. While we have laid out an approach to evaluate the pattern of buying time that relies on gathering new data, we note that it might also be feasible to perform such a comparative analysis with data in-hand by using publicly available databases of trait plasticity (e.g., Gunderson and Stillman 2015) and occurrence records (e.g., iNaturalist, GBIF).

8.7.2 Establishing a Causal Link between Plasticity and Persistence Using Reciprocal Transplant Experiments

Although the correlation of plasticity with urban success is suggestive of buying time, manipulative experiments are needed to establish a causal link. At either the intra- or inter-specific level, genotypes or species with varying levels of plasticity could be transplanted from the nonurban to urban habitat and monitored with respect to population size. Such an approach might be possible for only a subset of the species that could be assessed for the pattern of buying time, but transplantation would be necessary to directly establish that the plasticity caused population persistence.

8.7.3 Assessing the Consequences of Buying Time: Does Plasticity Buy Time for Populations to Adapt to Urbanization?

Finally, once the pattern of buying time was established, and, ideally the causal link between plasticity and persistence as well, these species that were able to persist in urban habitats would be excellent candidates to examine whether plasticity was able to buy time for urban adaptation. Common garden experiments could again be used, but in this case, with both urban and nonurban populations being reared for a complete generation and assessed for divergence in their trait values.

Here, the expectation would be that species for which plasticity allowed persistence in the novel urban environment also showed adaptive trait divergence from their nonurban ancestors. The magnitude of divergence might also be expected to scale with time since urbanization occurred. That is, more recently urbanized populations would have had less time to adapt.

8.8 CONCLUSIONS

Theoretical expectations of plasticity's role in buying time for populations—with or without subsequent evolutionary change—are supported by a number of empirical studies. As a consequence, it seems that there is consensus that buying time via plasticity can occur in natural populations undergoing environmental change. However, there are many unanswered questions in the details of this process (see Box 8.1), and this area harbors most of the controversy surrounding buying time, though we note that there is disagreement among researchers regarding how frequently buying time occurs and for how many systems and taxa. Indeed, the most controversial areas appear to be focused on the subsequent processes following buying time, namely whether plasticity buys time for evolution to occur, and the nature of the plastic response (particularly maladaptive plasticity) and its effects on evolutionary responses.

BOX 8.1 SUGGESTIONS FOR FUTURE RESEARCH

- What is the relative importance of buying time?
  - How much time does plasticity buy for populations? When plasticity fails to buy time, is this simply too small a magnitude of plasticity for the rate of environmental change, maladaptive plasticity, or that plasticity is not associated with population persistence?
  - How do we improve tests of buying time? Could a causal link between plasticity and persistence be established with broader tests of the pattern such as via urbanization gradients coupled with manipulative experiments?
- What are the predictors of buying time?
  - Is buying time most effective (high population persistence) for developmental selection mechanisms, moderately effective for generalized physiological response mechanisms and least effective for phenotypic switch? Do increasing costs of phenotype for developmental selection mechanisms temper the high probability of being adaptive in the novel environment, such that generalized physiological response mechanisms become most effective?
  - What are the costs of phenotypic plasticity in novel environments? Does this vary for different mechanisms of plasticity (i.e., developmental selection, generalized physiological response, phenotypic switch)?

  - What is the distribution effects of fitness effects for plasticity in novel environments? Is plasticity more often adaptive or maladaptive?
  - Is buying time more common with directional shifts in the environment versus exposure to completely novel environments?
- What are the consequences of buying time?
  - Does plasticity rescue populations outright, i.e., does plasticity buy 'infinite' time? Does plasticity buy time for adaptive evolution to occur? Or does buying time via plasticity simply lead to time lags in population extinction? How often does each of these outcomes occur?
  - Is buying time via plasticity rescue more gentle than evolutionary rescue which incurs demographic loss due to selection?
  - Is buying time via plasticity effective across a broader range of taxa than evolutionary rescue as it is released from constraints on rapid generation time and the presence of heritable variation?
  - Can purely buying time be separated from the evolutionary response after a single generation?

ACKNOWLEDGMENTS

We thank David Pfennig and two anonymous reviewers for helpful comments on a previous version of this manuscript. SED and RAM were supported by the Oglebay Fund Trust. SED was also supported by an NSF CAREER award (DEB-1845126).

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9 Innovation and Diversification Via Plasticity-Led Evolution

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CONTENTS

9.1 INTRODUCTION

Biodiversity requires explanation. Why are there so many different kinds of living things, and where do their unique features come from? Historically, biologists have attempted to address these questions by focusing on the ecological and genetic factors that foster evolutionary diversification and innovation. By contrast, less attention has been paid to development. Yet, a key feature of development—its tendency to be adaptively responsive to changes in an organism's environment—might play a critical role in the origins of new traits and new species.

In this chapter, we explore these issues by introducing the concept of plasticity-led evolution—adaptive evolution that is set in motion by phenotypic plasticity. As we describe, there are numerous ways that plasticity can contribute to the origins

DOI: 10.1201/9780429343001-9

of new traits and new species, suggesting that plasticity-led evolution may play a largely underappreciated role in driving evolutionary innovation and diversification. We then briefly discuss how to test for plasticity-led evolution in natural populations and summarize its empirical support. Finally, we consider where the study of plasticity-led evolution currently stands. In doing so, we highlight both areas in which a consensus has been reached and areas in which controversy remains. We close our chapter by providing answers to some commonly asked questions about plasticity-led evolution and offering suggestions for future research.

We begin, however, by placing the study of plasticity-led evolution in a historical context before providing a modern description of the concept.

9.2 PLASTICITY-LED EVOLUTION: HISTORICAL AND CONTEMPORARY PERSPECTIVES

Even before the term 'phenotypic plasticity' was coined in 1914 by the Swedish biologist Herman Nilsson-Ehle, biologists recognized that a change in the environment could trigger pronounced variation (that is, diversity) within species. For example, 'polyphenism'—the occurrence of alternative environmentally induced phenotypes within the same population (Mayr 1963; Nijhout 2003; West-Eberhard 2003)—fascinated many of the early evolutionists, such as August Weismann, Conrad Waddington, and Richard Goldschmidt. As they and others since came to appreciate, these environmentally induced morphs can be as different in behavior, morphology, physiology, and/or life history as different species (see Figure 9.1). Thus, an important question arose: were the forces that generate such diversity within species the same as those that generate diversity *between* species? Weismann (1882), for example, was convinced that the study of polyphenism was key to understanding species differences (see the Preface of this volume).

At the same time, biologists began to ask whether environmentally induced changes to an organism's behavior preceded and facilitated evolutionary change in *morphology* (e.g., Lamarck 1809; Baldwin 1896; Morgan 1896; Osborn 1896). Such a change in morphology could arise, the argument went, if a new behavior altered the selective pressures an organism experienced (e.g., by subjecting it to a new food source or competitive environment), and/or if the new behavior itself brought about morphological changes that were somehow inherited (for details on how this process might occur, see Bonduriansky and Day 2018). In either case, the change in behavior could cause the organism's genes to be 'pulled' into the next generation, thereby creating evolutionary change (see also Chenard and Duckworth 2021 in this volume).

Behaviors are not the only phenotypes that are environmentally responsive. Indeed, during the mid-twentieth century, researchers proposed that environmentally initiated change in diverse morphological and physiological traits could precede and facilitate genetic change (e.g., Schmalhausen 1949; Waddington 1953; Bateman 1959; see Box 3.2 in Pfennig 2021 in this volume). However, much of this work was ignored or dismissed by most evolutionary biologists (Simpson 1953a; Williams 1966; Orr 1999; see also Futuyma 2021; Pfennig 2021).

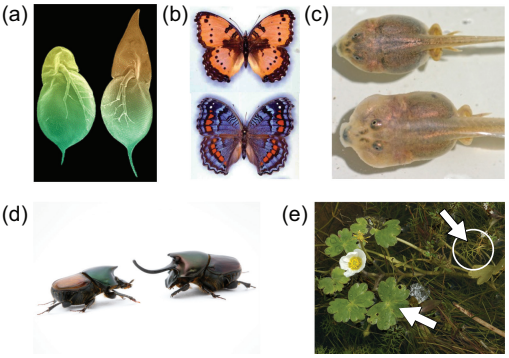

FIGURE 9.1 Phenotypic plasticity can generate diversity within species that is sometimes as great (or greater) than that normally seen *between different species*. To illustrate this point, we show examples of environmentally induced alternative phenotypes ('polyphenism'). (a) Normal (left) and predator-induced (right) morphs of water fleas, *Daphnia cucullata*; (b) wet-season (top) and dry-season (bottom) gaudy commodore butterflies, *Precis octavia*; (c) omnivore (top) and carnivore-morph (bottom) spadefoot toad tadpoles, *Spea multiplicata*; (d) small-horned (left) and large-horned (right) dung beetles, *Onthophagus nigriventris*; (e) broad, aerial leaves and narrow, submerged leaves (circled) on the same water crowfoot plant, *Ranunculus aquatilis*. (Reproduced from Pfennig et al. [2010], with the kind permission of the publisher.)

A key breakthrough came at the end of the twentieth century. During this time, Mary Jane West-Eberhard published two seminal works: a review paper (West-Eberhard 1989) and a highly influential book (West-Eberhard 2003). In these (and other) publications, she argued that phenotypic plasticity can promote adaptation, novelty, and both intra- and inter-specific diversity. The ensuing decades witnessed the emergence of what has become known as the plasticity-led evolution hypothesis (sometimes called 'PLE'; sensu Levis and Pfennig 2019).

According to the plasticity-led evolution hypothesis, adaptive evolution generally and the evolution of novelty and diversity specifically are set in motion—and potentially directed—by phenotypic plasticity. This view differs from the traditional view in which adaptive phenotypic evolution is triggered by a change in the genome (Figure 9.2a) and where the sole role of environmental change is to alter patterns of selection. With plasticity-led evolution, adaptive phenotypic evolution is triggered by a change in the environment (Figure 9.2b) that simultaneously alters patterns of selection while generating novel developmental variants upon which selection can act.

More precisely, the process of plasticity-led evolution starts when a genetically diverse population experiences a new environment. Because natural environments change constantly, and because these changes are often harmful to the individual (it reduces the match between its phenotype and its environment), organisms have evolved plasticity to produce phenotypes that are better suited for any new conditions they might encounter. In most natural populations, however, different genotypes vary

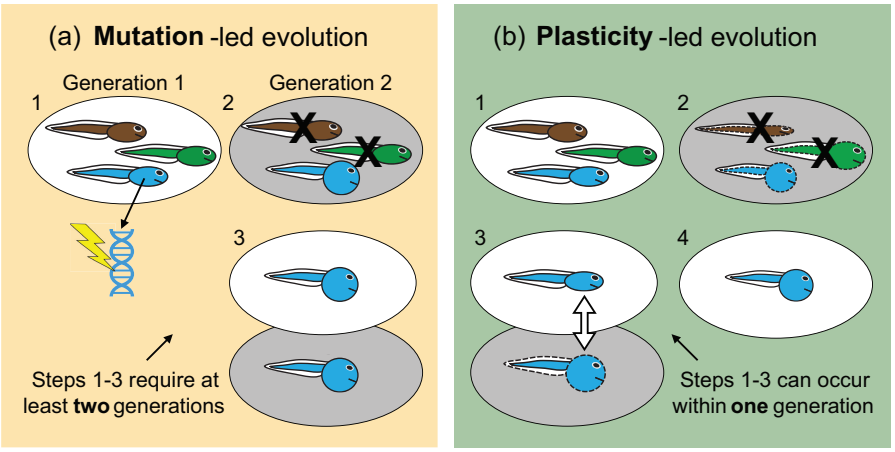

FIGURE 9.2 Alternative routes to novelty. (a) With mutation-led evolution, phenotypes change following a change in the genome. (Step a1) This process begins with a genetic mutation (here, different colors signify different genotypes). (Step a2) Assuming this mutation is not lethal, in the next generation it might cause any individual that inherits it to express a new phenotype (e.g., a 'bulgy' body tadpole form). Moreover, if the population experiences a new environment (indicated by the shaded background), where this form is now favored, selection can disfavor those genotypes that produce poorly adapted phenotypes for the current environment (indicated by an 'X'). (Step a3) Over evolutionary time, such selection might result in a novel phenotype that is produced regardless of the environment (indicated by different shading). (b) With plasticity-led evolution, phenotypes change following a change in the environment. (Step b1) This process begins when a genetically diverse population (once again, different colors signify different genotypes) (Step b2) experiences a change in its environment (once again, indicated by shading), which induces novel phenotypes (dashed lines). However, different genotypes typically differ in how they respond to an environmental change (indicated by different shapes). Selection can therefore act on such formerly 'cryptic genetic variation' and disfavor those genotypes that produce poorly adapted phenotypes for the current environment (indicated by an 'X'). (Step b3) If, depending on their environment, individuals produce either this novel phenotype or the ancestral phenotype, then the result is a novel 'polyphenism.' (Step b4) Over evolutionary time, selection might even favor the loss of plasticity (i.e., 'genetic assimilation'), resulting in a novel phenotype that is produced regardless of the environment (indicated by the loss of dashed lines).

in whether and how they respond to any given environmental change (Schlichting and Pigliucci 1998). Once this previously cryptic genetic variation is uncovered by plasticity, selection can remove from the population those phenotypes (and associated genotypes) that are poorly suited for the new environment. If the new environment persists, selection might promote the evolution of quantitative genetic changes that stabilize, refine, and/or extend those phenotypes that are best suited to the new environment. Since environmentally triggered traits are always associated with the environment that triggered their development, they are likely to experience consistent selection and directional modification. This association allows new environments to immediately produce and select among new phenotypes to rapidly refine their expression.

The refinement process can cause a change in the regulation of plasticity and also in the form of the phenotype, ultimately leading to a better match between phenotype and environment. In the end, plasticity-led evolution results in a new phenotype that was not present in the ancestral population, at least not in a well-adapted form. Thus, *plasticity-led evolution can be defined formally as the evolutionary process that occurs when a change in the environment triggers a change in phenotype via phenotypic plasticity, and following the uncovering of cryptic heritable variation in how different individuals respond to this environmental change, the environmentally induced phenotype is refined by selection into a new adaptive form*. In other words, plasticity-led evolution is adaptive evolution initiated by an environmentally induced change in a phenotype (plasticity).

The plasticity-led evolution hypothesis explicitly affirms that adaptive phenotypic evolution is not solely a consequence of selection acting on genetic variation. Instead, it highlights that adaptive evolution results from selection acting on heritable *developmental* variation. This developmental variation, of course, arises as a consequence of both gene products *and* environmental inputs. Moreover, by placing a major focus on the *initial* development of new phenotypes, the plasticity-led evolution hypothesis re-orders the sequence of events leading to adaptive evolution (see Section 9.4). Although the plasticity-led evolution hypothesis recognizes genetic variation as a necessary pre-requisite for evolutionary innovation and diversification, it emphasizes how such variation might only be revealed to selection when a change in the environment induces a change in phenotype through plasticity. In doing so, a plasticity-led evolution perspective shifts the focus away from viewing plasticity's role in evolution as a constraining force (sensu Huey et al. 2003) to a *constructive* one.

But how does plasticity generally, and plasticity-led evolution specifically, promote innovation and diversification? We turn to this topic next.

9.3 HOW PLASTICITY-LED EVOLUTION PROMOTES INNOVATION AND DIVERSIFICATION

Phenotypic plasticity—as mediated by plasticity-led evolution—can have far-reaching impacts on the origins of diversity, from promoting diversity and novelty within populations to promoting diversity between species. To discuss these impacts, we will examine plasticity-led evolution's contributions to evolutionary innovation and diversification separately.

9.3.1 Plasticity-Led Evolution and Innovation

When discussing the origins of major new organismal features (i.e., evolutionary innovation), it is generally assumed that new traits require genetic changes (Carroll 2008). Indeed, many new features can be traced to genetic changes, including single-gene mutations (e.g., Hoekstra et al. 2006), duplications of large regions of the genome (e.g., Conant and Wolfe 2008), alterations in regulatory sequence (e.g., Chan et al. 2010), and/or a variety of other changes in DNA sequence or content.

Yet, as mentioned above, biologists have long questioned whether novel features arise solely from genetic changes (West-Eberhard 1989, 2003; Moczek et al. 2011). According to this alternative view, phenotypic plasticity is an important instigator of evolutionary innovation, and plasticity-led evolution should therefore be considered a valid mechanism for generating evolutionary innovation. Here, we discuss three non-mutually exclusive pathways by which plasticity can foster evolutionary innovation: (1) by uncovering cryptic heritable variation; (2) by providing rich targets on which selection can act; and (3) by using exploratory developmental processes.

First, as highlighted earlier in Section 9.2, plasticity can initiate novelty by uncovering cryptic heritable variation (typically, such variation is genetic, but it might also be *epi*genetic; see Bonduriansky and Day 2018). Cryptic variation is not observed under normal conditions but has observable phenotypic effects under novel (or stressful) conditions (e.g., Gibson and Dworkin 2004). The uncovering of this cryptic variation is crucial for fueling plasticity-led evolution because selection operates on phenotypes, not genotypes, but it can only act on those phenotypes that are actually expressed. This cryptic variation arises as a result of dominance, epistasis, or gene-by-environment interactions/conditional trait expression (Paaby and Rockman 2014). The latter, conditional trait expression (where only a fraction of individuals in a population express a particular trait) is most relevant to plasticity-led evolution. Conditional trait expression often arises when individuals change their phenotype in direct response to different environments (West-Eberhard 2003; i.e., when they express plasticity; Van Dyken and Wade 2010). A common view is that when alternative traits are produced through plasticity, each such trait involves either different genes being expressed or the same gene(s) being expressed at different levels (Snell-Rood et al. 2010). Since phenotypes (and their corresponding genes) are only exposed to selection when expressed, genetic variation accumulates in genes that underpin any phenotype that is not expressed frequently (Paaby and Rockman 2014). Following a change in the environment, this formerly cryptic genetic variation can be uncovered (e.g., by perturbation of silencing/buffering mechanisms; Rutherford and Lindquist 1998), resulting in a novel phenotype. If this environmental change is novel (i.e., it has not been previously experienced by the focal lineage), then many of these new phenotypic variants will likely be maladaptive. However, some such variants will likely be adaptive in the novel environment just by chance (West-Eberhard 2003). In contrast, if the new environment is not novel, then some phenotypic variants will likely be biased toward being adaptive in the altered environment (Parsons et al. 2020). Whether adaptive or not, environmentally mediated changes to development (i.e., plasticity) generate a pool of heritable phenotypic variation on which selection can act. Such selection can then further refine those variants that happen to be well-suited to the new environment.

Second, plasticity can also foster evolutionary innovation by providing rich targets for diversifying selection. In particular, because plasticity typically results from a complex interaction between both gene products and environmental inputs—i.e., it involves a gene-by-environment interaction—it provides numerous targets on which selection can act to promote novelty. Indeed, from recent studies into the developmental underpinnings of complex traits (reviewed in Glazier et al. 2002; Wu and Lin 2006), it is clear that many phenotypic outcomes are the result of multifaceted

regulatory interactions during development. It is also clear that evolutionary mechanisms operating on these regulatory interactions can lead to novel traits (Carroll 2008; Moczek et al. 2011; Levis and Pfennig 2020). Yet, while the complexity of developmental systems is relevant to the evolution of any novel trait, it may be particularly important in the context of plasticity because of *additional interactions with the environment*. The number and diversity of interactions among regulatory factors on the one hand (such as hormones, receptors, transcription factors) and environmental conditions on the other provide numerous opportunities for development to be perturbed and new phenotypic variants to arise (e.g., from changes in the timing, location, or strength of interactions). Consider, for example, that many signaling molecules and signal transducers can modify, inhibit, or promote (i.e., regulate) activities performed by other molecules, and that these regulatory molecules typically have numerous targets (e.g., Payne et al. 2014). This diversity and abundance of targets may increase the probability of a trait becoming decoupled from its environmental cue, establishing new connections with an environmental cue, and/or experiencing various other modifications to its expression (Ehrenreich and Pfennig 2016).

Moreover, because development tends to be 'modular'—meaning that it typically entails multiple sets of interacting parts or 'modules' that are relatively autonomous with respect to each other (Schlosser and Wagner 2004)—ample opportunity exists for regulatory networks to evolve (semi-)independently of each other. Such semi-independent evolution enables some modules (and their associated phenotypes) to develop greater elaboration and refinement with minimal (or no) effects on other modules in the same organism (West-Eberhard 2003; Snell-Rood et al. 2010; e.g., the fore and hindwings of a butterfly or the different types of teeth in a mammal). This means that selection can simultaneously refine multiple plastic phenotypes: each phenotype is only subject to selection when it is expressed, and its refinement may have little impact on other phenotypes (Levis and Pfennig 2019). Thus, network complexity (e.g., number and types of regulatory connections), in conjunction with semi-independent evolution afforded by modular development, provides ample opportunity for the generation of novel phenotypes that can subsequently be refined by selection into adaptive traits.

Finally, plasticity can contribute to novelty through exploratory mechanisms (Levis and Pfennig 2020). These mechanisms typically operate through a process of developmental selection in which some form of environmental sampling is followed by developmental reinforcement of those responses that are most beneficial (Snell-Rood et al. 2018). The 'environment' being sampled may be internal (e.g., cytoskeleton formation) or external (e.g., an animal sampling various foods). Such mechanisms include but are not limited to (Kirschner and Gerhart 1998): cytoskeleton formation, neuron growth and development, plant stem and root growth, vertebrate adaptive immunity, habitat choice, and trial-and-error learning. In general, exploratory mechanisms can be highly sensitive to local conditions and thereby produce adaptive developmental outcomes even in novel environments (Snell-Rood 2012).

Exploratory mechanisms are a powerful force during plasticity-led evolution. Because they use fine-grained local responses generated by subunits of the larger phenotype, exploratory mechanisms can yield phenotypes that are well-suited to current

conditions (Kirschner 1992; Snell-Rood 2012; Snell-Rood et al. 2018; Ducatez et al. 2020). Indeed, exploratory mechanisms can produce appropriate phenotypes even under novel conditions (Lande 2014). Subsequently, the high costs typically associated with exploratory mechanisms may make the phenotypes they produce more likely to be fixed by selection (Scheiner et al. 2017; Snell-Rood et al. 2018) so that any burden of maintaining plasticity can be lessened.

To illustrate how exploratory mechanisms can generate novelty, recall from above that behavior, in particular, has long been recognized as playing a key role during plasticity-led evolution (e.g., Baldwin 1896; Wcislo 1989; Bateson 2004; Duckworth 2009; Lister 2014). Indeed, behavior is often described as 'hyperplastic.' This is because of the wide array of behavioral changes that many organisms can exhibit depend on environmental context. (As an aside, even organisms lacking brains can assess and respond to different environmental conditions. For example, bacteria and plants are known to move consistently toward or away from specific environmental stimuli.) While behavior may play a leading role in generating new selective pressures that foster evolutionary innovation (see Section 9.2), behavior (or any exploratory mechanism for that matter) may also be important for *reducing* the amount of environmental variation that an individual experiences. For example, a reptile behaviorally shifting to a shaded habitat during the hottest time of day reduces the temperature variation it experiences, and consequently, influences how selection acts on heat tolerance physiology (Huey et al. 2003). By narrowing the range of environments an organism experiences, behavior can help drive specialization to a particular environment. Specialization can, in turn, channel selection to favor other aspects of the phenotype (e.g., physiological or morphological) that work well in the new environmental conditions.

Ultimately, such specialization wrought by plasticity-led evolution might lead to a polyphenism (Figure 9.1). Indeed, the alternative phenotypes that characterize many polyphenisms are each tightly coupled to specific environmental conditions (Nijhout 2003). Although the evolutionary importance of these phenotypic alternatives has been underestimated, the evolution of a polyphenism may have important implications for the origins of major new features. This is because many (if not all) novel features likely first appeared evolutionarily as an alternative phenotype within a population (West-Eberhard 2003). Thus, as first suggested by Weismann (1882), the alternative phenotypes that constitute polyphenism might be an important phase in the evolution of major adaptive novelties that characterize different species and higher taxa.

In short, plasticity-led evolution may play a key role in evolutionary innovation at *all levels* of biological organization.

9.3.2 Plasticity-Led Evolution and Diversification

Phenotypic plasticity can contribute to diversification by promoting intra- and inter-specific divergence, speciation, and adaptive radiation (West-Eberhard 2003; Schlichting 2004; Pfennig et al. 2010). Below, we discuss how plasticity-led evolution promotes diversification at each level separately.

First, plasticity-led evolution contributes to intraspecific diversification by fostering the evolution of novel phenotypes. Once new phenotypes are produced through plasticity, plasticity-led evolution can result in their further elaboration. Specifically, when plasticity is favored, plasticity-led evolution can promote polyphenism, thereby generating pronounced diversity within species (Figure 9.1). Conversely, when plasticity is not favored (as when its costs exceed its benefits or when reliable cues for adaptive plasticity are absent; see Berrigan and Scheiner 2004), then plasticity-led evolution can result in a constitutively expressed (i.e., 'fixed') trait evolving from a previously environmentally induced trait. If different populations of the same species fix alternative forms for the trait, and especially if these populations differentially elaborate and refine these forms, then intraspecific diversity has increased. The loss of plasticity might occur for two reasons (Pigliucci et al. 2006; Ehrenreich and Pfennig 2016). First, when plasticity is costly (Murren et al. 2015), selection can actively eliminate it, leading to fixation of the favored phenotype (i.e., genetic assimilation occurs; sensu Waddington 1953; see also Scheiner and Levis 2021 in this volume). Second, plasticity can be lost through mutational degradation or genetic drift (Masel et al. 2007), as might occur when non-favored phenotypes are seldom expressed and thereby experience relaxed selection (Van Dyken and Wade 2010). However, regardless of whether plasticity-led evolution results in a new genetically fixed trait or a polyphenism, it can enhance intraspecific diversity.

Plasticity-led evolution can also facilitate divergence *between* species. Consider this passage from E. O. Wilson (1992, p. 174):

> *Imagine a case in which two such species have been squeezed together in the same communities long enough for evolution to occur. When they first came into contact, they were elastic and could diverge in their habits enough to lessen competition. The differences were phenotypic, the result of environment and not genes. The compression occurred in traits that were relatively easy to change, most likely by a retreat from parts of the habitat and diet by one or both of the species. As the generations passed, genetic differences arose and hardened the distinction between the two species.*

Wilson is proposing a scenario in which adaptive differences between competing species are initially mediated by plasticity. Later, these differences become fixed ('hardened'). In essence, he is describing plasticity-led 'character displacement'; that is, trait evolution that arises as an adaptive response to competition between species (Pfennig and Pfennig 2012). In a number of taxa, such competitively induced plasticity appears to have preceded the evolution of fixed genetic differences between populations in sympatry with a heterospecific competitor versus populations in allopatry (reviewed in Pfennig and Pfennig 2012, pp. 98–102). Because plasticity can generate rapid, widespread, and adaptive changes in resource-use traits, it might play a general and important role in character displacement specifically and population divergence generally.

Essentially, plasticity promotes diversification because it facilitates a population's ability to cross selective valleys and thereby access nearby selective peaks (Price et al. 2003; Schlichting 2004; selective 'valleys' and 'peaks' refer to phenotypes for which the population mean fitness is at a minimum and maximum, respectively). To reach such a peak through the traditional process involving accumulation of small genetic

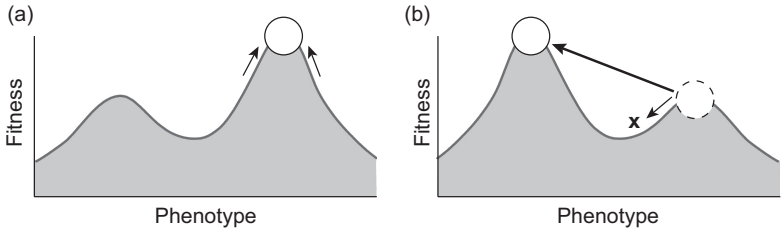

FIGURE 9.3 How plasticity promotes diversification by facilitating 'valley crossing.' (a) A well-adapted population should evolve to produce a mean phenotype that is associated with highest fitness on the adaptive landscape (the 'fitness maximum,' the position of which is indicated here by a circle). (b) However, when the environment changes, the adaptive landscape is likely to change also, such that the population mean phenotype is no longer at the new fitness maximum. This new fitness maximum would be inaccessible through incremental evolution because of an intervening fitness valley; selection should generally not take a population through such a valley. However, in a population that has evolved plasticity (particularly polyphenism; see Figure 9.1), the *individual members* of the population could cross this fitness valley in 'developmental time' by facultatively expressing an alternative phenotype that is associated with higher fitness; including one at the new fitness maximum.

changes, a population must first cross a fitness valley (i.e., it must produce offspring with phenotypes that are associated with lower fitness than the parents). Such valley crossing via the evolution of small genetic changes is normally prevented by selection. However, with plasticity (and especially with polyphenism), valley crossing unfolds in *developmental* time rather than in evolutionary time (Figure 9.3). By facultatively expressing an alternative phenotype closer to the fitness optimum, a population can traverse a valley rapidly—potentially in one generation. Consequently, in a population experiencing rapidly changing environmental conditions, plasticity enables populations to explore the fitness landscape (initially, through development; potentially via exploratory mechanisms described in Section 9.3.1), increasing its chances of diversifying.

Moreover, because plasticity can promote population divergence, and because the same factors that promote population divergence should also foster speciation (Schluter 2000), plasticity may thereby facilitate speciation (Pfennig et al. 2010). For example, there is evidence that matings between populations that differ in the expression of environmentally induced alternative phenotypes result in offspring of low fitness (Pfennig and Rice 2007). This reduced fitness could favor reproductive isolation between such populations and thereby possibly lead to speciation. Indeed, speciation may proceed especially rapidly when alternative phenotypes are environmentally induced. This is because a sudden change in the environment can simultaneously induce and favor a single alternative phenotype. Furthermore, any population differences that initially arose through plasticity might undergo genetic assimilation. This process might thereby contribute to the rapid accumulation of genetic differences between populations that, in turn, enhance reproductive isolation. Consistent with these arguments, there is growing support for the hypothesis that plasticity—particularly polyphenism—contributes to speciation (Meyer 1993; Smith

and Skúlason 1996; Pfennig and McGee 2010; Pfennig et al. 2010; Hendry 2016). For example, clades in which resource polyphenism has evolved are more species rich than sister clades (Pfennig and McGee 2010). However, since polyphenism is a potential *outcome* of plasticity-led evolution, plasticity-led evolution's main contribution to speciation may be facilitating the evolution of refined plastic responses that can subsequently generate the conditions favoring reproductive isolation and speciation.

Lastly, plasticity-led evolution might promote adaptive radiation (West-Eberhard 2003; Wund et al. 2008; Schneider and Meyer 2017), which occurs when the members of a single phylogenetic lineage diversify rapidly into numerous descendent lineages that occupy a wide variety of ecological niches (Simpson 1953b; Schluter 2000). Adaptive radiation mediated by plasticity-led evolution is sometimes referred to as the 'flexible stem hypothesis' (West-Eberhard 2003). Under this hypothesis: (1) the diverse forms produced by an adaptive radiation started out as environmentally induced forms; and (2) the nature of this ancestral plasticity can influence the nature of the radiation (West-Eberhard 2003; p. 565). This model further predicts that ancestral, plastic responses within a species should reflect the fixed trait differences observed between closely related species within the same clade (Gomez-Mestre and Buchholz 2006; Wund et al. 2008; Gibert 2017). This mirroring occurs because of divergent selection acting on the alternative phenotypes induced by plasticity and ultimately driving fixation of the alternative phenotypes in different lineages.

This model can help explain a characteristic of many adaptive radiations: 'parallelism,' in which similar traits have been repeatedly derived from the same ancestral population or phenotypic form (the resulting pattern is sometimes dubbed 'replicate radiation'; Schluter and Nagel 1995; Losos et al. 1998). A plasticity-led evolution framework emphasizes that the environment not only exerts parallel selective pressures but also generates parallel distributions of traits—owing to parallel induction of ancestral plastic responses—which, together, can lead to highly deterministic outcomes (Parsons et al. 2020). Thus, plasticity-led evolution may not only promote biodiversity but also govern the shape that biodiversity takes.

9.4 EVALUATING PLASTICITY-LED EVOLUTION

Obviously, one can test the plasticity-led evolution hypothesis experimentally by evolving populations under controlled laboratory conditions; that is, using an 'experimental evolution' approach (Garland and Rose 2009). A major benefit of experimental evolution is that it can be designed to test specific hypotheses. This approach was employed by Waddington in his classic studies of genetic assimilation (Waddington 1942, 1952, 1953). Ideally, one would choose to study subjects that can also be assayed in the wild in order to corroborate laboratory findings (e.g., see Suzuki and Nijhout 2006). Nevertheless, experimental evolution has limitations. First, it can only be used with organisms that have short generation times (e.g., bacteria, yeast, fruit flies). Second, some evolutionary processes might be too slow to observe within the span of a research grant or even a researcher's lifetime (a case in point is the limited insights that experimental evolution has provided into speciation). Finally, one is always left with the uncertainty of whether evolution in the laboratory reflects how it actually unfolds in natural populations.

Using natural populations to evaluate the origins of extant traits (or taxa) is challenging because only the final products of the evolutionary process are likely to still exist. In these cases, the main difficulty is that once a trait has evolved, its evolution cannot be studied *in situ*. To circumvent this problem, two general approaches could be employed.

First, phylogenetic methods (e.g., ancestral state reconstruction) could provide information on the timing of trait evolution and identify the order of shifts in trait regulation from environmental induction to genetic control (and vice versa) among lineages (Schwander and Leimar 2011; Schlichting and Wund 2014). In this way, one could determine if a trait has shifted from being plastic to being genetically fixed in some lineages that are phylogenetically embedded in a larger clade where plasticity is retained. This approach requires a well-resolved phylogeny and understanding of the degree of plasticity in those taxa included in the phylogeny.

A second widely used approach (Schlichting and Wund 2014; Levis and Pfennig 2016) is to compare the reaction norms (i.e., the trait's plasticity as estimated from the shape or slope of the line between trait values in alternative environments) of two different types of lineages: one that possesses the focal, potentially canalized, trait (representing the 'derived' condition) and one that is closely related to the former lineage but that lacks the focal trait (representing the 'ancestral' condition and that can therefore serve as an 'ancestor-proxy'). For example, one could study natural populations that have undergone a range expansion (Badyaev et al. 2002). In such cases, one could compare individuals from the 'sink' population (representing the derived condition) to those in the 'source' population (representing the ancestral condition). Reaction norms of these two types of lineages (or across a group of lineages ranging from 'more ancestral' to 'more derived') can be compared when both lineages are reared in both the derived environment (i.e., the environment in which the novel trait is associated) and the ancestral environment. Such a comparison can help infer what the common ancestor of the groups might have looked like in terms of its trait values and plasticity and how these features were elaborated in the focal lineage. Using this approach, one could then test the critical predictions of plasticity-led evolution (Table 9.1; Levis and Pfennig 2016). Indeed, in recent years, such methods have been used to evaluate plasticity-led evolution in a wide variety of taxa and traits (Table 9.2).

Finally, if plasticity-led evolution is being implicated in speciation and/or adaptive radiation, then there should be evidence that: (1) reproductive isolation evolved *after* lineage divergence, and (2) lineage divergence occurred (at least in part) *because of* phenotypic divergence via plasticity-led evolution (i.e., predictions 1–4 in Table 9.1 have support). Ideally, one should also show that interspecific (fixed) differences mirror intraspecific (environmentally induced) differences (Gomez-Mestre and Buchholz 2006; Wund et al. 2008).

9.5 PLASTICITY-LED EVOLUTION: CONSENSUS, CONTROVERSY, AND CHALLENGES

In all fields of research, it is important to take stock of where the field has been, where it stands presently, and where the field needs to go to continue to make progress. Here, we highlight areas of consensus surrounding plasticity's role in evolution before

TABLE 9.1
Key Predictions of the Plasticity-Led Evolution Hypothesis

Prediction	Explanation
1. The focal trait can be environmentally induced in lineages showing the ancestral state	The fundamental prediction of plasticity-led evolution is that the novel trait should exhibit ancestral plasticity. In other words, the derived trait (or components thereof) should be environmentally induced in lineages that normally lack expression of the trait, but only when they experience the derived environment (West-Eberhard 2003). This would suggest that the common ancestor between the ancestral and derived lineage(s) likely possessed such plasticity as well.
2. The focal trait exhibits evidence that heritable variation (i.e., variation upon which selection could act to refine the trait) has been uncovered by the novel conditions	Confirmation of heritable variation is necessary to help eliminate the possibility that the novel trait arose solely through lineage-specific mutations (as in Fanti et al. 2017; Santos et al. 2017), and instead, is the product of ancestral developmental plasticity. Such evidence could be inferred if the trait (or its components) have higher additive genetic variance/heritability when ancestral lineages are reared under derived conditions (Gibson and Dworkin 2004; Ledón-Rettig et al. 2014; Noble et al. 2019).
3. The focal trait has undergone an evolutionary change in its degree of plasticity and/or form in lineages with the derived trait	Evolutionary change in plasticity should manifest as changes to the slope, curvature, and/or elevation of the reaction norm (Crispo 2007). Finding that selection has led to the complete loss of plasticity would imply that the trait has undergone genetic assimilation. Whereas finding that plasticity has increased might point toward the possible evolution of a polyphenism. Either outcome (or something in between) could be expected depending on the rate of environmental change, the strength of selection acting on plasticity, and/or the phenotypes induced by plasticity.
4. The focal trait has experienced adaptive refinement as it is repeatedly induced and exposed to selection	The focal trait should exhibit a pattern of frequency-dependent adaptation (sensu Levis and Pfennig 2019) such that the more frequently it is induced, the greater (and potentially more rapid) refinement it should experience. Finding such a difference is likely when using distinct species for ancestor-derived comparisons. Intraspecific (e.g., among population) comparisons can help corroborate that this same process has operated at both the microevolutionary and macroevolutionary levels.

discussing some of the reasons why the topic remains controversial. We then devote most of this section to addressing two key challenges to plasticity-led evolution.

9.5.1 Plasticity-Led Evolution: Where Is the Consensus?

Although the notion that plasticity can impact evolution is not new (see Section 9.2; see also Futuyma 2021; Pfennig 2021 in this volume), the field has undergone a renaissance over the past two decades (as evidenced by a dramatic uptick in publications; see Forsman 2014). Here, we call attention to three areas of consensus between plasticity researchers and evolutionary scientists.

TABLE 9.2
Examples of Species, Conditions, and Traits for Which Two or More Key Predictions of Plasticity-Led Evolution Have Support in Naturally Occurring Systems

Species	Derived Condition(s)	Trait	Predictions[a] Supported	Reference(s)
		Bacteria		
Fischerella spp.	Nutrient availability	Colony morphology	1, 3	Koch et al. (2017)
Fischerella thermalis	High temperature	Heterocyst composition	1, 2(?), 3, 4	Miller et al. (2020)
		Plants		
Acacia spp.	Ant guards	Ant mutualism	1, 3, 4	Heil et al. (2004)
Arabidopsis thaliana	Shade, Hsp90 inhibition	Morphology; growth	1, 2	Pigliucci et al. (1999) and Queitsch et al. (2002)
Helianthus tuberosus (Jerusalem artichoke)	Water availability	Clonality; invasion ability	1, 2, 3	Bock et al. (2018)
Solanum spp.	Resource status	Staminate flower production	1,3	Diggle and Miller (2013)
		Nematodes		
Rhabditina spp.	Alternative diets	Mouth morphology	1, 3	Susoy et al. (2015)
		Crustaceans		
Daphnia melanica	Fish predators	Melanization	1, 3	Scoville and Pfrender (2010)
		Trilobites		
Agnostus pisiformis	Dysoxic stress	Morphology	1, (2?), (3?)	Jackson and Budd (2017)
		Insects		
Drosophila mojavensis	Alternative hosts	Host preference	1, 3	Matzkin et al. (2006) and Matzkin (2012)
Hymenoptera and *Isoptera* (ants, bees, wasps, and termites)	Social environment	Caste differentiation; eusociality	1, 2(?), 3, 4	Jones and Robinson (2018)
Nymphalis antiopa (mourning cloak butterfly)	Low temperatures	Wing patterning; coloration	1, 3	Shapiro (1981)

(*Continued*)

TABLE 9.2 (*Continued*)
Examples of Species, Conditions, and Traits for Which Two or More Key Predictions of Plasticity-Led Evolution Have Support in Naturally Occurring Systems

Species	Derived Condition(s)	Trait	Predictions[a] Supported	Reference(s)
Onthophagus taurus (bull headed dung beetle)	Altered population density	Various	1, 3	Casasa and Moczek (2018)
Polistes fuscatus and *P. metricus* (paper wasps)	Identity signals	Individual recognition	1, (3?)	Tibbetts et al. (2018)
Polites sabuleti (skipper butterfly)	Low temperatures	Wing patterning; coloration	1, 3	Shapiro (1975)
		Fishes		
Astyanax mexicanus (Mexican tetra)	Caves	Eye loss	1, 2, 3, 4(?)	Rohner et al. (2013)
Cyprinodon diabolis (Devils Hole pupfish)	High temperatures; reduced resources	Pelvic fin loss	1, 3	Lema and Nevitt (2006); Martin et al. (2016)
Fundulus spp. (killifish)	Various salinities	Salinity tolerance	1, 3	Whitehead et al. (2011) and Whitehead (2012)
Gasterosteus aculeatus (threespine stickleback)	Alternative resources	Resource use ecotypes	1, 3	Wund et al. (2008)
Gasterosteus aculeatus (threespine stickleback)	Fresh water	Growth (size); salinity tolerance	1, 2, 3, 4	McCairns and Bernatchez (2010), Mcguigan et al. (2011), and Robinson (2013)
Gasterosteus aculeatus (threespine stickleback)	Higher predation risk	Parental care	1, 2(?), 3, 4	Stein and Bell (2019)
Gasterosteus aculeatus (threespine stickleback)	Reduced cannibalism	Antipredator and courtship behavior	1, 3	Foster (1994) and Shaw et al. (2007)
Poecilia reticulata (Trinidadian guppies)	Lower predation	Size at maturity; head shape	1, 3	Torres-Dowdall et al. (2012)
Poecilia reticulata (Trinidadian guppies)	Lower predation	Gene expression	1, 3	Ghalambor et al. (2015)
		Amphibians		
Lithobates sylvaticus (wood frog)	Insecticide	Insecticide tolerance	1, 3, 4	Hua et al. (2015)
Notophthalmus viridescens (eastern newt)	Altered pond hydroperiod	Developmental strategy	1, 3, 4	Takahashi and Parris (2008)

(*Continued*)

TABLE 9.2 (*Continued*)
Examples of Species, Conditions, and Traits for Which Two or More Key Predictions of Plasticity-Led Evolution Have Support in Naturally Occurring Systems

Species	Derived Condition(s)	Trait	Predictions[a] Supported	Reference(s)
Salamandra salamandra (fire salamander)	Pond habitat/higher temperature	Gene expression	1, 3	Czypionka et al. (2018)
Spadefoot toad spp.	Ephemeral ponds	Development time	1, 3, 4	Gomez-Mestre and Buchholz (2006)
Spea spp. (spadefoot toad)	Alternative diets; competitors	Resource use Ecomorph	1, 2, 3, 4	Ledón-Rettig et al. (2010), Levis et al. (2018), and Levis and Pfennig (2019)
		Reptiles		
Anolis spp.	Alternative perch diameters	Hindlimb length	1, 3(?), 4(?)	Losos et al. (2000)
Crotalus spp. and *Sistrurus* spp. (rattlesnakes)	Predation pressure	Rattle	1, 3	Allf et al. (2016)
Notechis scutatus (tiger snake)	Alternative diets	Head size	1, 3, 4	Aubret and Shine (2009)
Uta stansburiana (side-blotched lizard)	Lava flow substrate	Melanization	1, 3, 4	Corl et al. (2018)
		Birds		
Agelaius phoeniceus (red-wing blackbird), *Parus major* (great tit), and other urban birds	Urban landscapes	Song	1, 3	Slabbekoorn (2013)
Haemorhous mexicanus (house finch)	Various	Reproductive attributes; offspring morphology	1, 3, 4	Badyaev (2009)
Haemorhous mexicanus and *Platalea ajaja* (house finch and roseate spoonbills)	Metabolized or biochemically redundant carotenoids	Feather structure; pigmentation	1, 3	Badyaev et al. (2017)

[a] For descriptions of the four predictions, see Table 9.1.

First, a quick review of the literature reveals that most evolutionary biologists appreciate that plasticity is often beneficial. Indeed, most scientists seem to recognize that plasticity provides an important mechanism for organisms to deal adaptively with the stress accompanying changing environments (Losos 2014). Second, precisely because of this first point, most evolutionary biologists also appear to appreciate that plasticity can 'buffer' organisms against environmental change (Fox et al. 2019) and thereby enable populations to persist in the face of novel or changing environments (Ducatez et al. 2020; see also Diamond and Martin 2021; Pfennig 2021 in this volume). Such persistence is important because it allows lineages to 'buy time' until they can evolve further (Pennisi 2018). Finally, most evolutionary biologists acknowledge that plasticity can readily evolve, as long as there is genetic variation in the response. Appreciation of these three facts is critical, for they form the bases for plasticity-led evolution.

BOX 9.1 ANSWERS TO NINE COMMONLY ASKED QUESTIONS ABOUT PLASTICITY-LED EVOLUTION

1. **What is plasticity-led evolution?** Plasticity-led evolution is the evolutionary process that occurs when a change in the environment triggers a change in phenotype via phenotypic plasticity, and following the uncovering of cryptic heritable variation in how different individuals respond to this environmental change, the environmentally induced phenotype is refined by selection into a new adaptive form.
2. **Isn't plasticity-led evolution just selection promoting evolutionary change by acting on quantitative genetic variation?** Yes, but under plasticity-led evolution, a change in the *environment* exposes that quantitative genetic variation to selection (via *plasticity*) in the first place.
3. **Is the basic idea behind plasticity-led evolution really new?** Not really. As we highlighted in Section 9.2, the rudiments of the idea go back over a century. However, the modern framing of the hypothesis—with its emphasis on environmental induction and uncovering of previously cryptic genetic variation—is relatively new.
4. **If genetic variation must be present before plasticity-led evolution can occur, how can one say that genes are 'followers?'** Because genes are necessary for producing phenotypes, they obviously cannot be 'followers' in the strictest sense. The 'genes as followers' perspective emphasizes that environmental induction and uncovering of previously cryptic genetic variation may have been the trigger that instigated many examples of phenotypic evolution. This viewpoint differs from a strict mutation-led evolution viewpoint, in which genes can be more clearly described as 'leaders.'
5. **Isn't plasticity-led evolution another way of saying 'plasticity evolves,' which we already knew about? What's new?** Plasticity-led

evolution is not synonymous with 'plasticity evolves.' Plasticity-led evolution does involve the evolution of plasticity (see Figure 9.2b), which researchers have indeed long known about. What is new is that the evolution of plasticity can have important ramifications: in particular, it might set in motion a sequence of events leading to the origin of new traits and even new species, as we describe in this chapter.

6. **Doesn't plasticity-led evolution merely describe the evolution of incomplete penetrance?** No. In genetics, 'penetrance' refers to the proportion of individuals carrying a particular allele that express the trait normally associated with that allele. An allele exhibits 'incomplete penetrance' if some individuals carrying the allele do not express the trait. Because incomplete penetrance can arise owing to environmental factors, it could be said to be caused by phenotypic plasticity. However, not all cases of incomplete penetrance are due to plasticity; incomplete penetrance can also be caused by other factors, such as genetic modifiers. Moreover, as noted in our answer to the previous question, plasticity-led evolution entails more than just the evolution of plasticity.
7. **Is plasticity-led evolution the only way that plasticity can impact evolution?** No, it is not. In fact, in some cases, plasticity might actually impede evolution and in other cases have no impact at all. Moreover, plasticity-led evolution is not the only way that plasticity can facilitate evolution (see also Pfennig 2021 in this volume).
8. **Does plasticity-led evolution contradict existing evolutionary theory?** Not at all. As with every case of adaptive evolution, plasticity-led evolution entails selection acting on underlying heritable (e.g., genetic) variation (see also Futuyma 2021; Pfennig 2021; Schlichting 2021 in this volume). However, plasticity-led evolution does place greater focus on the proximate mechanisms by which phenotypic variation arises and on the evolution of development.
9. **Are there any examples of a trait that has arisen via plasticity-led evolution?** There are numerous *possible* examples, including many from natural populations (see Table 9.2). However, without a time machine, one can never be certain that a particular trait arose via plasticity-led evolution rather than via mutation-led evolution; of course, the converse is also true!

9.5.2 Plasticity-Led Evolution: Where Is the Controversy?

Where consensus over plasticity's role in evolution starts to break down is when talking about whether plasticity can promote evolution *directly*, i.e., via plasticity-led evolution. One problem here is that the rationale behind plasticity-led evolution has generally not been articulated clearly. This lack of clarity has fostered confusion over what is meant by plasticity-led evolution (Wray et al. 2014), when, in fact, plasticity-led evolution fits comfortably within modern evolutionary theory (see Box 9.1; see also Futuyma 2021; Pfennig 2021; Schlichting 2021; and West-Eberhard 2021 in this volume). An unfortunate consequence of this confusion is

that many textbook authors (whether intentionally or not) have simply ignored the topic altogether. For example, very few college-level evolution textbooks even list the terms 'phenotypic plasticity' and 'genetic assimilation' in their indices [for an exception, see Futuyma and Kirkpatrick's (2017) textbook, in which they discuss plasticity-led evolution on pp. 394–395]. Genetic assimilation, in particular, has long been controversial (Simpson 1953a; Williams 1966; Orr 1999; de Jong 2005; Wray et al. 2014; see also Scheiner and Levis 2021 in this volume). Thus, because students are not being exposed to the plasticity-led evolution hypothesis (and related ideas), ignorance of the topic persists.

However, perhaps the main impediment to a greater appreciation of plasticity-led evolution is the perceived lack of empirical evidence in support of it (Levis and Pfennig 2016). We say "perceived lack" because empirical support for plasticity-led evolution has increased dramatically in recent years (e.g., see Table 9.2). Nevertheless, because "the best way to elevate the prominence of genuinely interesting phenomena such as phenotypic plasticity … is to strengthen the evidence for their importance" (Wray et al. 2014, p. 164), in the long run, devising and implementing rigorous tests remains essential to widespread acceptance of plasticity-led evolution.

9.5.3 Plasticity-Led Evolution: Where Are the Challenges?

We now address two clear challenges to the plasticity-led evolution hypothesis. As we describe, these challenges focus on whether or not a plasticity-led evolution interpretation is necessary and/or sufficient to explain evolutionary innovation and diversification.

One key challenge to the plasticity-led evolution hypothesis is a debate over the *order* of events versus the *importance* of those events (e.g., Futuyma 2015). We refer to this debate as being over 'sequence' versus 'significance,' respectively. This debate specifically takes issue with a claim made by Mary Jane West-Eberhard (2003) when discussing plasticity-led evolution in her influential book, *Developmental Plasticity and Evolution* (pp. 157–158):

> *Most phenotypic evolution begins with environmentally initiated phenotypic change… Gene-frequency change follows, as a response to the developmental change… Genes are followers, not necessarily leaders, in phenotypic evolution.*

Of course, as we have stressed throughout this chapter, pre-existing genetic variation is the starting point for plasticity-led evolution. This means that genes cannot be followers in the strictest sense. Or as Douglas Futuyma (2017, p. 6) has put it:

> *Genes are 'followers' only to the extent that genetic assimilation… 'fine-tunes' an adaptation that had already evolved by selection and genetic variation.*

It is important to note that both the sequence and significance viewpoints emphasize how downstream quantitative genetic changes modify and refine an environmentally induced phenotype. The major difference between these two perspectives is that the sequence viewpoint (as articulated by Futuyma) emphasizes the role of past selection and existing variation, whereas the significance viewpoint (as articulated by West-Eberhard) emphasizes the relative importance of different processes during adaptive evolution: development of novel phenotypic variants versus selection on those variants. Thus, the challenge is that plasticity-led evolution may not constitute a

significant departure from, or extension of, evolutionary theory: selection and genetic variation precede any plastic responses that ignite a sequence of plasticity-led evolution (see also Futuyma 2021 and West-Eberhard 2021 in this volume). However, even if past selection may have acted and/or genetic variation already exists, the significance view would argue that these factors are not developmentally or phenotypically important until an environmental change makes them so. In this way, the environmental induction and uncovering of previously cryptic variation (regardless of how that variation accumulated) play the major (i.e., leading) role during a particular bout of phenotypic evolution.

The significance viewpoint also elevates the importance of environmental change during the initiation of novelty by giving it equal footing with mutation. Indeed, as we describe below, environmentally induced phenotypic change may have even *greater* evolutionary potential than a mutation. Whereas novel *genotypic* variation ultimately stems from new mutations, novel *phenotypic* variation stems from developmental innovation, which itself depends on both genetic *and* environmental factors. Therefore, when discussing the origin of novel phenotypes, one must consider the roles of both gene products and environmental inputs. Furthermore, these two inputs are potentially evolutionarily interchangeable, meaning that selection can slide trait regulation anywhere along a continuum from greater environmental control (potentially resulting in a polyphenism) to greater genetic control (potentially resulting in a fixed trait via genetic assimilation). Moreover, because phenotypic variation is required for evolution by natural selection, environmental change is similar to a new mutation in that it can be considered a first-order cause of evolutionary novelty. That is, environmental inputs during development give rise to new phenotypes *before* natural selection sifts among those phenotypes. Importantly, this view re-orients how we think of environmental change by highlighting its dual role as a *generator* and then *selector* of phenotypic variation.

In addition to being partners with gene products during developmental—and thus phenotypic—innovation, environmentally induced phenotypic change may harbor greater evolutionary potential than mutationally induced change for at least three, non-mutually exclusive reasons (West-Eberhard 2003; Levis and Pfennig 2016). First, changes in the environment often affect many individuals simultaneously. This situation is in contrast to a genetic mutation, which initially affects only one individual and its immediate descendants. Such widespread impact of environmental change enables a newly induced trait to be tested among diverse genetic backgrounds, thereby increasing the opportunity for selection to act and for subsequent adaptive refinement to occur.

Second, although the chance that a particular mutation will occur is not influenced by whether or not the organism is in an environment in which that mutation will be advantageous—in other words, adaptively directed mutation does not occur (Sniegowski and Lenski 1995)—environmentally triggered traits are always associated with a particular environment: the one that triggered it. Therefore, environmentally induced traits are more likely than mutationally induced novelties to experience consistent selection and directional modification (West-Eberhard 2003). This constancy allows new environments to immediately produce *and* select among new phenotypes and rapidly refine their expression (Badyaev 2005). While transmission of environmentally induced novelties across generations requires some mechanism of inheritance, which is most likely genetic, the same is true of mutationally induced

novelties: both require a pre-existing genetic (or otherwise heritable) background in which to integrate and transmit novel information.

Third, the waiting time for a new mutation to arise and increase in frequency in a population can be prohibitively long (Charlesworth 2020). In contrast, new phenotypic variants triggered by the environment are produced over developmental time, i.e., over a single individual's lifetime. The speed with which environmentally induced change can arise might prove critical in the face of a rapidly changing environment, where a delay in producing adaptive new phenotypic variants might result in a population going extinct (Pfennig and Pfennig 2012; Ducatez et al. 2020).

A second major challenge to plasticity-led evolution is its alleged inability to discriminate among alternative hypotheses (Kovaka 2019; Noble et al. 2019). This problem largely stems from the methodological approaches that have been used to study plasticity-led evolution. Specifically, since most studies focus on characteristics of extant taxa using ancestor-derived comparisons of phenotypic responses, it is difficult (or perhaps, in some cases, impossible) to determine if ancestral plasticity did indeed drive evolutionary change or if a *de novo* lineage-specific mutation did. That is, without knowing the molecular basis of a plastic response, one cannot determine if a new mutation preceded and drove the development of the derived phenotype or, conversely, if cryptic genetic variation—coupled with environmental change—did.

Notably, the key predictions outlined in Table 9.1 do not address these difficulties. Yet, these concerns are irrelevant if the key predictions are not supported. Thus, while the predictions outlined in Table 9.1 are necessary to demonstrate plasticity-led evolution, they may not be sufficient to rule out alternative hypotheses. We speculate that a discriminatory signature of plasticity-led evolution might combine evidence of: (1) a soft selective sweep (i.e., adaptation from standing genetic variation; Messer and Petrov 2013); (2) conditional expression (Van Dyken and Wade 2010); and (3) phenotypic, developmental, and environmental change (e.g., support for the predictions in Table 9.1). A good example that extends beyond the key predictions described in Table 9.1 comes from a study of craniofacial morphology in cichlids by Parsons et al. (2016). In this study, researchers followed the framework outlined in Section 9.4 for evaluating plasticity-led evolution. However, they went a step further by identifying environmentally sensitive quantitative trait loci and determining that the derived allele of one of these loci exhibited markedly reduced environmental sensitivity than the ancestral allele. That is, they were able to identify a particular regulatory locus whose evolution matches predictions of genetic assimilation via plasticity-led evolution. Recently, more attention has been paid to the evolution of plasticity mechanisms and how various mechanisms might influence plasticity-led evolution in different ways (Snell-Rood et al. 2018; Levis and Pfennig 2020; see also Goldstein and Ehrenreich 2021; Ledón-Rettig and Ragsdale 2021 in this volume). This focus on mechanisms, coupled with additional theoretical and modeling efforts (e.g., Scheiner 2014; Scheiner et al. 2017), may provide tractable approaches for dealing with the difficulty of discriminating among alternative hypotheses (Kovaka 2019).

9.6 CONCLUSIONS

Explaining the origins of diversity is a longstanding problem in evolutionary biology. As we have seen, phenotypic plasticity can have far-reaching impacts on the

origins of diversity, ranging from promoting diversity and novelty within species (Figure 9.1) to promoting population divergence, speciation, and even adaptive radiation. Accordingly, incorporating plasticity generally and plasticity-led evolution specifically into evolutionary thinking provides a richer understanding of how and why living things diversify. Finally, in Box 9.1, we provide answers to some commonly asked questions about plasticity-led evolution, and in Box 9.2, we provide some suggestions for future research.

ACKNOWLEDGMENTS

For commenting on earlier drafts of this chapter, we thank Emily Harmon, Andrew Isdaner, Pat Kelly, Ryan Martin, and Carl Schlichting.

BOX 9.2 SUGGESTIONS FOR FUTURE RESEARCH

- Identify any molecular or otherwise evolutionary signature(s) of plasticity-led evolution that differentiate it from mutation-led evolution. A key impediment to expanding the plasticity-led evolution research program is that the methods traditionally used to study it (see above) may not apply to many taxa or traits. Laboratory experiments and/or additional modeling efforts could be used to identify a signature of plasticity-led evolution that can be applied to diverse taxa, and more importantly, that rules out alternative evolutionary explanations.
- Clarify how different developmental processes, sources of genetic variation, and plasticity mechanisms influence the mode and tempo of plasticity-led evolution. Current efforts have focused primarily on gathering any evidence of plasticity-led evolution. A next step involves comparisons among traits, taxa, and developmental mechanisms to determine if, and how, their propensity to evolve via plasticity-led evolution differs. Comparisons among so-called exploratory processes and other mechanisms of plasticity will likely be fruitful.
- Determine whether particular traits or developmental processes are more or less interchangeable between genetic and environmental control than other such categories. What types of genetic architecture, features of gene regulatory networks, and/or what other aspects of phenotype development make some traits more or less likely to undergo shifts in primary control of development?
- Evaluate under what conditions, if any, polyphenism promotes speciation and identify the mechanisms by which it does so. Polyphenisms have long been theorized to promote speciation and diversification, but there is little direct empirical evidence supporting this idea. Clarifying how speciation via polyphenism differs from other models of speciation, if at all, would be useful.
- Clarify whether and how ancestral plasticity influences the nature of phenotypic diversity during adaptive radiations. Do plastic responses

tend to bias the phenotypes that arise via radiation? Are plastic traits more likely to drive radiations than non-plastic traits?
- Continue evaluating plasticity-led evolution in diverse traits and taxa.

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10 Phenotypic Plasticity and Evolutionary Transitions in Individuality

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CONTENTS

10.1 INTRODUCTION

Evolutionary transitions in individuality occur when the unit of selection and adaptation, the evolutionary individual, changes from one level of organization to another (Buss 1987; Maynard Smith and Szathmáry 1995; Michod 1999; Hanschen et al. 2017a). Examples involve some of the key events in the history of life, including the evolution of the cellular genome from groups of cooperative genes, the evolution of eukaryotic cells from bacterial and archaeal cells, the evolution of multicellular organisms from unicellular ancestors, and the evolution of eusocial societies from solitary ancestors. These transitions in individuality are rare evolutionary events, having occurred dozens of times, as compared to the millions of speciation events that have occurred throughout the history of life. Although rare, these evolutionary transitions have bestowed on life one of its most familiar characteristics, its hierarchical organization, in which biological individuals—genomes, cells, multicellular organisms, eusocial societies—are comprised of groups of cooperating individuals from lower levels.

In this chapter we ask how phenotypic plasticity affected evolutionary transitions in individuality (see Table 10.1 for a summary of evolutionary transitions in individuality and the possible importance of phenotypic plasticity in each transition).

DOI: 10.1201/9780429343001-10

TABLE 10.1
The Potential Role of Phenotypic Plasticity in Four Key Evolutionary Transitions in Individuality

Evolutionary Transition in Individuality	Potential Role of Plasticity
Origin of the genome in cells. The genome is a cooperative group of genes that can be selected for if it resides in a compartment, or cell-like structure (Michod 1983, 1999; Szathmary and Demeter 1987; Maynard Smith and Szathmáry 1995).	Authors have hypothesized mobile genetic elements were important in the origin of genomes (Brosius 1999; Durand and Michod 2010; Ågren 2014; Koonin 2016). Genomic rearrangements, including the movement of transposable elements, can be induced by environmental stress and contribute to genomic plasticity (Wessler 1996; Bennett 2004; Leitch and Leitch 2008).
Origin of eukaryotes through endosymbiosis.	Some endosymbioses can be facultative and induced by environmental stress (Lamelas et al. 2011; Bellantuono et al. 2019). It is unclear whether the endosymbiotic origin of mitochondria and plastids in early eukaryotes was similar.
Origin of multicellularity	In this chapter, we develop the hypothesis that plasticity played a role in key steps such as the origin of group formation and the origin of cellular differentiation.
Origin of eusociality	Plasticity is important in caste development and may have played a role in the origin of groups with a simple division of labor.

Plasticity research has predominantly focused on unicellular and multicellular organisms (Schlichting and Smith 2002), and researchers have hypothesized that phenotypic plasticity affected the origin of novel traits in both multicellular and unicellular organisms (West-Eberhard 2003; Levis and Pfennig 2016, 2021). What role did phenotypic plasticity play in the evolution of novel traits required for the transition from unicellular to multicellular organisms?

We break this question down by considering the role of phenotypic plasticity in the different stages of an evolutionary transition in individuality. For our purposes, the stages discussed in more detailed presentations of this theory (Michod 1999; Hanschen et al. 2015) may be condensed into two stages: (1) the formation of groups, and (2) the evolution of cooperation and conflict (including the evolution of division of labor or specialization in the fitness components of the group). Cooperation benefits the group but can be costly to individuals within the group. Group members may benefit from cheating and consuming common resources without contributing to the group, leading to the evolution of mechanisms that inhibit cheating and promote cooperation (Michod 1999; Michod and Roze 2001). Division of labor in the basic components of fitness, survival and reproduction, increases cooperation, decreases cheating, and, as a result, increases the degree

of integration of the group so that group fitness is no longer the average of the fitnesses of group members. We focus on the evolutionary transition from unicellular individuals to multicellular individuals in the volvocine green algae, but briefly discuss the evolution of multicellularity in animals and the evolution of eusocial insect societies.

10.2 MULTICELLULARITY

The evolution of multicellular organisms from unicellular organisms is one of the most common evolutionary transitions in individuality and has occurred dozens of times. Multicellularity is distributed throughout eukaryotes and also occurs in bacteria and archaea (Bonner 1998; Fisher et al. 2013). Multicellularity may be obligate or facultative (Grosberg and Strathmann 2007; Resendes De Sousa António and Schulze-Makuch 2012; Fisher et al. 2013). Obligate multicellularity occurs when a protracted multicellular stage is always part of the life cycle, whereas facultative multicellularity occurs when the life cycle can be completed in the unicellular state, and multicellular structures only develop under certain environmental conditions. Both obligate and facultative multicellularity have evolved independently in multiple clades, including the lineage leading to vascular plants, green algae (including the volvocine green algae), bacteria, archaea, animals, brown algae, red algae, and fungi (Bonner 1998; Grosberg and Strathmann 2007; Fisher et al. 2013; Herron et al. 2013).

Facultative multicellularity occurs in response to environmental conditions in a variety of species (Resendes De Sousa António and Schulze-Makuch 2012; Fisher et al. 2013). In taxa such as the social amoeba, *Dictyostelium*, the facultatively multicellular stage is an integrated part of their life history and has been shaped by selection (Bonner 2003). In cases of facultative multicellularity in which the multicellular forms exhibit multiple cell types, it is not the specific stages of the evolutionary transition in individuality (such as group formation or division of labor) that are environmentally induced. Rather, it is the integrated multicellular organism itself that is environmentally induced, complete with division of labor and other properties characteristic of multicellular individuality.

Facultative multicellularity is different from the environmental responses that are the focus of our inquiry in the coming sections. As already mentioned, facultative multicellularity involves an integrated suite of traits that includes all the steps to multicellularity mentioned above. In the coming sections, we break down the evolution of multicellularity into these smaller steps and ask whether plasticity played a role in the evolution of group formation and division of labor. In that way, we aim to focus more on the origins of the steps to multicellularity rather than on their environmental regulation in extant facultatively multicellular species.

We focus on the volvocine green algae, which is a model system for studying the evolution of multicellularity. The volvocine green algae are a monophyletic clade of chlorophycean green algae found in freshwater lakes, streams, and ponds. Extant species range from unicellular to differentiated multicellular and exhibit different degrees of cellular differentiation in the multicellular state, including undifferentiated colonial species, species with sterile somatic cells, and species with

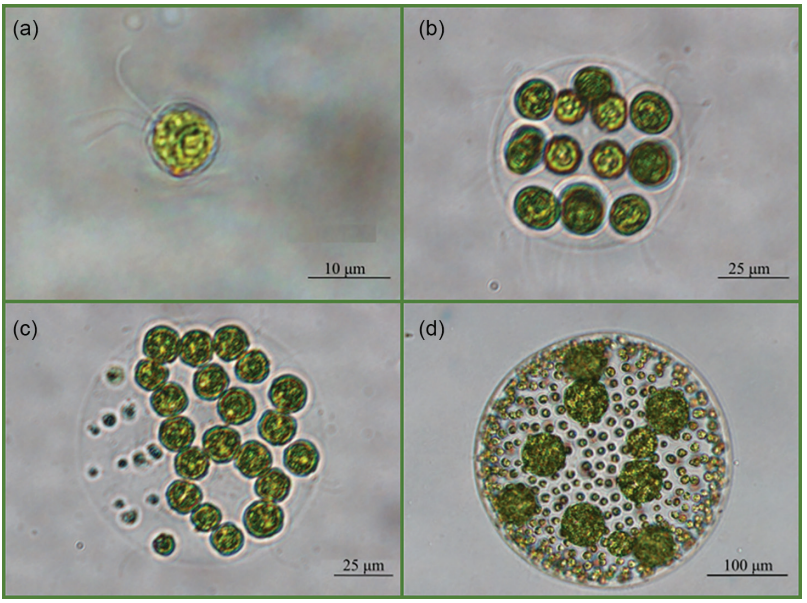

FIGURE 10.1 Four volvocine green algae species that exhibit differences in group formation and cellular differentiation. (a) Unicellular *Chlamydomonas reinhardtii* strain CC 124. (b) *Eudorina elegans* strain UTEX 1201, in which member cells are typically undifferentiated, but cooperate in flagellar action and building a structured spherical colony with a colony boundary. (c) *Pleodorina starrii* strain NIES 1362, which possesses both undifferentiated cells and small somatic cells that do not reproduce and specialize in flagellar action and survival. (d) *Volvox carteri* strain Eve, which consists of large germ cells specializing in reproduction and small somatic cells specializing in survival.

both specialized germ and specialized somatic cells (Kirk 1999; Michod 2007). Representative species referred to in this chapter are shown in Figure 10.1. Since extant species are hypothesized to have characteristics similar to ancestral species that represented intermediate stages in the evolution of multicellular individuality, the clade is used as a model system to study the evolution of multicellularity (Herron and Michod 2008). The transition to multicellularity in the volvocine green algae occurred approximately 230 mya (Herron et al. 2009), which is relatively recent when compared to transitions in other clades, such as the animals and plants. Some of the genes necessary for somatic cell development in the multicellular volvocine alga *Volvox carteri* have been identified (Kirk et al. 1999), and the transcriptomic profiles of somatic and germ cells have been characterized (Matt and Umen 2018). The genomes of many of the key species have been sequenced (Merchant et al. 2007; Prochnik et al. 2010; Hanschen et al. 2016; Featherstone et al. 2018; Hamaji et al. 2018). Developmental, physiological, phylogenetic, ecological, molecular, and fitness-based approaches have all been employed in understanding the evolution of multicellularity in this clade (Desnitski 1995; Kirk 1999; Herron et al. 2009; Coleman 2012; Herron 2016).

10.3 PLASTICITY AND GROUP FORMATION IN THE VOLVOCINE GREEN ALGAE

We are using the volvocine green algae to ask whether the initial stage in the evolution of multicellularity, group formation, was an ancestrally plastic response to the environment that later came under developmental-genetic control. Group formation may occur through two routes: (1) via aggregation of cells from different sources, or (2) via daughter cells staying together after cell division, which gives rise to clonal groups of genetically related cells.

In the first route, aggregation typically occurs in response to an environmental cue. The group phenotype only forms following an environmental signal, and at other times the unicellular stage can reproduce in the absence of a group stage. In *Chlamydomonas*, the close unicellular outgroup to the multicellular volvocine green algae, these environmental cues include changes in nutrient availability and the presence of predators (Iwasa and Murakami 1969; Nakamura et al. 1975; Lurling and Beekman 2006; Khona et al. 2016). Environmental cues such as predation have been hypothesized to selectively favor the evolution of obligate multicellularity (Herron et al. 2019), so the use of this cue for group formation in *Chlamydomonas reinhardtii* suggests that group formation in this species has previously been acted on by selection.

The second route to multicellularity (clonal group formation) occurs when daughter cells fail to separate after cell division and stay together (Bonner 2000; Fisher et al. 2013; Olson 2013). This can be due to an environmental cue (Tecon and Leveau 2016) or genetic mutation (Ratcliff et al. 2015), and depending on the mechanism, may or may not persist in descendants. Clonal group formation can be either obligate, meaning that it always occurs as part of the life cycle of an organism, regardless of environmental conditions, or facultative, meaning that it only occurs in response to environmental stimuli (Olson 2013). In *Chlamydomonas*, facultative clonal group formation can occur in response to abiotic and biotic stressors and does not persist in descendants (Mikheeva and Kriuchkova (1980) as cited in Lurling and Beekman (2006); Khona et al. 2016).

Phylogenetic analysis indicates that obligate clonal multicellularity evolved once in the volvocine green algae (Herron and Michod 2008). The ancestor of this clade is inferred to have resembled its close unicellular relative, *C. reinhardtii*, which diverged from its multicellular relatives approximately 255 mya (Herron et al. 2009). *Chlamydomonas reinhardtii* cells are haploid, flagellated, free-swimming, and photosynthetic. They are phenotypically and genetically similar to the cells comprising the colonial and multicellular species (Merchant et al. 2007; Prochnik et al. 2010; Hanschen et al. 2016; Featherstone et al. 2018; Hamaji et al. 2018).

Chlamydomonas cells form groups called ‘palmelloids’ in response to environmental stressors, such as predation or nutrient deprivation (Figure 10.2). Palmelloids can be formed by the same two processes by which groups are formed generally: via aggregation or via clonally related cells staying together during development (Olsen et al. 1983; Lurling and Beekman 2006; Khona et al. 2016). Despite these differences, the unifying feature of all palmelloids is that they are cell groups that form in response to *environmental* cues.

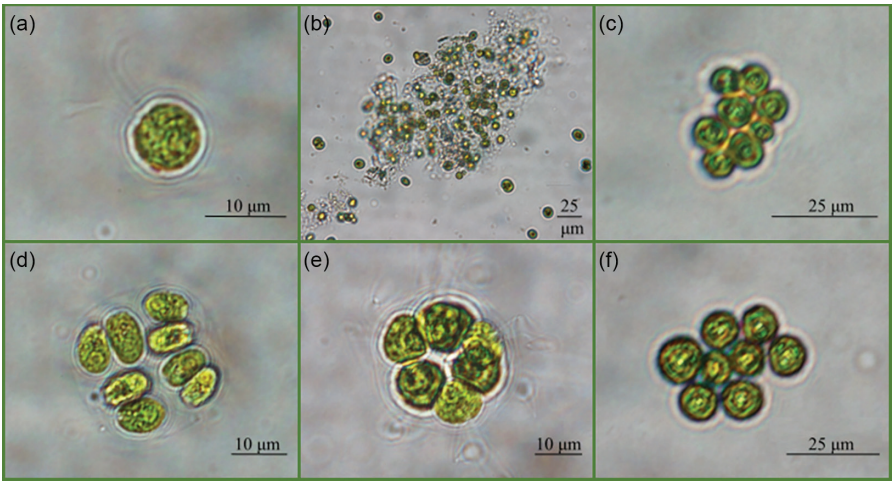

FIGURE 10.2 Unicellular *C. reinhardtii* along with examples of facultative and obligate cell groups discussed in the text. (a) Unicellular *C. reinhardtii* strain CC 124. (b) A large, disorganized *C. reinhardtii* CC 124 palmelloid induced by 20 hours of culture in a 150 mM saline solution. (c) A small, more organized *C. reinhardtii* CC 124 palmelloid induced by 20 hours of culture in a 150 mM saline solution. (d) A *C. reinhardtii* cell group from population B2-04 experimentally evolved in the presence of a ciliate predator, provided courtesy of M. D. Herron (described in Herron et al. [2019]). (e) The undifferentiated colonial species *Pandorina charkowiensis* UTEX LB 840. (f) The undifferentiated colonial species *Gonium pectorale* K4-1 '+'.

Palmelloids formed by aggregation have been described as disorganized and consist of 10–100,000 cells that can be genetically distinct and can even be comprised of cells from different species (Sathe and Durand 2016). These aggregative palmelloids are held together by the secretion of a gelatinous extracellular matrix. For example, the palmelloid in Figure 10.2b appears to be aggregative. In contrast, palmelloids that develop clonally are made up of smaller, organized clusters of 4–16 cells (Harris 2009). The cells that make up clonal palmelloids are contained within the mother cell wall. The palmelloid in Figure 10.2c appears to be phenotypically consistent with clonal palmelloids, although this needs to be confirmed by developmental studies. This type of palmelloid shares similarities in cell number and spherical cell arrangement with simple colonial volvocine algae species, including *Tetrabaena* and *Pandorina*. That said, both types of palmelloids are made up of cells that lose fully functional flagella when in a group, causing the group to lack motility (Lurling and Beekman 2006; Khona et al. 2016), which differentiates them from their obligately colonial relatives. We now discuss the results of several experimental evolution studies which indicate that plastic palmelloid formation may precede the evolution of obligate clonal group formation (Ratcliff et al. 2013; Herron et al. 2019).

Ratcliff et al. (2013) selected *C. reinhardtii* for increased settling rate by propagating algae that settled at the bottom of a centrifuge tube after centrifugation, thereby selecting for large, less motile clusters of cells. The multicellular clusters that evolved

consisted of up to hundreds of cells, with a mean of 58 per cluster, that reproduced via motile unicells and persisted after the removal of settling selection, indicating the group phenotype came under genetic control. These clusters differed from palmelloids in that they were not transient and instead were stable and heritable. Moreover, the group phenotype was present for most of their life cycle each generation. Also, unlike in aggregative palmelloids, cells within a cluster were clonally related and stayed together after division. The latter characteristic is surprising given that the group phenotype was more similar to aggregative palmelloids than clonal palmelloids. Despite their different developmental program, these multicellular forms shared phenotypic similarities with aggregative palmelloids in that they had similar numbers of cells, were not motile, and were held together by the extracellular matrix.

Herron et al. (2019) experimentally evolved *C. reinhardtii* cell groups in the presence of the unicellular predator *Paramecium tetraurelia*. The predator could consume the unicells but not the larger cell groups. Group formation was thereby selected for over hundreds of generations. While stable group phenotypes without flagella and with a degree of clonal development evolved in two of the five populations, there was variation in the group morphology and development among isolates from these populations. Cell groups developed in some lines via a mix of aggregation and clonal development; the maternal extracellular matrix kept daughter colonies and unicells together while simultaneously also trapping any unicells that were nearby. The large size, mechanisms underlying group formation, and lack of flagella and clear organization were similar to aggregative palmelloids in these lines. However, such lines differ from palmelloids: group formation has persisted for more than 4 years in the absence of selective reinforcement, and development could be both clonal and aggregative. Other lines evolved obligate cell groups that were phenotypically distinct from the ones held together by extracellular matrix. These lines developed clonally and were held together by the maternal cell wall, and daughter colonies (when released out of the mother colony) typically consisted of 4, 8, or 16 cells. The cells within the group were arranged consistently; this arrangement caused them to morphologically resemble the smaller volvocine green algae and the clonally developing palmelloids.

There are clear phenotypic similarities and key differences among: (1) the clonally developing palmelloids; (2) the clonally developing experimental lines with 4, 8, or 16 cells; and (3) the smaller colonial species with 4, 8, 16, or 32 undifferentiated cells (Herron et al. 2019). In all three cases, development is clonal, cells are enclosed by a cell wall, and cell number is primarily constrained to powers of two. Moreover, predation, the agent of selection in the Herron et al.'s (2019) experimental evolution study, can also induce the development of clonal palmelloids (Lurling and Beekman 2006). Predation is also hypothesized to be a selective pressure that favored the evolution of multicellularity (Stanley 1973; Bonner 1998). However, while colonial volvocine algae species are motile, experimentally evolved *Chlamydomonas* and *Chlamydomonas* palmelloids are not (Kirk 1998; Khona et al. 2016; Boyd et al. 2018). Experimentally evolved *C. reinhardtii* cell groups differed from palmelloids in that they remained stable in the absence of predators, whereas palmelloid development occurs in response to environmental cues. It is possible that the experimentally evolved forms began as an adaptive plastic response to the environment and

were then modified by mutations that spread due to adaptative evolution (Levis and Pfennig 2021). The observations above raise the question of whether group formation in the volvocine green algae evolved via the loss of ancestral plasticity (genetic assimilation (see Scheiner and Levis 2021 in this volume).

In summary, facultative group formation is present in a unicellular outgroup to the multicellular volvocine green algae, with palmelloids sharing phenotypic similarities with experimentally evolved cell groups and simple, obligately multicellular algae species. The observations above raise the question of whether group formation in the volvocine green algae evolved through the loss of ancestral plasticity (genetic assimilation. The plastic phenotype could have come under developmental-genetic control when obligate cell groups evolved in this clade, with secondary modifications affecting traits such as motility.

10.4 PLASTICITY AND DIVISION OF LABOR IN THE VOLVOCINE GREEN ALGAE

After cell groups form, cooperation may evolve and along with it, the possibility of defection and the evolution of conflict mediation; that is, developmental mechanisms that enhance cooperation by diminishing the opportunity for, or benefits of, cheating (Michod 2003, 2021). One critical developmental process involving both the evolution of cooperation and the mediation of conflict is reproductive division of labor. Reproductive division of labor occurs when cells specialize in either survival or reproduction, the two basic components of fitness in any organism. The evolution of reproductive division of labor often precedes the evolution of other forms of cellular differentiation (Simpson 2012). The evolutionary priority of reproductive division of labor can be understood in terms of conflict mediation. For other types of somatic cells to evolve, it must first be settled which cells are going to reproduce; otherwise, cells will continually compete for access to the next generation.

During the evolution of multicellularity, reproductive division of labor takes the form of specialized reproductive (germ) and non-reproductive (somatic) cells. Germ and somatic cells are specialized in one of the two basic components of fitness and therefore would have low fitness were they to leave the group and occur in the absence of the other. Consequently, the fitness of groups of specialized cells may be high, even though specialized cells would have low fitness were they alone. As a result, the individuality of the group is enhanced as the fitness at the group level is decoupled from fitness at the cell level (Shelton and Michod 2020). The transfer of fitness to the new level results in a new unit of selection and adaptation—that is, the multicellular individual. After the evolution of germ-soma division of labor, additional cell types can evolve and have evolved in multiple clades. However, in the volvocine green algae additional cell types have not evolved, possibly due to the mechanism of soma determination (i.e., the inhibition of chloroplast development) that prohibits somatic cells from replicating post embryonically (Nedelcu and Michod 2004).

Studies suggest that plasticity has played a role in the evolution of cellular differentiation (Schlichting 2003; Nedelcu and Michod 2006, 2020). Ancestral pathways

induced by stressful environments in unicellular organisms have been co-opted for use in cellular differentiation during the evolution of multicellularity (Nedelcu and Michod 2006, 2020; Nedelcu 2009). Unicellular organisms, like many organisms, may be expected to downregulate reproduction to survive stressful environments. Such regulation is an example of plasticity that has previously been shaped by adaptive evolution. The same pathways that downregulate reproduction in a unicellular ancestor may be activated in a cell group to produce cells with reduced effort at reproduction; in other words, to produce somatic cells (see discussion of *regA* in the volvocine algae below). Previously plastic responses to the environment may later become part of normal development and cease being regulated by environmental inputs (Schlichting and Smith 2002; Nedelcu and Michod 2006, 2020; Wagner et al. 2019; see also Levis and Pfennig 2021 in this volume).

The range of cellular differentiation seen in the volvocine green algae make them a useful model system for studying the evolution of cellular differentiation. Multicellular volvocine green algae are typically characterized as having three different categories of cellular differentiation: undifferentiated colonies (where each cell is undifferentiated, and could, in principle, survive and reproduce were it alone), soma-differentiated colonies (with both non-reproductive somatic cells and undifferentiated cells), and germ-soma differentiated colonies (with specialized germ and somatic cells; Kirk 1999). Undifferentiated cells reproduce and have flagella that allow them to swim as well as mix water around the colonies for more efficient nutrient uptake and disposal of waste. Flagella are attached to the cell via basal bodies, which also play an important role in cell division. As a result, flagellar function decreases as cell division proceeds, with flagellar function being lost after about the fifth division (the 32 cell stage) (Koufopanou 1994). Consequently, colonies with 32 or more undifferentiated cells cannot swim and reproduce simultaneously (Koufopanou 1994). However, the evolution of somatic cells with permanent flagella allows large colonies to swim while reproducing (Solari et al. 2006).

Somatic and germ cells have both evolved multiple times within the volvocine green algae (Herron and Michod 2008). Moreover, within one volvocine clade (the Eudorina clade), somatic cells have been gained and/or lost multiple times (Grochau-Wright et al. 2017) (Figure 10.3). When a trait is gained multiple times within a diversifying clade, one possible mechanism underlying such recurrence is that the common ancestor possessed developmental plasticity in the trait of interest (West-Eberhard 2003; Scoville and Pfrender 2010). The recurrent evolution of the trait would then involve plasticity followed by repeated genetic assimilation rather than *de novo* gains of the trait. Could the repeated evolution of somatic cells shown in Figure 10.3 be explained by the presence of plasticity in the development of somatic cells in the common ancestor of the clade followed by the repeated loss of this plasticity?

To explore this question, we focus on species within the Eudorina clade. The Eudorina clade contains species with all three categories of cellular differentiation: undifferentiated polyphyletic *Eudorina* species with 8–32 cells, soma-differentiated polyphyletic *Pleodorina* species with 32–128 cells, and germ-soma differentiated

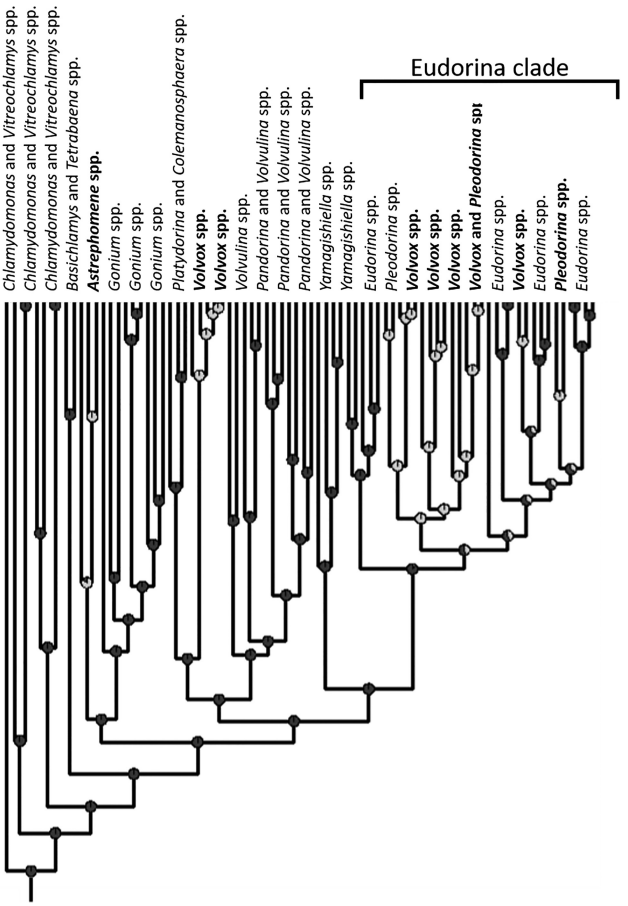

FIGURE 10.3 Volvocine green algae phylogeny illustrating a maximum-likelihood ancestral state reconstruction of obligate somatic cells using an equal rates model. The Eudorina clade, which likely underwent multiple gains and/or losses of obligate somatic cells, is bracketed. Species in bold possess obligate somatic cells and species not in bold are historically characterized as being undifferentiated. Pie charts at nodes indicate support for undifferentiated and soma-differentiated state at that node. Light gray corresponds to support for that node possessing obligate somatic cells and dark gray corresponds to support for that node being undifferentiated. Figure modified from Grochau-Wright et al. (2017).

polyphyletic *Volvox* species with hundreds to thousands of cells (Figures 10.1 and 10.3). The gene *regA*, which is necessary for the development of somatic cells in *Volvox carteri*, is found in both species with and without somatic cells (Grochau-Wright et al. 2017). The recurrent gains and/or losses of somatic cells, the diversity of cellular differentiation, and the characterization of part of the underlying genetic pathway make the Eudorina clade an ideal system for examining the potential

importance of plasticity in the evolution of cellular differentiation and evolutionary transitions in individuality.

Research examining the origin of *regA* indicates that environmental regulation may have played an important role in the evolution of somatic cells. The gene *regA* encodes a transcription factor and is necessary for somatic differentiation in *V. carteri* (Kirk et al. 1999; Meissner et al. 1999). In *regA* mutants, cells that otherwise would have remained somatic de-differentiate during development and become capable of reproduction (Kirk 1997).The homolog of *regA* in the unicellular *C. reinhardtii* is *rls1.* When expressed in *C. reinhardtii, rls1* causes cells to cease reproduction, similar to the lack of reproduction seen in volvocine algae somatic cells (Nedelcu and Michod 2006; Nedelcu 2009). Although both somatic cells and stressed *C. reinhardtii* cells lack reproduction, this state is transient and reversible in *C. reinhardtii*, whereas somatic cells in *V. carteri* are terminally differentiated and permanently smaller than reproductive cells. Researchers have hypothesized that the ancestral stress-response of down-regulating reproduction in response to the environment in a unicellular ancestor was co-opted for a new function in a new context: the origination of non-reproductive somatic cells in a multicellular organism (Nedelcu and Michod 2006, 2020; Nedelcu 2009).

The co-option of a life history stress-response gene described above indicates that phenotypic responses to environmental stress may have played a role in the origin of cellular differentiation in the volvocine green algae. In the multicellular context, the previously environmentally regulated response was co-opted to produce the development of somatic cells. While adaptive plasticity played a role in the evolution of somatic cellular differentiation in the volvocine lineage, the intermediate steps are unclear. During the evolution of multicellularity, the origin of undifferentiated cell groups preceded the origin of cellular differentiation. Although we know that the transient down-regulation of reproduction in response to environmental stress was present in unicellular organisms, it remains unclear if somatic-like cells—small, terminally differentiated cells that do not reproduce—developed in simple colonial organisms in response to stress prior to the evolution of obligate soma.

To determine if somatic-like cells could develop plastically in an undifferentiated species, we recently subjected undifferentiated *Eudorina* species to an environmental stressor. The genus *Eudorina* is paraphyletic, but all *Eudorina* species fall within the Eudorina clade, with some undifferentiated *Eudorina* species being more closely related to *Volvox* species than they are to other members of their nominal genus. We focused primarily on *E. elegans* strain UTEX 1201, which is part of the undifferentiated outgroup to the rest of the Eudorina clade and likely never had a differentiated ancestor (Grochau-Wright et al. 2017). We used 2 hours of cold shock as a stressor, as temperature variation is ecologically relevant for freshwater algae (Similä 1988; Kremer et al. 2018).

We found that cold-shocked colonies developed cells that resembled somatic cells (Figure 10.4); we refer to these cells as somatic-like. Somatic-like cells are living cells with beating flagella that do not reproduce and are smaller than reproductive cells (unpublished data). Somatic-like cells are a plastic response to the environment

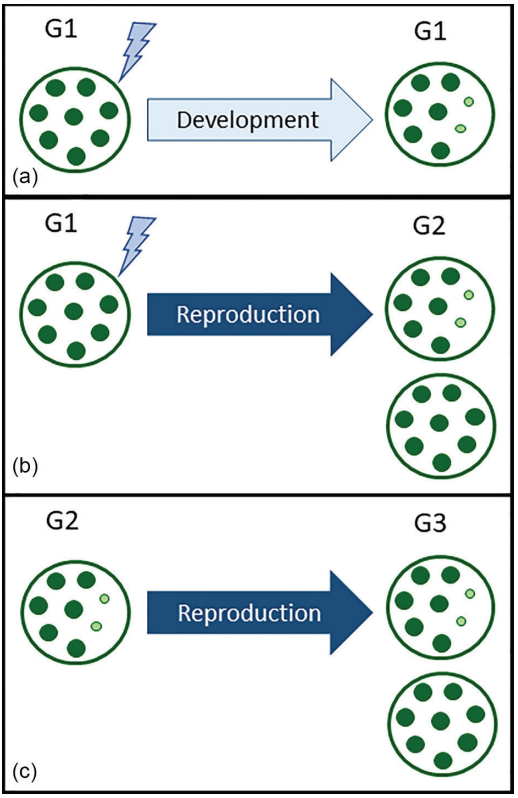

FIGURE 10.4 A schematic representing the production of somatic-like cells in generations 1 (G1), 2 (G2) and 3 (G3). (a) Undifferentiated G1 colonies can develop somatic-like cells after cold shock. (b) Cold-shocked G1 colonies give rise to offspring with and without somatic-like cells. (c) The G3 grand-offspring of cold-shocked G1 colonies can also have daughters with and without somatic-like cells. The proportion of these G3 colonies with somatic-like cells does not differ from that of controls (unpublished data).

and do not persist for more than two generations after cold shock. New cells do not emerge in response to the stressor—instead, previously undifferentiated cells alter their phenotypic state (unpublished data). Full characterization of somatic-like cells is ongoing and will allow us to determine if there are morphological differences between somatic-like cells in generations 1 and 2.

Somatic-like cells that develop in cold-shocked colonies appear to be a cell-level plastic response to the cold shock. These cells develop within the first generation (G1) when formerly undifferentiated cells alter their phenotypic state in response to the environment. Since cell-level plasticity refers to immediate cell responses to the environment in G1, we hypothesize that cell-level plasticity is present when somatic-like cells are formed in G1.

We also found that cold-shocked colonies produced more daughter colonies with somatic-like cells than do control colonies (Figure 10.4). This indicates that stressed colonies reorganize development to produce somatic-like cells in their offspring

(transgenerational plasticity; see Bonduriansky 2021). Consequently, we hypothesize that developmental plasticity is present at the level of the colony in G2 colonies. The mechanism underlying cell-level differentiation in direct response to cold shock likely differs from that underlying the development of somatic-like cells in colonies produced by mothers exposed to cold shock. As a result, group-level plasticity is not simply a scaled-up version of cell-level plasticity but is instead a mechanistically distinct property of group development.

Finally, when cold shocking other *Eudorina* species, we found that phylogenetically distinct *Eudorina* species developed somatic-like cells following a cold shock. While some of the examined *Eudorina* species are members of an outgroup to the rest of the Eudorina clade, others fall within the largest part of the clade and are closely related to obligately differentiated species (Figure 10.3). This suggests that the ancestor of the Eudorina clade possessed plastic somatic-like cells that later came under developmental-genetic control.

Taken together, our ongoing work supports the hypothesis that the development of cells that resemble soma are a plastic response to environmental stress and an intermediate step in the evolution of obligate cellular differentiation (Figure 10.5). Interestingly, this plastic response exists at both levels of organization that are central to this evolutionary transition in individuality: the cell and the cell-group or colony level. Cold-shocked cells become somatic-like (cell-level plasticity), and the offspring of cold-shocked colonies develop somatic-like cells (colony-level plasticity). It is unclear whether the occurrence of plastic somatic-like cells is an adaptive response to environmental stress or a by-product of developmental interactions with cold shock. It is also unclear if this response utilizes the same pathway to downregulate reproduction in response to stress as *C. reinhardtii* does. Current work is underway to characterize developmental changes that occur following cold shock and to determine if the expression of regA or related genes is upregulated during the development of somatic-like cells.

10.5 OTHER EVOLUTIONARY TRANSITIONS IN INDIVIDUALITY

While this chapter has focused on multicellularity and the volvocine green algae model system, the question remains as to whether plasticity plays a role in other evolutionary transitions in individuality. We conclude by briefly discussing two examples of other evolutionary transitions in individuality in which plasticity is present: the evolution of multicellularity in animals and the evolution of social insect eusociality. Here, we predominantly focus on group formation in choanoflagellates and division of labor in eusocial insects.

10.5.1 Group Formation in Choanoflagellates and Animals

Choanoflagellates are the closest relatives of animals and are used as a model system to study the evolution of animal multicellularity (Ruiz-Trillo et al. 2007; King et al. 2008). Choanoflagellates and metazoans likely shared a common ancestor more than 600 mya (King et al. 2008). Multicellular animals have diversified substantially since this time, evolving a range of developmental programs, cell types, tissues, and

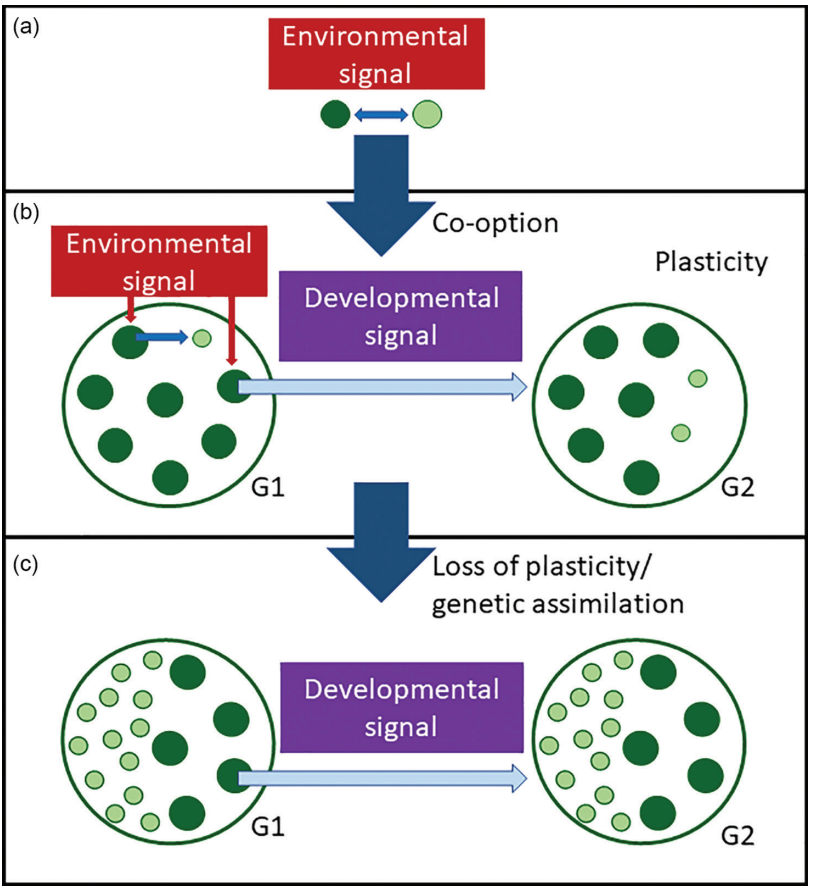

FIGURE 10.5 A schematic illustrating a hypothesized stage in the origin of somatic cells in the volvocine green algae. Nedelcu and Michod (2006) hypothesized that an environmentally regulated gene was co-opted to developmentally produce soma in differentiated species. Figure 10.5 presents a hypothesis for a key intermediate step in this co-option pathway in undifferentiated species. (a) *Chlamydomonas* reversibly alters its state from reproductive (shown in dark green) to non-reproductive (shown in light green) in response to an environmental signal. (b) The hypothesized intermediate step for the origin of plastic somatic cells in normally undifferentiated species, similar to what is seen in *E. elegans* (Figure 10.4) and discussed in the text. An environmental signal can cause somatic-like cells (shown as small and light green) to develop from undifferentiated reproductive cells (shown as larger and dark gray) in the generation 1 colony exposed to the stressor, the environmental signal. The red arrow on the left points to a reproductive cell that undergoes cell-level development and becomes a somatic-like cell. The red arrow on the right points to a reproductive cell whose colony-level development is altered. This cell develops into a G2 colony, shown at the right, that can have both reproductive and somatic-like cells. (c) *Pleodorina*-like colony in which the environmental cue is no longer necessary to induce the development of somatic cells (also shown as small and light green), and that a developmental cue is sufficient.

morphologies. Morphological similarities between Choanoflagellates and the early branching sponges are apparent and have been used to bolster our understanding of the evolutionary origin of metazoans (Brunet and King 2017).

Choanoflagellates such as *Salpinoeca rosetta*, *Codosiga botrytis*, and *Desmarella kent* exhibit diverse forms of facultative group formation, making multicellular structures in response to environmental conditions, such as an abundance of bacterial prey (Cavalier-Smith 2017). This response allows them to more efficiently capture and consume bacterial prey (Woznica et al. 2016) and involves the integration of multiple traits, indicating that these plastic responses have previously been shaped by adaptive evolution. The multicellular structures are the result of clonal multicellularity: they arise when cells fail to separate after division and remain attached to one another (Fairclough et al. 2010). Two colonial forms (rosettes and chains) can develop in *S. rosetta*.

The earliest multicellular animals may have resembled choanoflagellate rosettes, which are colonial forms in which cells develop in a spherical arrangement, are morphologically distinct from unicells, and are held together by multiple mechanisms (Brunet and King 2017). Several lines of evidence support this. First, the collar complex (a single, apical flagellum surrounded by microvilli) seen in choanoflagellates is present in many animal phyla as part of epidermal, nephridial, or epithelial cells (Salvini 1978; Rieger 2009). This is consistent with animals possessing an ancestral choanoflagellate-like morphology. Second, these collar cells function in nutrient absorption (Takashima et al. 2013), similar to how the collar complex in a rosette sweeps in nutrients. Furthermore, rosettes resemble the blastula (an early developmental stage) in diverse animal phyla. The blastula is a ball of cells that are held together by some of the same mechanisms that hold rosettes together (Dayel et al. 2011, Roberson and Barondes 1983, Lee et al. 1997, Salas-Vidal and Lomeli 2004).

Studies of choanoflagellate multicellularity suggest that the most recent common ancestor of animals exhibited simple, facultative multicellularity, forming multicellular structures that resembled choanoflagellate rosettes (Nielsen 2008, Arendt et al. 2016). In choanoflagellates, this multicellularity remained inducible by environmental cues. In most animals, multicellularity became obligate and a diverse range of complex morphologies evolved (Brunet and King 2017). Future research should elucidate the novel traits involved in the origin of facultative multicellularity to gain a more complete picture of how multicellularity originated in animals.

10.5.2 Plasticity in Eusocial Insect Societies

The evolution of eusocial societies is an evolutionary transition in individuality in which multicellular organisms form highly integrated social groups called eusocial colonies. As in all transitions in individuality, the transition from solitary living to sociality involves the formation of groups and the evolution of cooperation and conflict and conflict mediation, including through division of labor. Division of labor takes the form of castes, in which organisms give up their own reproduction and specialize in facilitating the survival of the group; these organisms are generally

called workers. Other organisms (typically referred to as queens) specialize in reproduction. Here we focus on the role plasticity plays in the evolution and maintenance of division of labor in eusocial insects.

Eusociality has evolved multiple times in insect clades (Cronin et al. 2013). In Hymenoptera, an insect order containing bees, wasps, and ants, eusociality has evolved at least ten times in the last 100 million years (Bourke 2011). Hymenoptera contains obligately eusocial species with a complete reproductive division of labor between queens and workers. This order also contains solitary species and species with facultative group formation (Hines et al. 2007). A degree of division of labor may have been present as a plastic response in the solitary ancestors of eusocial Hymenoptera. When solitary bees are experimentally placed in a group, they exhibit rudimentary division of labor. Some bees direct their activity towards guarding, while others focus on foraging or colony-building (Wcislo and Danforth 1997; Jeanson et al. 2005). This rudimentary division of labor, expressed in solitary bees in response to changes in their social environment, may have been a preadaptation that set the stage for the evolution of complex division of labor in some lineages (Nowak et al. 2010). In other words, the tendency for behavior to be flexible in different social contexts may have led to the origin of division of labor (Jeanson et al. 2005; Quiñones and Pen 2017).

Studies examining facultatively eusocial species have also shed light on the potential importance of plasticity in the evolution of eusociality. Species such as the facultatively eusocial bee *Megalopta genalis* will form solitary and social nests in the same population. Solitary nests arise when the female produces all sons in her brood, while social nests arise when at least one daughter stays at the nest as a worker instead of dispersing (Wcislo et al. 2004; Wcislo and Gonzalez 2006; Chambers et al. 2007). Gene expression in the abdomens of facultatively eusocial *M. genalis* is caste-specific, and upregulated genes are rapidly evolving and have likely been targets of selection in obligately eusocial species (Jones et al. 2017). Eusociality may have been ancestrally plastic, with this plasticity later followed by changes in gene regulation and adaptive refinement (plasticity-led evolution; see Levis and Pfennig 2021).

In addition to being important to caste development in species with facultative eusociality, plasticity is also central to caste differentiation in obligately eusocial insects (Nijhout 2003; Corona et al. 2016). In many species, caste determination is triggered primarily by environmental cues, although the relative importance of genes and the environment varies across species (Schwander et al. 2010). Caste development can be affected by nutrition (Smith et al. 2008), temperature, and pheromones (Libbrecht et al. 2013) and is often mediated by the expression of conserved genetic pathways (Berens et al. 2015; Corona et al. 2016). Castes themselves are complex alternative phenotypes and can exhibit distinct morphology, physiology, and behavior. Castes confer benefits to the colony as a whole, as division of labor increases the efficiency of labor and defense, with life history strategies affecting the strength of selection (Fjerdingstad and Crozier 2006).

There are clear parallels between the evolution of eusociality and the evolution of multicellularity. Facultative eusociality is similar to facultative multicellularity in that it is a complex, integrated response to environmental cues. This response

does not correspond to group formation as is seen in *Chlamydomonas* palmelloids; instead, it is more similar to facultative multicellularity in *Dictyostelium*, in which the development of an integrated individual is triggered by environmental cues. Facultative group formation in social insects often includes division of labor, whereas *Chlamydomonas* palmelloids consist of undifferentiated cells without division of labor. Despite these differences, both evolutionary transitions in individuality are characterized by (1) the presence of plasticity in at least one step, and (2) evidence of ancestral plasticity followed by increasing genetic control of the trait and adaptive refinement.

10.6 CONCLUSIONS

This chapter discusses whether phenotypic plasticity played a role in evolutionary transitions in individuality, using the transition from unicellular to multicellular volvocine green algae as a case study. We focused on key steps in the evolution of multicellular individuality—group formation and the evolution of cooperation and conflict through cellular differentiation—and found plasticity to be present throughout those stages. Additional work is needed to determine the extent to which plasticity may have played a role in the origin of novel traits underpinning these steps and to identify the mechanisms involved.

We started by discussing the plastic cell group formation that occurs in response to environmental cues in *Chlamydomonas*, the unicellular outgroup to the multicellular volvocine green algae, and predicted to be similar to the unicellular ancestor to this clade. We highlighted the phenotypic similarities and differences between *Chlamydomonas* facultative cell groups called palmelloids, experimentally evolved cell groups, and extant volvocine algae species. The results discussed here provide preliminary support for the hypothesis that group formation was ancestrally plastic in the volvocine green algae before coming under genetic control (genetic assimilation; see Scheiner and Levis [2021]).

We then considered the evolution of cooperation and conflict via division of labor, a key step in the transition from unicellular to multicellular individuality. We presented unpublished, ongoing work finding that undifferentiated species can develop somatic-like cells in response to environmental stress, and hypothesized that plastic cellular differentiation may have been ancestral to a clade in which somatic cells have been gained and/or lost multiple times. This work also reveals that plastic cellular differentiation is likely present at both the level of the cell and the level of the cell group or colony. This is significant for understanding how transitions in individuality proceed because a central component of the evolution of multicellularity is the transition from cell-level traits to group-level traits (Hanschen et al. 2017a). We also reviewed studies showing that *Chlamydomonas* exhibits temporal changes in reproductive state in response to environmental cues, and that the ancestral gene involved was likely duplicated and co-opted into a gene family necessary for the development of obligate somatic cell differentiation in *Volvox* (Nedelcu and Michod 2006, 2020; Nedelcu 2009).

While plasticity is present in both group formation and cell differentiation, the extent to which it played a role in the evolution of novel traits underpinning these

steps remains unclear. During the evolution of group formation in the volvocine green algae, adaptive plasticity could have preceded the fixation and subsequent evolutionary modification of cell groups, similar to what may have occurred in experimental evolution studies (Ratcliff et al. 2013; Herron et al. 2019). More research is needed to determine how the plastic phenotype itself arose. Similarly, the plastic development of somatic cells in response to stress preceded the evolution of obligate somatic cells in the Eudorina clade. This plasticity may have been inherited from stress responses present in a unicellular ancestor and later co-opted for plastic cellular differentiation.

We concluded by briefly discussing the role plasticity may have played in other evolutionary transitions in individuality, focusing on the evolution of animal multicellularity and eusociality in insects. Choanoflagellates, the closest relative of animals, exhibit facultative multicellularity in the presence of bacteria. Plasticity may have also been important in the evolution of eusociality. Some species exhibit facultative group formation with a primitive division of labor; this facultative eusociality is likely ancestral to obligate eusociality. Caste differentiation in obligately eusocial species is also plastic, with caste development utilizing conserved pathways. Additional research is needed to better understand the extent to which plasticity may have facilitated these and other transitions in individuality.

The role of plasticity in evolutionary transitions in individuality remains largely unexplored and may be controversial. As discussed above, researchers have characterized facultative cell group formation in multiple species; however, research examining the evolution of multicellularity has predominately focused on the genetic changes involved in producing multicellular structures (e.g., Ruiz-Trillo et al. 2007; King et al. 2008; Rokas 2008; Cock et al. 2010; Prochnik et al. 2010; Suga et al. 2013; Hanschen et al. 2016). Less attention has been paid to how plasticity may have contributed to the origin of group formation (but see Van Gestel and Tarnita 2017). The role of plasticity in the evolution of cellular differentiation is better understood but is also controversial. While some researchers have argued that plastic responses to the environment may have been instrumental in the origin of new cell types (Schlichting 2003; Gavrilets 2010; Wagner et al. 2019; Nedelcu and Michod 2020), others have emphasized the importance of genetic changes to gene regulatory networks (reviewed in Arendt et al. 2016).

The potential importance of plasticity in two other evolutionary transitions in individuality introduced in Table 10.1—namely, the origin of the genome and origin of eukaryotes—also requires additional research. Concerning the evolution of eusocial insect societies, researchers have posited that eusociality was ancestrally facultative before coming under genetic control in some lineages (e.g., Jones et al. 2017; Jones and Robinson 2018). Plasticity in caste development has been well-characterized and is uncontroversial (reviewed in Corona et al. 2016).

This chapter lays out the groundwork for examining how plasticity may have contributed to evolutionary transitions in individuality, with a focus on the evolution of multicellularity in the volvocine green algae. Additional research is needed to better understand the role of phenotypic plasticity in evolutionary transitions in individuality (see Box 10.1).

BOX 10.1 SUGGESTIONS FOR FUTURE

- Determine if plasticity contributed to the origin of novel traits that underpin evolutionary transitions in individuality ('ETI'). While we discussed evidence that plasticity was present in several key ETI steps, it remains unclear whether plastic responses were responsible for the origin of novel traits that mediate these steps.
- Determine if plasticity was present in other ETI steps. While we focused on group formation and division of labor in this review, there are several other steps in an ETI that we did not discuss here. Plasticity could be present in other traits that mediate ETIs, including those that promote cooperation and mediate conflict.
- Identify the molecular mechanisms underpinning plastic group formation and cellular differentiation in the volvocine green algae. Are the genes and pathways underlying plastic responses the same ones that are responsible for obligate group development and cellular differentiation in closely related species?
- Determine if plastic cellular differentiation at the cell and group levels are examples of adaptive plasticity. Has plastic cellular differentiation been shaped by adaptive evolution or is it is a by-product of interactions between developmental-genetic processes and temperature stress? Additionally, cell-level and group-level plasticity may be underpinned by different pathways and may have different evolutionary implications.
- Use phylogenetic reconstructions to determine when plastic cellular differentiation arose and compare that origin to the origin of key genes necessary for obligate cellular differentiation. Such studies should include reconstructing the evolutionary history of somatic-like cells and will provide insight into evolutionary processes responsible for the evolution of cellular differentiation.
- Examine plasticity in group formation and cellular differentiation in additional ETIs. Work similar to the volvocine algae case study described here should be carried out in other taxa to determine the extent to which the case study can be generalized.

ACKNOWLEDGMENTS

We thank Aurora Nedelcu, Matthew Herron, De Andre Onyi, Andrew Vizzerra, David Pfennig, and two anonymous reviewers for their helpful comments and suggested changes. We also thank Pierre Durand, Erik Hanschen, and Zach Grochau-Wright for their feedback on and assistance with the figures and tables. This research was supported by NSF grant DEB-2029999.

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11 Phenotypic Plasticity in the Fossil Record

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CONTENTS

DOI: 10.1201/9780429343001-11

11.1 INTRODUCTION

Phenotypic plasticity, the ability of an individual organism to express different phenotypes in response to varying environmental conditions, is ubiquitous across life's diversity. It is also increasingly recognized as fundamental to the survival and adaptive capacity of organisms, as well as forming an integral part of the evolutionary process (West-Eberhard 2003; see also Pfennig 2021 and Sultan 2021 in this volume). We can suppose as a working assumption that what is true today must have also been true for organisms in the past (Chauffe and Nichols 1995). While almost all research on phenotypic plasticity has been based on living organisms, the fossil record has the potential to test models of biological processes with resources not amenable to the biology of the present-day:

- Long time-series to trace phenotypic change and variability on timescales ranging from 10^0 to 10^9 years
- Extinct relatives of living taxa, as models of ancestral phenotype and variation
- Quantification of diversification and extinction in clades through time

Evolutionary hypotheses that could in principle be tested in the fossil record include the origin of adaptive plasticity in heterogeneous environments; the reduction of plasticity in more stable environments; the role of plasticity (including behavioral plasticity) in promoting and guiding evolution and dispersal; and the part played by plasticity in major evolutionary transitions. Addressing such questions, however, makes high demands of the fossil record (Webster 2019). A more-or-less continuous fossil sequence through an adequate period of time would be required to track changes in phenotype and its plasticity, with associated evidence of ancient environments to test for potentially causal or adaptive factors. Ideally, we would also require time-correlated sequences in different areas to account for habitat variation and dispersal. And each time/space unit should be represented by sufficient fossils of the lineage under study to represent variation within the population and allow statistical testing between samples. Addressing these questions in the fossil record is usually not, therefore, a matter of choosing one's preferred taxonomic group and getting to work, but carefully selecting a taxon and geological setting that fulfills, or partly fulfills, the above criteria.

Moreover, testing these ideas presupposes that it is possible to determine how far morphological change was generated by developmental plasticity or genetic differentiation. Often challenging in living species, for fossils it is harder still (Newell, 1947; Dynowski and Nebelsick 2011; Webster 2019). Unlike present-day studies, we cannot undertake field transplants, laboratory manipulation of conditions, breeding experiments, or (except in very rare cases) access the genotype. All we have is morphology, normally restricted to hard parts, and sometimes traces of the organism's activity in life. These challenges, and the solutions that have been proposed to deal with them, will first be surveyed (Section 11.2). We will then explore to what extent fossil data can be brought to bear on the major evolutionary questions where phenotypic plasticity has been implicated (Section 11.3).

11.2 IDENTIFYING PHENOTYPIC PLASTICITY IN THE FOSSIL RECORD

Many paleontological papers, acknowledging that observed variation might be due either to plasticity or to genetic differentiation, explicitly decline to choose between them. Yet a variety of distinguishing criteria have been proposed (e.g., Lister 1992; McKinney and McNamara 1991; Chauffe and Nichols 1995; Schoch 2014; Jackson 2020), and an account of them illustrates the great diversity of fossil examples in which phenotypic plasticity (often referred to as ecophenotypy in the paleontological literature) has been explored, from protists to humans, and from the earliest known fossils up to the historical period (Box 11.1). Note that in many cases several of the factors listed below are taken together to support the identification of phenotypic plasticity.

BOX 11.1 THE GEOLOGICAL TIMESCALE: MYR, MILLIONS OF YEARS AGO

Eon	Era	Period		Epoch	Start Date (Myr)
Phanerozoic	Cenozoic	Quaternary		Holocene	0.012
				Pleistocene	2.6
		Neogene	Tertiary	Pliocene	5.3
				Miocene	23
		Paleogene		Oligocene	34
				Eocene	56
				Paleocene	66
	Mesozoic	Cretaceous			146
		Jurassic			200
		Triassic			251
	Paleozoic	Permian			299
		Carboniferous			359
		Devonian			416
		Silurian			444
		Ordovician			488
		Cambrian			542
Proterozoic	Neoproterozoic	Ediacaran			635
	Mesoproterozoic				
	Paleoproterozoic				2500

11.2.1 Correlation with Paleoenvironmental Variation

Fossil species are often found across a range of locations or time-intervals with evidence of variation in factors such as temperature, salinity, water depth, or predation pressure (Figure 11.1).

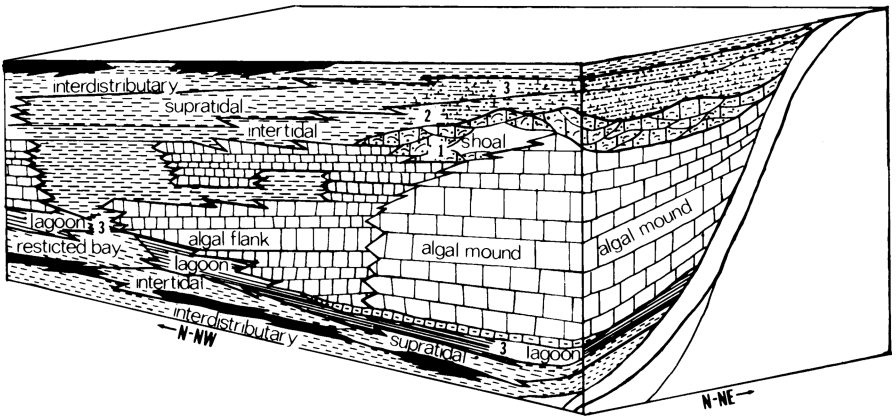

FIGURE 11.1 An example of the reconstruction of paleoenvironmental variation in time and space from rocks deposited in different settings. The block represents an area of approximately 20 × 10 km in north-central Illinois during Pennsylvanian (Upper Carboniferous) times; vertical scale greatly exaggerated. Algal mound: a mat of algae in shallow seas that aggrades limestone; algal flank: side of algal mound; interdistributary: area between the channels of a delta; intertidal: shore between high and low tides; shoal: sandbanks off the coast; supratidal: shore above high tide. (Reprinted with permission from Hickey, D.R., *Journal of Paleontology*, 61, 290–311, 1987.)

These paleoenvironmental factors can be assessed via a variety of proxies, including stable isotopes, the composition of the faunal community, and the nature of the enclosing sediment that reflects aspects such as water flow rate and the nature of the ancient substratum. If across samples with constant environment, the phenotype of the target species remained the same, but with observed environmental differences it varied, phenotypic plasticity is suspected, and in many cases a likely adaptive basis for the correlation can be suggested.

For example, Witts et al. (2020) examined shell shape in Late Cretaceous ammonites (a group of extinct molluscs with coiled shells, related to squid). Tracing the species *Hoploscaphites nicolletii* across its entire spatial and temporal range, the authors found no directional trend but variations in shell shape correlated with those of paleoenvironmental proxies. Thus, the shells were more compressed in areas of faster water flow where this shape is believed to improve hydrodynamic efficiency, and the authors interpret this as adaptive phenotypic plasticity. In a species of Triassic crinoid (sea lilies, Phylum Echinodermata), Dynowski and Nebelsick (2011) found that deeper-water populations had longer feeding arms than shallow-water ones, and suggested a link to lesser predation pressure and slower water (and nutrient) flow in deeper-water environments. The authors pointed out, interestingly, that "echinoderms can modify their internal skeleton throughout their entire life. The modular construction of the skeleton, consisting of distinct plates on which there is ongoing addition and resorption of material, leads to an extensive ability of the echinoderms to reflect the ambient habitats by ecophenotypic [i.e., plastic] adaptations."

Transitions in the time dimension hold particular interest. In a celebrated example, many lineages of marine invertebrates became dwarfed after the end-Permian Mass

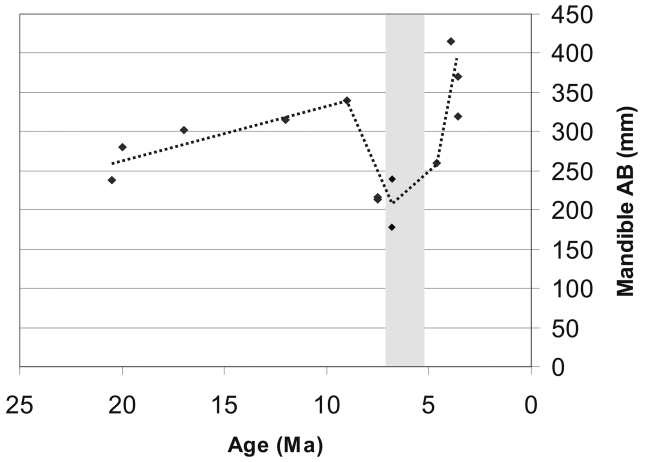

FIGURE 11.2 Variation in mandible length (AB, a measure of body size) in the fossil dugong *Metaxytherium*. The shaded band marks the Late Miocene Messinian Age (7.24–5.33 Ma). (Reprinted with permission from Bianucci, G. et al., *Garyounis Scientific Bulletin*, Special Issue 5, 145–157, 2008.)

Extinction, and authors have argued that this was at least in part a result of phenotypic flexibility, particularly in response to known globally low oxygen conditions (Twitchett 2007). Subsequent size increases coincide with increased oxygenation, ocean circulation, and food supply, that would have promoted more vigorous growth (Foster et al. 2020). In mammals too, nutrition is a key determinant of body size attained during growth, and in a Mediterranean lineage of dugong (sea cow) fossils, reduced body size is seen at exactly the time of the 'Messinian salinity crisis' ca. 7–5 million years ago (Myr), when a reduction in the quality and quantity of the sea-grasses, on which dugongs depend, has been inferred (Bianucci et al. 2008; Figure 11.2). The authors propose a direct nutritional cause of the 'dwarfed' phase that lasted some two million years.

An immediate counter to such examples would be that correlation of morphology to environment, especially if adaptive, might be due to natural selection rather than phenotypic plasticity. Additional evidence, beyond mere correlation with paleoenvironment, is desirable to support the interpretation of fossil variation as induced by environmental, rather than genetic, change.

11.2.1.1 Analogy with Living Relatives

Modern experiments and field observations on the plastic response can aid the interpretation of related fossil forms (Hageman and Todd 2014). If the pattern of differences between fossil samples corresponds to known plasticity in closely related extant taxa, plasticity is plausible in the fossil example, especially if there is correlation with the same environmental factors. For example, oysters from relatively crowded situations tend to be more elongate, irregular, and laterally compressed than their non-crowded counterparts; similar observations in fossil oysters are therefore likely to be plastic in origin (Johnson 1981). Individuals of living marine species in brackish-water conditions are often of reduced body size; in a fossil setting, evidence

for brackish conditions would suggest this cause for observed small individual size. Restricted growth can also be caused by oxygen shortage; this would be suggested by anoxic markers in the rocks enclosing the fossils: fine-grained sediments and the chemical products of sulfur-reducing bacteria (Hallam 1965).

To aid the interpretation of variation in ancient hominins (species closer to humans than to other apes), Anton and Kuzawa (2017) reviewed evidence from contemporary human populations for phenotypic plasticity in traits responsive to variation in climate or nutrition that could be preserved in the fossil record. For example, individuals raised at high altitude have increased lung volume, accommodated by increasing chest dimensions, a pattern potentially visible in fossil remains. In a range of living mammal species, individuals in colder environments tend to have shorter legs, reducing heat loss, and this is at least partly due to phenotypic plasticity; mammals ranging from mice to pigs reared in cold conditions show stunting of appendages and shorter tail lengths (Lister 2004). Analogous variation in fossil species, seen for example in the relatively short limbs of European red deer (*Cervus elaphus*) in the last glaciation, might therefore be a plastic response (Lister 1997).

The criterion of analogy is clearly less applicable to fossil groups with no living relatives. Even where living relatives are available, caution is warranted, since closely related species do not always respond analogously to the same environmental factors. Transplantation experiments in the living coral *Montastrea annularis* showed that variation in numerous characters can be completely explained by environmental factors. However, comparing *M. annularis* with a related Pliocene species *Solenastrea fairbanksi*, Foster (1979) found that the two species responded to different environmental factors, and highly flexible characters in one species are inflexible in the other (Table 11.1).

Further, the same kinds of differences may reflect evolution in one species and environmentally induced change in another (Raup, 1972), likely by modification of the same developmental pathway. In several lineages of coccolithophores (a major component of phytoplankton), variations of form in time and space, that had been

TABLE 11.1
Approximate Correlations between Various Morphological Features and Environmental Factors in Two Species of Scleractinian Corals: Pliocene *Solenastrea fairbanksi* and Living *Montastraea annularis*

	M. annularis	*S. fairbanksi*
Band thickness	(+) light intensity	(+) nutrient supply
Endotheca spacing	(+) light intensity	(+) light intensity
Corallite diameter	(+) light, nutrients	inflexible
Theca or exotheca thickness	(−) nutrient supply	(+) light intensity
Septum thickness	(+) water energy	(+) light intensity
Near neighbor distance	(−) light intensity	(+) water energy
Coenosteal void shape	(+) water energy	(+) water energy
Columella thickness/diameter	(−) nutrient supply	(−) nutrient supply

Source: Data from Foster, A.B., *Lethaia* 12, 245–264, 1979.

proposed as phenotypic plasticity within single species, have been shown in the living representatives to be invariant under experimentally varied conditions, and/or to be genetically distinct, thus identifying them as separate species (Geisen et al. 2004).

These considerations apply equally to plants. Barclay et al. (2007) and Royer et al. (2009) summarize phenotypically plastic features in modern leaves with potential use as paleoenvironmental indicators in fossils. These include the dissection of leaves, inversely correlated to temperature; the relative thickness of different cell layers that reflects light intensity; papillae that enhance photosynthesis in deeply shaded habitats; and cuticle thickness as an indicator of aridity. The stomatal density of leaves, in particular, is known to be phenotypically plastic and has been extensively employed in fossil plants to estimate past CO_2 levels; the greater the density, the lower the CO_2 (Chaloner 1994; Cristophel and Gordon 2004; Figure 11.3). However, the relationship in modern plants is often species-specific, so accurate estimation of paleo-CO_2 concentration relies on an ability to assign ancient leaves to modern families, genera, or ideally species, not always possible with fossil material.

11.2.1.2 The Pattern of Variation

In living species, the relationship between an environmental variable and the resultant phenotype of a developmentally plastic character is known as its reaction norm (Sultan 2021). In fossils, a broad reaction norm has been inferred in cases of high level of intraspecific variability, especially in a variable environment (e.g., Hughes 1991 on trilobites; Taylor 2005 on bryozoans; Anton and Kuzawa 2017 on hominins).

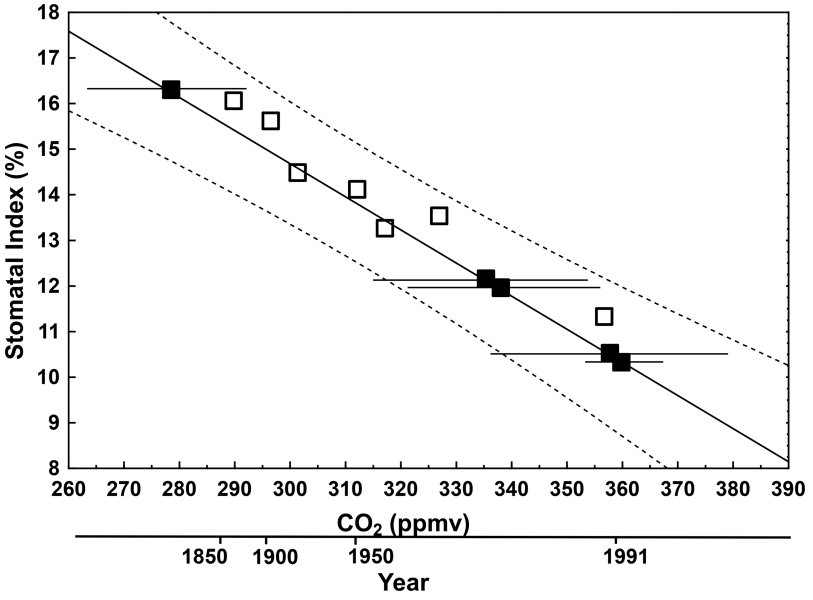

FIGURE 11.3 Stomatal index (density of stomata) in leaves of the oak *Quercus petraea* in relation to atmospheric CO_2. White squares, historical samples since 1850; black squares, fossil samples spanning 10–2 Myr, plotted on modern line to estimate ancient CO_2 concentrations. (Adapted from Van der Burgh, J. et al., *Science*, 260, 1788–1790, 1993.)

In a stable environment, conversely, these same species should show uniformity as only a small part of the reaction norm would be expressed. Continuous rather than discrete variation is also sometimes taken as a signal of plasticity. In the Cretaceous ammonite *Schloenbachia varians*, Wilmsen and Mosavinia (2011) found that shell form varied with water depth in a continuously covarying fashion, supporting a phenotypically plastic effect (Figure 11.4).

Caution again attaches to this criterion taken alone, as genetic variation (especially for polygenic characters) can also produce continuous variation, and this can be expressed clinally. Moreover, phenotypic plasticity may be expressed as discontinuous variation producing discrete phenotypes (Pfennig 2021). The possibility that observed variation across conspecific samples reflects genetic differentiation is minimized if samples are both contemporaneous and geographically close. At a given stratigraphic level (i.e., time period), past environmental gradients can often be traced over very short distances in rock exposures, tracking, for example, deeper and shallower parts of a former lake, or nearshore to offshore marine environments (Figure 11.1). In some cases, different paleoenvironments may form a local patchwork. In this case, correlation of intraspecific morphology with environment is likely

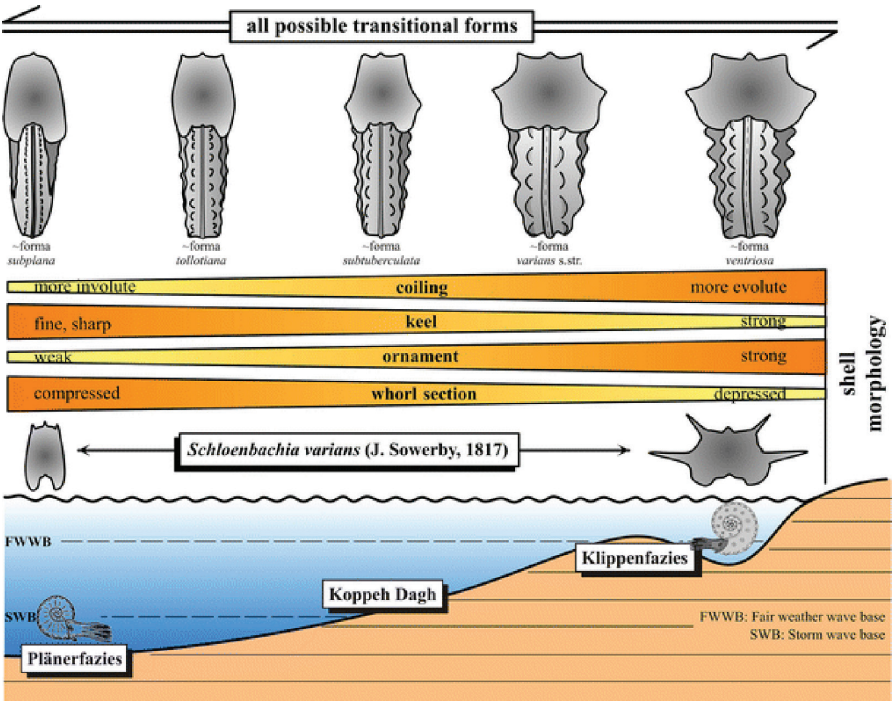

FIGURE 11.4 Schematic representation of clines in the proportion of shell morphs within contemporaneous samples of the Cretaceous ammonite *Schloenbachia varians* across different paleoenvironments. (Reproduced with permission from Wilmsen, M., and Mosavinia, A., *Paläontologische Zeitschrift,* 85, 169–184, 2011.)

due to phenotypic plasticity, since the population as a whole can be inferred to have been interbreeding so local genetic differentiation is unlikely. Distinct morphs that are truly sympatric on the other hand, with no evidence of environmental partitioning, are likely to represent dimorphism or separate species (Geary, 1992).

11.2.1.3 Parallel Change across Species

The simultaneous and similar morphological response of an array of fossil species, particularly when mirroring known common reaction norms in living taxa, has been taken to suggest phenotypic plasticity, especially where the species co-exist (Chauffe and Nichols 1995; Schluter 2016). For example, the increase in size of bivalves in the early Triassic, in combination with evidence for increased oxygen and food availability (see above), led Foster et al. (2020) to suspect phenotypic plasticity because it occurred in parallel across multiple species. Another widely discussed generalization is the 'temperature-size rule': the finding that 80% of living ectothermic animal species grow to larger size in colder temperatures (Atkinson 1994). Mechanistic explanations have been elusive but life-history modeling suggests that most ectotherms mature at a larger adult size at lower temperatures because of shifts in relative growth rate at different developmental stages (Walters and Hassall 2006). In the fossil record, Hunt and Roy (2006) showed that body size in marine ostracods (tiny crustaceans enclosed in an ovoid shell) of the genus *Poseidonamicus*, spanning 40 million years of the Cenozoic, was inversely correlated to proxies for ocean temperature. The trends occurred in parallel across species of the genus so that plasticity within similar reaction norms likely contributed to the trends. In bryozoans (sea-mats, a phylum of colonial animals composed of individual zooids), an inverse correlation between water temperature and zooid size in many living species, determined as a phenotypically plastic response, has been utilized in estimating paleotemperatures from multispecies fossil assemblages that show parallel change (Okamura and Bishop 1988).

The degree of parallelism (or divergence) may vary across characters. Thus Sheldon (1987), studying 15,000 trilobites spanning 3 Myr of the Ordovician in Wales, found that eight lineages increased the number of tail ribs, but that times of increase were uncorrelated across taxa, implying selection, whereas fluctuations in body size correlated across taxa and were considered likely phenotypic flexibility.

11.2.2 Ontogeny and Growth

11.2.2.1 Magnitude of Change

Authors have assumed that the more profound, complex, or unique a modification, the less likely it is to have arisen by environmental effects on a single genotype, and the more likely to be the result of genetic change (Lister 1992; Anton and Kuzawa 2017). In the celebrated case of the rapid morphological transitions seen in Cenozoic molluscs from Lake Turkana, Kenya, various authors had invoked 'ecophenotypic' (plastic) change, but Williamson (1982) argued for species-level distinctions since "the magnitude of the changes documented in both bivalves and gastropods was generally far greater than that observed in the ecophenotypic transformations of even the most plastic of modern African freshwater molluscs." On this point, he was proven correct although the replacements turned out to be by invasion rather than intra-lineage

evolution (Van Bocxlaer et al. 2008). Further examples are given for bryozoans (Budd et al. 1994) and deer (Lister 1995) below. This criterion cannot demonstrate plasticity but may suggest its exclusion. Note, however, that several cases of profound morphological alteration are known to have resulted from developmental plasticity (West-Eberhard 2003).

11.2.2.2 Relative Growth

Chauffe and Nichols (1995) suggested that if a single element of a complex structure changes, genetic change is probably involved. Environmental effects on development act more broadly; for example, rickets in humans (due to nutritional deficiency) affects many bones of the skeleton. Along similar lines Dececchi et al. (2018), assessing whether related Ediacaran fossils were separate species or 'ecophenotypic' variants, assumed that plasticity would affect developmentally correlated characters, and that individuals differing in a series of uncorrelated characters do so from genetic differentiation. From an ontogenetic perspective Schoch (2014), considering the growth of early fossil amphibians, suggested that in cases of simple truncation or extension of the ontogenetic trajectory, without changes in the sequence of developmental events, plasticity forms as likely an explanation as evolution. If the structure of the trajectory had altered, however, or events producing substantial morphological changes had been added or omitted, genetic innovation was more likely.

By analogy with living representatives, some body parts are expected to be more canalized than others. In mammals, the size and shape of teeth are fixed prior to their eruption, whereas the bony skeleton continues to grow later into the individual's life. The effect of factors such as nutrition, therefore, is most pronounced in the skeleton, whereas variance in tooth size more closely reflects genetic differences (Caumul and Polly 2005). Marshall and Corrucini (1978) suggested that environmentally determined dwarfing could be identified in this way: the animals should retain relatively large teeth for their small skeletons. Many fossil mammals that became dwarfed on islands show this condition, although selective change cannot be ruled out; small breeds of domestic dog, for example, have relatively large teeth (Lister 1995; Lister and Hall 2014).

11.2.2.3 Growth Influenced by Substrate

Modification of growth is sometimes clearly driven by environmental factors. Schrøder et al. (2018) studied the Paleocene brachiopod *Obliquorhynchia flustracea*, whose larvae settled between the branches of the coral *Dendrophyllia candelabrum*. Brachiopods are a phylum of marine invertebrates with a laterally-symmetrical double-valved shell, but in *O. flustracea* many adult shells exhibit an asymmetry that the authors attribute to phenotypic plasticity rather than genetic determination because: (1) the asymmetry can be seen to develop with ontogeny (see Section 11.2.5); and (2) left-twisted and right-twisted morphologies occur in equal numbers (assumed to depend randomly on the constraints of the space and the side the larva settled on). Taylor and Schindler (2004) described fossil bryozoans symbiotic with hermit-crabs, that constructed a gently curved tube extending for up to 50 mm outward from the aperture of the gastropod shell that was the crab's home. The varying shape of the tube is concluded to be a plastic response to the shape of the crab's body and the curved path of its movement in and out of the shell.

11.2.3 Behavioral Traces

Behavioral flexibility is a 'special case' of phenotypic plasticity as it can be enabled either by pre-programmed alternative behaviors (in most invertebrates) or by 'cognitive intelligence' (in higher vertebrates especially) although the distinction is not absolute and some form of 'learning' is almost universal (Wright et al. 2010; Plotnick 2012; see also Chenard and Duckworth 2021 in this volume). In either case, however, flexible behavior to a given end (e.g., two methods of feeding) falls under the definition of phenotypic flexibility if it is performed by a single genetic individual. Across individuals or populations, the same issues that affect morphological characters also apply to behavior: is observed variation due to genetic differentiation or phenotypic plasticity? Here the difference between 'programmed' and cognitive flexibility (e.g., switching food plant choice in an insect versus in an elephant) becomes significant. In the case of the insect, it would be difficult in the fossil record to know if we are looking at two fixed behaviors in different individuals, or two parts of a common behavioral reaction norm. In the case of a fossil elephant, we may assume individual choice because of the known cognitive flexibility of the family; even if the individuals differed genetically in cognitive ability it would likely not specify particular food species. We are here relying on analogies with living representatives to guide our judgment, as for morphological characters.

Behavioral data comes from traces such as tooth wear or preserved stable isotopes as indicators of feeding preference; skeletal modifications due to use or disuse of body parts; traces of locomotion left on the substrate (if they can be reliably related to the animal that left them); or 'extended phenotype' like burrows and nests (ditto). Examples of behavioral flexibility in fossil populations are numerous and varied (Boucot and Poinar, 2010; Lister, 2014). For instance, feeding switches in sticklebacks, from mid-water to the lake bottom, were identified from tooth-wear and interpreted as likely flexible behavior on the basis of analogous variation in modern populations (Purnell et al. 2007). An unusual example of cognitive flexibility in a mammal was described by Figueirido et al. (2017) who observed dental caries in the population of Pleistocene short-faced bears (*Arctodus simus*) from Rancho La Brea (California), but not in populations from the north-western USA. They deduced a more omnivorous diet (including sugar-rich fruits) in the La Brea sample, based on the observation that in living bears caries are seen in herbivorous and omnivorous species but not in the largely carnivorous polar bear.

Behavior can exert a direct influence on morphology, a form of 'phenotypic accommodation' (West-Eberhard 2003, pp. 51–54). Many studies report alterations in skeletal morphology or bone density as a result of exercise—for example, left-right asymmetry in humerus dimensions in modern tennis players, and Trinkaus et al. (1994) reported greater robusticity in the right humeri of Neanderthals, attributed to strenuous, right-dominant activity.

11.2.4 Speed and Reversibility of Change

Since developmental plasticity can modify phenotype within a generation and can revert to the original form with equal speed if the environment reverses, very rapid and/or reversible change has been seen as a signal of plasticity, whereas genetic change is expected to take longer. However, the imperfect resolution of the fossil

record, especially in the light of known rapid evolutionary changes, militates against this simple 'rule of thumb.' Even the shallowest unit of rock from which we collect fossils is likely to represent a certain interval of time—hundreds, thousands, or even tens of thousands of years, and may also have incorporated dead animals washed in from a catchment including several habitat types (Huntley et al. 2018). Resolving the difference between a process that takes one or a few years (plastic response) from one that might take thousands of years or more (evolutionary response) is therefore difficult (Schoch 2014). The rock record also frequently incorporates gaps in deposition, sometimes invisible in the field, that can make a gradual process appear sudden.

In some situations, the fossil record can be resolved on an annual basis. These are layered sediments deposited at the bottom of lakes or on the seafloor, each layer being a visible record of one year's sedimentation (Schimmelmann et al. 2016), so year-by-year analysis of morphological variation is theoretically possible (Simola 2013). In a study of stickleback fish (*Gasterosteus*) fossils through an annually resolved sequence from the Miocene of Nevada, Bell et al. (2006) and Purnell et al. (2007) demonstrated an increase in body armor through roughly 150 years, fast but not instantaneous, and the authors concluded that selection was at work. Moreover, studies of modern sticklebacks demonstrate that a transformation of this rapidity is plausible; a newly colonizing lake population showed significant phenotypic change within only 20 years (Aguirre and Bell, 2012), and a notable increase or reduction in body armor can be achieved through mutation of a single control gene (Chan et al., 2010). Hence rapidity of change alone does not uniquely identify phenotypic plasticity in the fossil record; other lines of evidence must be invoked.

Over longer time-periods, fossil morphology constantly fluctuating around a mean has been interpreted as environmentally-induced plasticity. For example, in the Middle Devonian bivalve (clam) *Actinopteria boydi*, Nagel-Myers et al. (2018) found reversible changes of morphology with no overall trend though 3–4 Myr, and therefore interpreted the fluctuations as plastic in origin. Even here, genetic effects cannot be ruled out—reversals in allelic frequencies of a gene under selection could occur in a fluctuating environment. Selection is very likely in cases of long-term directional change (Anton and Kuzawa 2017). Some of the best examples come from microfossils preserved in deep-sea cores, where large samples may be available from finely spaced horizons. In the Eocene planktonic foraminifer *Turborotalia cerroazulensis* (Protista), gradual transformation of shell shape was observed across 51 horizons spanning some 10 Myr and was interpreted as an evolutionary rather than plastic transformation (Pearson and Ezard 2014).

11.2.5 Variation within a Single Genetic Individual

One way of excluding genetic differentiation is to examine variation within a single genetic individual. Any observed morphological or behavioral change is likely to have been a plastic response to the environment.

11.2.5.1 Variation of Growth Rate

Where stages in the ontogeny of an individual are visible in the adult, growth trajectories can be plotted. This can be achieved by following growth rings, or more generally mapping the direction of growth, in shells, bones, or the branches of organisms

such as corals or plants. Studying the Cretaceous bivalve *Actinoceramus sulcatus*, for example, Crampton and Gale (2005) found that shell shape through ontogeny did not just change according to a gradual allometric trajectory, but that intermittent switching of growth pattern had occurred, assumed to be driven by external influences during growth. Wang et al. (2004) found greatly varying growth form in the calcareous skeleton of the Carboniferous coral *Commutia exolete*, due to redirection that kept the oral surfaces of the polyps upward during growth in a muddy substratum.

The ability of an individual to alter its *rate* of growth through ontogeny, in response to more or less favorable environmental conditions, is a form of phenotypic plasticity. Sanchez and Schoch (2013) compared bone histology across individuals of the Triassic amphibian *Gerrothorax pulcherrimus*, finding great variability of growth rate as well as in age at maturity and life-span, the variation appearing higher in fluctuating lake environments than in stable ones.

Growth variation can be periodic, suggesting cyclicity in environmental drivers. In the Carboniferous bryozoan *Rhombopora blakei*, Hageman et al. (2011) identified nested growth cycles that were annual (e.g., monsoon driven), subannual (e.g., periodic storm events), and biweekly (lunar tidal cycles). Indications of seasonal climate changes can also be seen in growth bands of shells, where summer and winter bands can be identified and their relative widths evaluated. Good (2004) analyzed growth bands in five bivalve species through the Late Jurassic of central North America and identified periods with minimal seasonal variation (no visible growth bands) and others with annual climatic cycles (regular growth bands).

11.2.5.2 Bilateral Asymmetry

Morphological variation between the left and right sides of a nominally bilaterally symmetrical organism (also known as fluctuating asymmetry) is considered as an index of developmental canalization (Tucić et al. 2018). It has the inbuilt control that both sides of the organism share the same genotype and generally developed in the same environment (Webster and Zelditch 2008). Hence fluctuating asymmetry is not an index of phenotypic plasticity per se, but a less canalized developmental program might be more likely to also exhibit environmentally controlled plasticity (Tucić et al. 2018). Polly et al. (2011) used left-right asymmetry in molar shape of vole species as a measure of the environmental (non-genetic) component of variance within species. They found that environmental variance accounted for about 10%–30% of within-species variance, suggesting heritabilities of 0.71–0.89 for molar tooth shape and implying that inter-population differences were likely largely genetically determined. This approach has been considered a major potential area of research into phenotypic plasticity in the fossil record (Webster 2019).

11.2.5.3 Variation within Colonial Organisms

Organisms such as corals and bryozoans form colonies of, respectively, polyps (housed in corallites) and zooids (in zooecia). These provide traction for identifying environmental effects on development, as all individuals of the colony share the same genotype (Hageman 1995). As stated by Budd et al. (1994), "morphologic differences among corallites built by genetically identical polyps at different positions within a colony can be used as a preliminary first step to estimate the magnitude of overall ecophenotypic plasticity within species and to identify the characters that vary" (Figure 11.5).

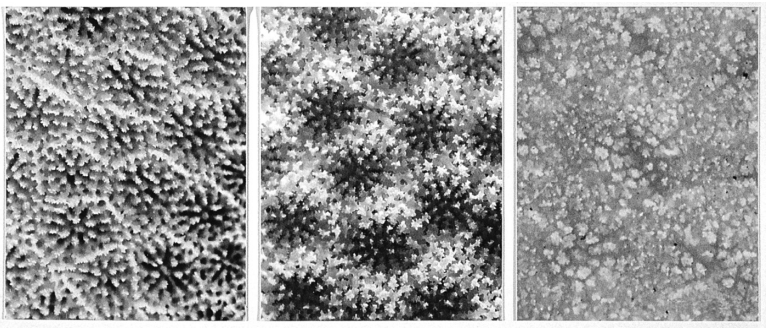

FIGURE 11.5 Variation within a single colony of the coral *Porites lutea*. Corallites (pouches in the limestone skeleton) are built by genetically identical polyps, revealing developmental plasticity. (Reproduced from Veron, J., *Zoological Journal of the Linnean Society,* 169, 485–508, 2013, under license CC BY 2.5.)

Thus, Hageman et al. (1999) placed living bryozoan colonies in different experimental conditions and found the form of the colony as a whole, and the size of individual zooids, to be strongly environmentally influenced, while the detailed anatomy of the zooids was less so, and utilized this in interpreting variation in fossil bryozoans.

11.2.5.4 Behavioral Variation of a Single Individual

Trace fossils can sometimes give clues to individual behavioral flexibility (Plotnick 2012). For example, in the Permo-Triassic of Antarctica, four types of trace had been named, but Miller (2003) showed that many individual traces change from one type to another, suggesting that they all represent the same taxon and that its behavior was flexible. Moreover, by analogy with living species, the forms of the traces suggested switching between feeding modes (suspension versus deposit feeding). In Miocene shrimp burrows studied by Miller and Curran (2001), the thickness and definition of burrow walls changed markedly within a single burrow, depending on variation in substrate, and the authors interpreted this as "behavioral plasticity intrinsic to the tracemaking organism."

11.2.6 Direct Genetic Evidence

The extraction and sequencing of DNA from fossil material has provided insights into population history and the evolution of functional genes, and more recently of epigenetic and regulatory factors (Linderholm 2015). This field holds a barely tapped potential for exploring evolutionary patterns in the fossil record, including those involving phenotypic plasticity, as both phenotype and genotype are potentially available for the same individuals. Its limits are molecular preservation in only a fraction of fossil material, determined by climatic and sedimentary context, and a shallow time-depth usually to 10^4–10^5 years, exceptionally to 10^6 years (Van der Valk et al. 2021). In European red deer (*Cervus elaphus*), size variation across the climatic cycles of the last glaciation (ca. 50–10 kyr [thousands of years ago]) was suggested to be driven by phenotypic plasticity and no correlation with genetic variation in

mitochondrial DNA was found (Meiri et al. 2018). A few remains from SE Europe, however, morphologically indistinguishable from red deer but of exceptional size exceeding the bounds of the other samples, were genetically highly divergent, mapping to the related, large Asian species *C. canadensis.*

11.2.7 Summary

Traditional methods of identifying plastic responses in the fossil record warrant caution: the speed and reversibility of plastic change can be mimicked by rapid genetic change, as can correlation of morphology with paleoenvironment. Analogies with living relatives are helpful but may be misleading if related species show different plastic responses. In combination, these factors may be suggestive of plasticity, for example in a case of rapid, parallel response across related species in concert with relevant environmental change, or spatial variation correlating with microenvironments in a single population. More promising for future research is the analysis of variation within single genetic individuals—tracking ontogenetic fluctuations, left-right asymmetry, or variation across a colony (Box 11.2). In the longer term, ancient DNA promises direct insights into the genetic architecture underlying the development of observed phenotypes.

11.3 PHENOTYPIC PLASTICITY AND EVOLUTION

Some key hypotheses for the significance of phenotypic plasticity in providing adaptation, and for impacting the process of evolution, are outlined below along with relevant fossil evidence. Insofar as these hypotheses can be tested in the fossil record, we need first to outline the predicted pattern in time and space that would corroborate each one. Figures 11.6–11.8 and 11.10–11.12 present schematic patterns of variation through time under different models. Black lines indicate some fluctuating environmental parameter(s). A, B, and C are expressed phenotypes of a species; if part of a common reaction norm they are marked $\tilde{A}$, $\tilde{B}$, and $\tilde{C}$. Morphological characters are enclosed in squares, behavioral ones in circles. Pentagons indicate novel morphology or a new species. The various processes are not mutually exclusive (for other summaries of potential mechanisms, see Pigliucci 2001; West-Eberhard 2003; Uller et al. 2019; Pfennig 2021).

11.3.1 Production and Maintenance of Adaptive Plasticity

Environmental variability in time and/or space is considered a key promoter and maintainer of phenotypic plasticity (Snell-Rood and Ehlman 2021 in this volume). The idea of selection by and for varying conditions in the fossil record was developed by Potts (1998), who coined the term ‘variability selection.’ His primary focus was the evolution of the human brain and its capacity for innovation and problem-solving, an adaptation permissive of behavioral flexibility rather than adaptively plastic in itself. However, the principle that environmental variability selects for individuals capable of flourishing in a range of conditions applies also to the origin of phenotypic plasticity (Scheiner et al. 2019; Figure 11.6).

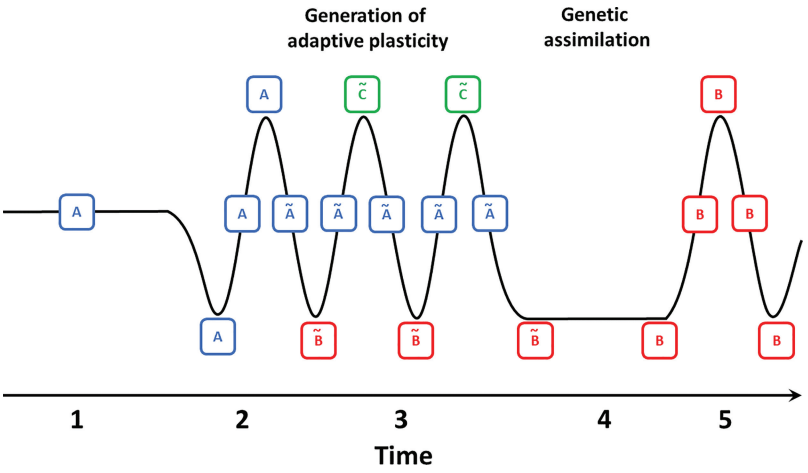

FIGURE 11.6 Generation of phenotypic plasticity followed by genetic assimilation. At time (1) the species begins with a single phenotype in a constant environment; (2) the environment begins to fluctuate but at first the phenotype remains fixed; (3) a range of adaptive phenotypes evolves; they are parts of a reaction norm Ã–C̃; (4) the environment becomes stable in the state where B is favored, so the 'B' phenotype becomes canalized; (5) as a result, only 'B' is expressed even if the environment begins to fluctuate again (stages 1–3 might begin again). Note that the variation during interval (3) could be expressed spatially as well as temporally.

In discussing the evolution of cognitive faculties in advanced hominins, Potts (1998) suggested the increase in amplitude of Pleistocene climatic cyclicity after *ca.* 750 kyr as the key stimulus. These climatic fluctuations were also invoked by Lister (1984, 2014) who suggested that the vegetational shifts to which mainland red deer was subjected, varying between cool grassland, savanna, and temperate woodland, selected for a genotype capable of adaptive developmental modulation. Hence, their living descendants show adaptive plasticity in their feeding adaptations both behaviorally and in their digestive anatomy (Hofmann 1983). By contrast, other species of deer, moose (*Alces alces*), and roe (*Capreolus capreolus*), which have more restricted ecological ranges today, tracked woodland habitats in the Pleistocene and are physiologically incapable of consuming large quantities of grass.

Highly variable environmental conditions often characterize small islands. In the dwarf goat *Myotragus* from the Pleistocene of the Balearic Islands (Spain), Köhler and Moyà-Solà (2009) found limb-bone histology indicating slow and flexible growth rates unlike that of any other mammal, and concluded that developmental and physiological plasticity had been crucial to its survival.

Comparisons across related taxa in the same stratigraphic context indicate different levels of evolved plasticity. Schneider et al. (2010) compared three bivalve genera from a Jurassic sequence in Portugal. In *Arcomylitus morrisii*, there is variation in shell shape between soft-bottom and sandy environments that may have been adaptive to differing modes of attachment to the substrate. In *Eomiodon securiformis* and two *Isognomon* species, by contrast, there was no correlation between shell shape and environmental conditions, implying that an adaptive reaction norm had not evolved in these forms.

11.3.2 Plastic Variation, Selection, and Assimilation

With the return to a more stable environment, phenotypic plasticity may be reduced by selection for an optimal phenotype, potentially leading to canalization through genetic assimilation (Scheiner and Levis 2021 in this volume; Figure 11.6). Modelling suggests that this process could take 10^4 generations (Chevin and Lande 2010) or even 10^5 generations (Scheiner et al. 2019)—implying long-term trends potentially traceable in many fossil sequences.

Several authors have suggested genetic assimilation to account for observed patterns of speciation in the fossil record. In Carboniferous crinoids studied by Holterhoff (1992), the species *Delocrinus subhemisphericus* displays significant phenotypic plasticity between habitats: smaller size in offshore populations, larger in nearshore ones. The author suggests that offshore, quiet bottom waters inhibited effective filtration, imposing a limit to viable size, while enhanced nutrition in nearshore populations produced larger body sizes. In later horizons, the ancestral *D. subhemisphericus* persisted in offshore areas but robust new species including *D. vulgatus* are found in nearshore areas. The author concludes: "Speciation may have involved the stabilization and subsequent diversification of the earlier nearshore phenotype."

In birds, a flexible rate of bone growth, with 'lines of arrested growth' formed during unfavorable intervals and visible in bone cross-sections, was primitive to the fossil stem group but has been lost in all living birds, possibly in parallel between different clades. Starck and Chinsamy (2002) suggest that reduced developmental plasticity may have been selectively favored in connection with the shortened development time of more derived birds, including all those now living.

While these examples assume that the initial plastic variation was adaptive, this is not a prerequisite for its further evolutionary potential. Species accumulate cryptic genetic variation in unexpressed parts of their reaction norm, and a subsequent environmental change beyond previous fluctuating boundaries can induce an array of novel, randomly adaptive phenotypes that provide raw material for selection, a process that has been termed 'plasticity-led evolution' (Levis and Pfennig 2016; see also Levis and Pfennig 2021 in this volume). The observation of a shift in mean phenotype following environmental change in the fossil record does not uniquely identify this process, but Jackson (2020) proposed a test for plasticity-led evolution: increased phenotypic *variability* in a population encountering a new environment, followed by reduction in variability as the environment and phenotype stabilise (Figure 11.7). In a fossil setting, excellent stratigraphic control would be required to convincingly demonstrate this pattern, as increased variability might result from time-averaging of fossils undergoing rapid phyletic change, or the immigration of a related form and its co-existence with the incumbent (Lister et al. 2005).

11.3.3 Guiding the Direction of Evolution

Because of its speed of reaction, phenotypic plasticity will often be the first response of species confronted with an environmental challenge, enabling survival while the slower process of selection takes effect, an effect known as 'buying time' (Fox et al. 2019; see also Diamond and Martin 2021 in this volume; Figure 11.8). Moreover,

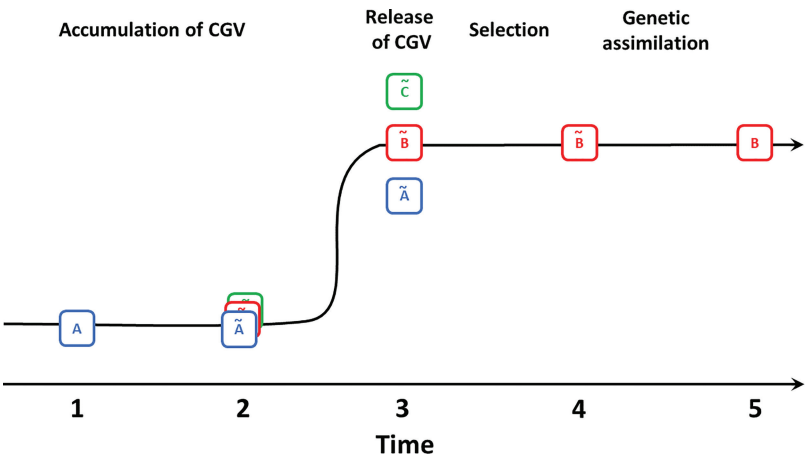

FIGURE 11.7 The 'Plasticity-Led Evolution' model. (1) Single canalized phenotype; (2) cryptic genetic variance (CGV) has accumulated, i.e., (hidden) reaction norms; (3) environment changes, development is altered, CGV is released, revealing an array of unselected phenotypes and reaction norms (e.g. Ã—C̃); (4) selection among genotypes; here, a reaction norm with B̃ expressed is favored; (5) new environment stabilizes, genetic assimilation narrows reaction norm to B. Assimilated forms of A, B, or C could seed new species as in Figure 11.10. Compare Jackson (2019, Figure 4).

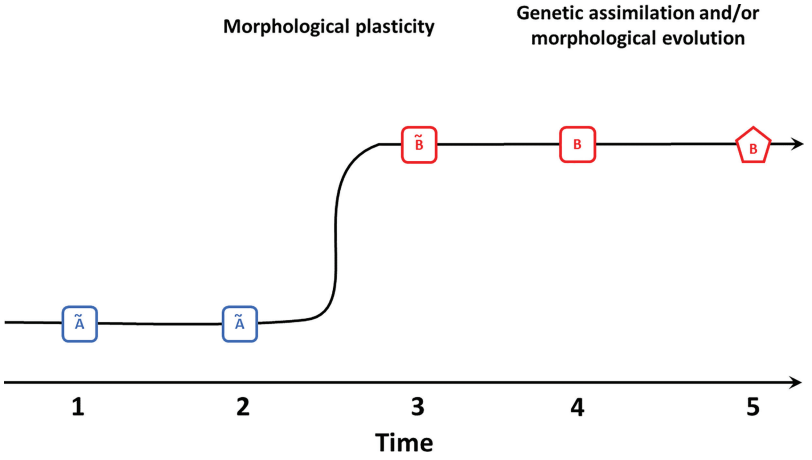

FIGURE 11.8 Guiding evolution with plastic morphological lead: (1)–(2) Ã, starting morphology, part of an adaptive reaction norm; (3) B̃, another part of the reaction norm is induced by, and allows survival in, new environment ('buying time'); (4)–(5) natural selection further adapts morphology B to fit new environment, possibly with genetic assimilation.

the mode of accommodation may influence the nature of subsequent morphological adaptation (Baldwin 1902). Observing this process in the fossil record is tricky because the transition from plasticity to genetic control as the developmental basis for observed morphological change is difficult to identify in most cases.

In a study of early multicellular animals known as rangeomorphs, Hoyal Cuthill and Conway Morris (2017) employed a modelling approach to suggest that phenotypic plasticity may have enabled the evolution of large body size in this group. Reaching up to 2 m in length, rangeomorphs from 570 Myr ago show a size increase of one to two orders of magnitude in comparison with earlier representatives. The authors assumed that growth in the frond-like animal was correlated to nutrient uptake and that this would be proportionately higher in the fine terminal branches because of their high surface area to volume ratio (Figure 11.9). This produced a positive feedback loop such that growth was always greater at the leading edge than the base, so that under optimal nutrient conditions size could increase dramatically. The plastic growth program thus provided a developmental mechanism that led to the appearance of large-bodied species at a time of increased regional nutrient supply.

In the opposite direction, phenotypic plasticity in response to food limitation may have initiated the dwarfing of mammals on islands in the Pleistocene, a process that occurred multiple times across taxa and was clearly adaptive. For example, translocation experiments with living red deer indicate plasticity in adult male body mass of ca. 90–250 kg, but Pleistocene red deer on the island of Jersey in the English Channel dwarfed to ca. 36 kg (Lister 1995). Since island dwarfs also frequently show specialized locomotory and feeding adaptations to their habitat, Lister (1996) proposed a staged process: initial very rapid size reduction through phenotypic plasticity, genetic assimilation and further reduction in size, and a longer period of selected change to fine-tune adaptation.

The 'flexible stem' hypothesis was proposed by West-Eberhard (2003, Ch. 28) and explored by many authors (Wund et al. 2008; Pfennig et al. 2010; Levis and Pfennig 2021), including some paleontologists (Figure 11.10). According to this model, the reaction norm of a phenotypically flexible species could provide the morphological basis for descendent species, including the repeated origin of similar morphologies—parallel evolution.

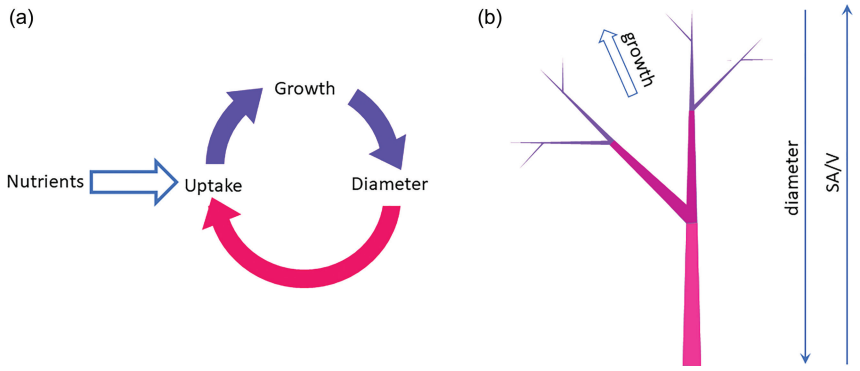

FIGURE 11.9 Computer simulation of rangeomorph growth. (a) Model of feedback between branch diameter, nutrient uptake, and growth. Arrows indicate positive (blue) versus negative (red) effects. (b) Proposed nutrient-dependent growth. Colors indicate the relative proportion of volume by which segments will grow at the next step (blue, high; red, low). SA/V, surface area/volume. (Adapted from Hoyal Cuthill, J.F, and Conway Morris, S., *Nature Ecology and Evolution* 1, 1201–1204, 2017.)

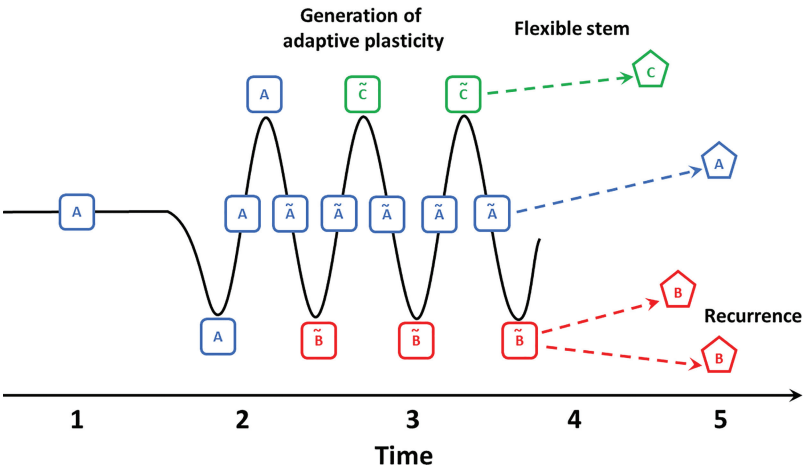

FIGURE 11.10 The 'Flexible stem' scenario: (1)–(3) as in Figure 11.6; (4) populations-bearing phenotypes Ã, B̃, and C̃ become isolated in, or move to, regions where that morphology is expressed or favored, and (5) may evolve into new taxa A, B, and C, their respective phenotypes possibly becoming fixed by genetic assimilation. Similar daughter taxa (e.g. B) may arise by recurrence (see text).

For example, living amphibians show substantial plasticity in the timing of metamorphosis (the transformation of an aquatic larva into a terrestrial adult), dependent on environmental factors. In Schoch's (2014) study of the Paleozoic to Mesozoic amphibian stem-group, similarly broad reaction norms were identified in many species, and changes in ontogenetic timing produced more strictly aquatic and more terrestrialized forms, that speciated in different environmental contexts. Moreover, phylogenetic analysis indicates that this happened multiple times, an example of parallel evolution likely due to descent from a plastic ancestor, termed 'recurrence' by West-Eberhard (2003).

Phenotypic flexibility should thus favor the exploration of, and survival in, new environments, as well as subsequent adaptation and speciation (West-Eberhard 2003; Sol 2007; Grove 2015; Levis and Pfennig 2021; Figure 11.10). Anton and Kuzawa (2017) outlined criteria for identifying this phenomenon in fossil hominins: the successful disperser should be quite plastic itself, more so than related species, and successive dispersers into similar environments should show parallel phenotypic responses. Grove (2015) developed a modelling approach, predicting that the most favorable situation for successful dispersal was a relatively calm interval following a period of significant environmental perturbation that had selected for enhanced plasticity (see Section 11.3.1). The model was tested on the dispersal of *Homo sapiens* out of Africa, using paleoclimatic proxies from an East African lake core spanning the last 250 kyr as the environmental signal. The model identified the interval c. 105–97 ka as the optimum for a significant dispersal event, corresponding very well with the earliest appearance of *H. sapiens* in the Middle East at ca. 100 ka.

Has the prevalence of plasticity changed through geological time, and if so, how may this have impacted life's diversification? It has been suggested that the

'Cambrian explosion' was facilitated by the developmental plasticity of early metazoans (McNamara 1983). In corollary, the decline in origination rate of new metazoan body plans following the early Cambrian might reflect developmental canalization in derived taxa, limiting their ability to evolve forms with radically different body plans. Evidence on this question has come primarily from studies of trilobites (dominant marine arthropods of the Paleozoic) where it has elicited much discussion. Early Cambrian trilobites often display high levels of intraspecific variability (for example, in the number of thoracic segments) that has been interpreted as due to weakly canalized growth, but during the Cambrian there was a trend toward increased regulation of ontogeny (McNamara 1983; Webster 2015). A similar pattern has been suggested for other groups of animals, but whether such 'developmental constraints' would actually inhibit the origination of new body plans is debated (Hughes et al. 1999).

11.3.4 Behavioral Plasticity

Behavioral accommodation is likely to be a key factor allowing dispersal into, and survival in, a new habitat. Wright et al. (2010) developed an 'adaptive flexibility hypothesis' for cognitively advanced species whereby a population entering a new habitat would show an increase in behavioral diversity as individuals apply innovative behavioral strategies. Learning by individuals and copying by others will lead to a reduction in behavioral diversity as optimal strategies are achieved. Lister (2013) used stable isotope analysis in African fossil elephants to identify a behavioral switch from consumption of trees and shrubs (browsing) to grasses (grazing) around 8 Myr ago. The switch coincided with the spread of grassland, and the earliest stages were marked by increased variation in feeding strategy within several taxa.

Moreover, behavioral change has long been considered a key initiator of evolutionary innovation (Baldwin 1902, Hardy 1965, Vane-Wright 2014, Chenard and Duckworth 2021). Not only may behavioral accommodation 'buy time' (Figure 11.11), but the manner in which it is achieved can guide the morphological adaptations that result (Hardy 1965; Lister 2014). Moreover, an initial behavioral change may be easier than a morphological one to identify as a plastic response since in cognitively advanced species it can be assumed likely the result of Plotkin's (1988) 'choosing intelligence' rather than genetic programming (see Section 11.2.3).

In order to determine whether behavioral change preceded morphological innovation in the fossil record, we need a time-series including independent evidence of both (Lister 2014). In the fossil elephants studied by Lister (2013), the behavioral switch from browsing to grazing was followed only after several million years by dental adaptations to grazing; a similar pattern is seen in other herbivorous mammals (Lister 2014). In hominins, the origin of bipedal locomotion has been posited to have commenced by facultative upright walking as seen in living chimpanzees (even though their locomotory morphology is adapted to quadrupedal walking), followed by morphological adaptation (Carvalho et al. 2012). In these mammalian examples, cognitive behavioral flexibility is assumed to have played a significant part. Moreover, the morphological changes were directly adaptive to the feeding or locomotory switch initiated in behavior. By contrast, in Miocene stickleback fish (see Section 11.2.4), body armor evolved over 150 years following the shift from

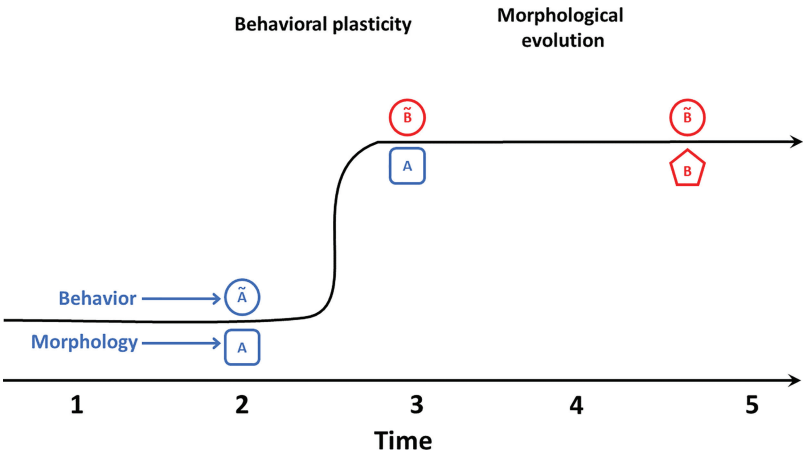

FIGURE 11.11 Guiding evolution with behavioral lead: (1)–(2) coadapted behavior Ã and morphology A; (3) behavioral flexibility ($\tilde{B}$) accommodates to new environment, 'buying time,' but morphology (A) stays the same; (4)–(5) natural selection adapts morphology (B) to fit new environment and behavior. Compare Lister (2014, Figure 4).

midwater to benthic feeding (Purnell et al. 2007). This is behavior-led evolution, but (a) the evolutionary response (predator protection) is only indirectly related to the initial behavioral change (location and feeding), and (b) it is unknown whether the behavioral shift was genetically selected or part of a pre-existing behavioral repertoire; only in the latter case would it fall strictly under the definition of phenotypic plasticity.

Behavioral change is also important as a direct inducer of morphological plasticity that is immediately adaptive (Figure 11.12). For example, the adoption of upright posture in hominins has been explored by a feature affected by activity in life: the orientation of bony struts deep within the hindlimb joints, which are known to respond to external loads by orienting their long axes along lines of principal stress. Analysis of these struts using micro-CT scans of fossil tibiae of the 3.5–2.0 Myr-old *Australopithecus africanus* indicates that these hominins primarily adopted an extended, bipedal posture like modern humans and unlike the knuckle-walking of chimpanzees (Barak et al. 2013). The alteration of jaw morphology in living and fossil hominins according to diet (Collard and Wood 2007) is another potential example and is widespread across vertebrates. It is likely that induced morphologies of this type have led to adaptive innovation via genetical assimilation and modification (Wimberger 1994; Figure 11.12).

Plasticity in living species that are relics of ancient ancestors can potentially provide clues to major evolutionary transitions seen in the fossil record. Standen et al. (2014) made an outstanding contribution to our understanding of the origin of tetrapods (land vertebrates) from their fishy ancestors (Figure 11.13). This transition, occurring approximately 400 million years ago, entailed terrestrial locomotion and the evolution of supporting limbs. As stem tetrapods are extinct, a basal member of the extant clade, *Polypterus*, was used to estimate ancestral

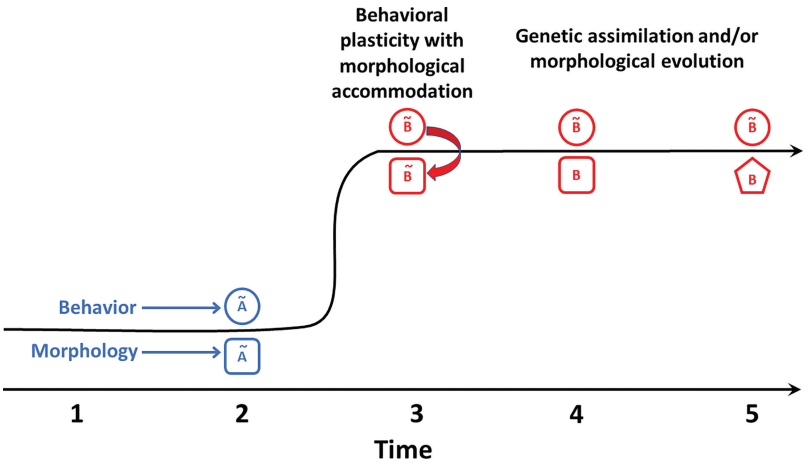

FIGURE 11.12 Behavioral lead with morphological accommodation. (1)–(2) Ã, coadapted starting behavior and morphology, both with reaction norms; (3) behavioral flexibility ($\tilde{B}$) accommodates to new environment, modifying morphology ($\tilde{B}$) through new usage; (4)–(5) natural selection further adapts and assimilates new morphology.

FIGURE 11.13 A modern analogue for the origin of tetrapods. (a) The living bichir *Polypterus* © Mirkorosenau/Dreamstime, with permission. (b) Reconstruction of early tetrapod from the Devonian, *Tiktaalik*. © Nobu Tamura, CC BY_SA.

plasticity; its morphology is in key respects comparable to that of stem tetrapods. This living fish is capable of surviving on land and can walk with its pectoral fins. Standen et al. (2014) found that fish raised on land walked differently from those raised in water, indicating behavioral plasticity. Their fins were planted closer to the body midline and spent less time on the ground, and there were smaller tail oscillations, all reducing energy expenditure. The skeletal anatomy also exhibited phenotypic accommodation in response to terrestrialization, as increased forces in the pectoral girdle induced a modelling response in the bones.

Crucially, Standen et al. (2014) found that the differences in bone morphology observed between water-raised and terrestrialized *Polypterus* bore a remarkable resemblance to the evolutionary changes of stem tetrapod pectoral girdles through the water-to-land transition in the Devonian period. With the proviso that *Polypterus* is only an approximate analogue for long-extinct tetrapod ancestors, and that developmental programs may have altered, it remains plausible that similar behavioral and anatomical changes took place and that phenotypically plastic traits became heritable through genetic assimilation. See Lister (2014) for discussion of earlier ideas on this topic.

11.3.5 Inhibition of Evolution and Extinction

Models predict that variability, whether underpinned by genetic variation or plasticity, promotes persistence of a population in the face of environmental change, thereby reducing the risk of extinction (Lande and Shannon 1996; Ducatez et al. 2020). Adaptive plasticity is also theorized, depending on circumstances, to sometimes inhibit evolution by providing immediate and flexible adaptation (Chenard and Duckworth 2021; Diamond and Martin 2021; Pfennig 2021).

In fossil time-series with sufficient resolution, first-order evolutionary trends, or the lack of a trend (stasis), are normally modulated by second-order, short-term fluctuations of form (Voje 2016), and phenotypic plasticity (as well as variation in gene frequencies) are likely mechanisms. Plastic morphological excursions may be adaptive, allowing the species to endure through minor environmental perturbations, and at the same time may dampen selection and thereby promote stasis (West-Eberhard 2003). Thus, Crampton and Gale (2005) documented fluctuation in the expression of radial folds on the shell of the Cretaceous bivalve *Actinoceramus sulcatus* over some tens of thousands of years, that the authors attribute to plasticity 'maximizing phenotypic adaptability.' Plasticity at the metabolic level in the Triassic amphibian *Gerrothorax pulcherrimus*, visible in the histology of fossil bones (see Section 11.2.5), allowed the species to survive shifting environments for 35 million years with little change in gross morphology (Sanchez and Schoch 2013).

A major feature of the geological record is the existence of 'reef gaps,' periods characterized by the absence of fossilized coral. This led to the hypothesis that corals have a means of alternating between soft bodies and fossilizing forms. Fine and Tchernov (2007) subjected living coral colonies to acidic pH values. After a month, their calcareous skeleton dissolved but the polyps survived as free-living individuals. After 12 months, when transferred back to normal pH conditions, the experimental soft-bodied polyps calcified and reformed colonies. The authors suggest that this phenotypic plasticity could have enabled reef corals to survive past episodes of acidic ocean conditions, accounting for 'reef gaps.'

Extinction resilience via plasticity can also be tested at the community level. In the Plio-Pleistocene extinction event, between ca. 4-1 Myr ago around the coasts of North America, bivalve species that survived were significantly more variable morphologically than victims (Kolbe et al 2011). The authors suggest that greater morphological variation promoted survivorship by enabling adaptation to changing conditions or by

permitting the occupation of a larger geographic range. The extent to which this variation reflects a plastic response to the environment or genetic diversity is unknown, but this could be in principle tested using the criteria outlined earlier (see Section 11.2).

11.4 CONCLUSION

Despite the limitations of fossil data, its time perspective can provide unique insights into the evolutionary process. The role of phenotypic plasticity is particularly challenging to identify in the fossil record as the environmental and genetic influences on morphological variation and change are difficult to separate. However, by bringing the full weight of modern biological understanding to bear on paleontological research, combined with the paleontologist's understanding of the context, limitations, and potential of fossil material, we can unleash great potential for illuminating the complex interaction of genetics, development, and environment in evolution (Box 11.2).

BOX 11.2 SUGGESTIONS FOR FUTURE RESEARCH

- Collaborative research projects between paleontologists and neontologists (biologists), using insights from living species to inform fossil transitions and vice versa
- Experimental modification of morphology in living species by behavioral change (e.g., of jaw shape by giving different foods, or locomotory apparatus by placing in different topographic settings), to test for replication of evolutionary changes seen in the fossil record
- Surveying for the rare, optimal cases of very finely stratified, richly fossiliferous deposits, with associated paleoenvironmental proxies. Collecting statistical samples of fossils of selected target species level-by-level for tracing patterns of change
- Targeting fossil species with 'inbuilt' indicators of developmental plasticity: where ontogenetic shape change is preserved in adult morphology, where clonal individuals vary, or where fluctuating (left-right) asymmetry can be quantified
- Quantitative testing of the predictions of alternative models involving phenotypic plasticity, such as increased variance in morphology (Jackson 2020) or behavior (Wright 2010) when a species encountered a new paleoenvironment
- Exploitation of behavioral proxies in the fossil record where they can be placed in a time-series with morphological change in the same species, to test for behavioral leads
- Comparison of rates of change in fossil record with population-genetic models of potential duration of genetic assimilation and other processes
- Targeting of functional and regulatory genes in ancient genomes, in tandem with living relatives where possible, to illuminate the developmental basis of phenotypic changes seen in the species' fossil morphology.

ACKNOWLEDGMENTS

I am most grateful to Katie Collins, Matt Grove, Jennifer Hoyal Cuthill, Illiam Jackson, and Jeremy Young for valuable discussion and commenting on parts of the draft manuscript. Feedback from David Pfennig and three anonymous referees substantially improved the final product.

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Section IV

Plasticity and Evolution

Controversies and Consensus

DOI: 10.1201/9780429343001-IV

12 The Special Case of Behavioral Plasticity?

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CONTENTS

12.1 INTRODUCTION

Is behavior special? This question has often had a dual meaning in evolutionary biology. Not only does it ask whether behavioral expression is different from other traits, but it also asks whether behavior plays a distinct role in the process of evolution. These two sub-questions are related because it is from the unique expression of behavior that its outsized role in evolution is supposed to derive.

Historically, there have been several ideas about why behavioral traits might differ from other aspects of the phenotype in influencing evolutionary change (see Bateson 1988; Wcislo 1989 for reviews). One general argument is that behavior, because it is more plastic than other traits, should be at the forefront of responses to environmental change (Baldwin 1896; West-Eberhard 2003). This idea emphasizes the 'flexibility' of behavioral responses (see Box 12.1 for distinctions between types of 'plasticity') and suggests that this unique aspect of behavior enables organisms to respond quickly when encountering a novel environment, survive in difficult circumstances, and essentially buy time for evolutionary changes in other traits (see Diamond and Martin 2021; Levis and Pfennig 2021; Pfennig 2021 in this volume). A second argument is that particular types of behavior play a unique role in evolution. These include habitat choice because it determines the selective environment

DOI: 10.1201/9780429343001-12

(Waddington 1959; Bateson 1988; Huey et al. 2003), learned behaviors that allow swift and adaptive responses to environmental change (Plotkin 1988a; Dukas 2013), and social behaviors that generate their own unique evolutionary dynamics (West-Eberhard 1983; Dunbar 1988; Davison and Michod 2021). In turn, these different perspectives have led to a debate about whether behavior primarily drives or inhibits evolution (Huey et al. 2003; Sol et al. 2005; Duckworth 2009; Munoz and Losos 2018; see also Pfennig 2021). In this chapter, we suggest that the perpetual search for "the role of behavior in evolution," reflects a general dissatisfaction with a static view of evolution (see Box 12.1)—a view on which we elaborate below. We argue that past perspectives on the role of behavior in evolution—whether viewing behavior as a driving or inhibiting force in evolution—can be reconciled through a systems approach in which the interactions of organisms and feedbacks between behavior, development, and natural selection are key to understanding mechanisms of evolution.

Before we elaborate on this perspective, we first discuss two major assumptions that have been implicit to this debate and have, to a large extent, hampered progress. The first assumption is that behavior is generally more plastic than other traits (West-Eberhard 2003). We argue that the range of plasticity and flexibility of behavioral traits cannot be generally described as more or less plastic than any other physiological or morphological trait. We suggest that this assumption has persisted because much of evolutionary theory has focused on the static expression of structural traits even though, across taxa, dynamic trait expression is the norm (Nijhout 2003; Sultan 2021). The second assumption is that any unique role of behavior in evolution can apply only to animals, and perhaps is even limited more stringently to animals with higher cognitive abilities (Plotkin 1988a). If this assumption were true, it would make the role of behavior in evolution relevant to only a small subset of the diversity of life on earth. To assess this assumption, we provide an overview of behavioral mechanisms across life, from protists and fungi to plants and animals, showing that there are ubiquitous principles of behavioral response across disparate taxa despite a diversity of mechanisms underlying these responses. Finally, after establishing that these assumptions are unwarranted, we suggest that the persistent fascination with the role of behavior in evolution reflects a need to integrate static and dynamic perspectives (Box 12.1) to better understand evolutionary dynamics.

12.2 IS BEHAVIOR MORE PLASTIC THAN OTHER TRAITS?

When biologists use the term 'behavior' it often evokes images of complex cognitive feats in animals, but it is really a more general term used across scientific disciplines to describe the movement and action (or inaction) of matter. Scientists apply this term to many different systems ranging from the movement of molecules across a concentration gradient to wave action on a sandy shore to a pair of breeding birds feeding their nestlings.

In organismal biology and evolution, the same general principle applies—behavior refers to movement or action—but, at this scale, it also refers to what an organism does. Behavioral traits are generally considered inherently flexible and reversible in expression, differing from physiological traits in that they are expressed at the level

BOX 12.1 GLOSSARY OF TERMS

- **Behavior:** *Across scientific disciplines*: the action of matter. *In evolution and ecology*: action (or inaction) that operates at a whole-organism level and so is visible to an external observer. This latter requirement is what differentiates behavior from physiology as well as thinking, the latter two being dynamic processes that occur at a lower scale.
- **Behavioral Flexibility:** A type of behavioral plasticity that occurs within an individual and is typically reversible. It differs from developmental plasticity in that behavioral changes occur post-maturity.
- **Developmental Plasticity:** A type of plasticity that occurs when the same genotype is capable of producing phenotypic variation as a result of variation in the environment during ontogeny. Developmental plasticity differs from phenotypic flexibility in that it is not reversible and refers to phenotypes that become fixed after maturity is reached.
- **Learning:** Acquisition of information about the environment through experience or observation, often resulting in modification of behavior.
- **Memory:** The storage of information about past events that can be accessed in the future to affect a behavioral response. Can be short-term and unstable or long-term and stable.
- **Niche Construction:** Modification of the environment by an organism. Can be active or passive.
- **Personality:** Consistent differences among individuals in behavior across time and/or contexts. Often measured as repeatability: an assessment of the relative among- and within-individual variance in a behavior. A low within-individual variance coupled with high among-individual variance means a behavior is highly repeatable.
- **Robustness:** The ability to maintain a steady phenotypic state in the face of environmental or genetic variation.
- **Static versus Dynamic Perspectives of Evolution:** Static perspective focuses on delineating patterns of variation in traits, fitness, and genomes and uses approaches such as measurement of selection, statistical partitioning of trait variation, and comparative studies. Dynamic perspective focuses on the underlying mechanisms that lead to these patterns and investigates the physiological, genetic, and behavioral bases of differences in reproductive success, how specific genes and environmental conditions interact to produce variation in traits, and the ecological interactions that underlie patterns of diversification and evolutionary change. The two approaches are complementary and one approach is incomplete without the other.
- **Systems Approach to Evolution:** A holistic approach to studying evolutionary dynamics. Views organisms and environments as complex systems and emphasizes feedbacks, nonlinear dynamics, and networks of interactions across scales.

of the whole organism. Their dynamic expression has contributed to the reputation of behavior for being highly plastic; however, while all behaviors are reversible in expression, many are also often stable in their level of expression across time and contexts (Sih et al. 2004; Duckworth 2009, 2015). Certain 'personality' traits, such as aggression, show particularly high repeatability (Bell et al. 2009; Duckworth 2014). Yet, even these relatively stable behavioral traits can be plastic, as their expression is often also influenced by environmental variation in adulthood or during development (Komers 1997; Stamps 2016). Therefore, behavioral plasticity encompasses a broad range of different types of behavior and timescales of expression from rapid actions to personality traits, and from the change in the level of expression of a behavior during an individual's life (known as behavioral flexibility) to maternally induced fixed behavioral phenotypes (Bonduriansky 2021 in this volume) that are determined early in ontogeny (known as 'developmental plasticity'; see Box 12.1).

Many morphological traits also span the range from stable to flexible and can even change on timescales similar to behavioral traits (Piersma and Van Gils 2011; see also Pfennig 2021 in this volume). Examples include the rapid increases in the gut size of snakes within minutes of feeding (Starck and Beese 2002), skin pigmentation changes within hours of sun exposure in mammals (Gilchrest et al. 1996), and muscle size changes within days to weeks in response to changes in use (see Piersma and Van Gils 2011 for review). Diverse multicellular organisms from plants and fungi, to cartilaginous fish, annelid worms, and echinoderms show indeterminate or flexible growth throughout life (Sebens 1987; Aizen et al. 2019). Moreover, all traits, even relatively stable skeletal traits, are dynamic during development and, during this time, are often more sensitive to environmental variation. Flexible responses to environmental variation are the default state for traits as the biochemical reactions and interactions that occur at the cellular level are affected by changes in temperature, pH, ion, and nutrient balance (Nijhout 2003). Thus, insensitivity of traits to environmental variation is a derived evolutionary state, as are adaptive plastic responses that are triggered by aspects of environmental variation that induce specific and directed phenotypic changes.

These observations emphasize that, even though it is often easier to focus on 'static' measures of traits (e.g., mean expression), all traits are 'dynamic' in their expression during ontogeny and many remain flexible in adulthood. However, because behavioral traits are ephemeral in their expression, they are more difficult to measure compared to traits that are relatively unchanging in adulthood, like structural size. This is undoubtedly the reason that such structural size traits (e.g., in determinate growers) are the focus of many evolutionary models even though across life they represent a minority of traits (Sebens 1987; Aizen et al. 2019). Heightened awareness of this issue has led to the development of new methodologies to measure and analyze dynamic traits in evolutionary and quantitative genetic frameworks (Wu and Lin 2006; Gomulkiewicz et al. 2018). The historical emphasis on static traits likely led to assumptions that behavior is more plastic than other traits even though, in reality, dynamic morphologies that are flexible in expression are relatively common. Thus, making broad statements about differences in plasticity between behavioral and morphological traits is misleading and unproductive. This doesn't mean that there are no differences in plasticity between specific traits, but these types of comparisons always have to be made on a trait by trait basis and cannot apply to a class of traits as a whole.

A final point is that, even though the expression of behavior is ephemeral at the whole-organism level, variation in its expression is underlain by physical components of the neuroendocrine system. Thus, behavioral traits are subject to the same sorts of constraints as any other morphological trait (Duckworth 2018; Duckworth et al. 2018). This is why we observe a range of flexibility in behavioral traits from imprinted preferences to open-ended 'learning' to relatively stable personality variation. All traits fall along a spectrum of flexible to stable in adulthood and it is often flexibility at one level that enables stable expression at a higher level. For example, organismal homeostasis emerges due to a myriad of physiological interactions and responses that constantly monitor and react to changing environmental conditions (Nijhout and Reed 2014). In turn, some stable structures are necessary to maintain organismal integration and to enable flexibility at a higher level. After all, it is the relatively stable structure of the skeletal system in vertebrates that enables an animal to walk, run, and do other flexible activities. Thus, organismal phenotypes are necessarily a mosaic of flexible and stable components and it is the integration of these components across levels that enables functioning organisms. Most importantly, because all traits span the range of flexibility, it is not fruitful to make assumptions about the plasticity of a group of organisms or an entire class of traits; the extent of plasticity and flexibility can only be assessed and compared on a trait by trait basis.

12.3 DIVERSITY OF BEHAVIORAL MECHANISMS ACROSS LIFE

Past discussions of the causal role of behavior in evolution have generally focused on animals (Wcislo 1989; Huey et al. 2003; Losos et al. 2004) and in some cases, even more stringently on animals with higher cognitive abilities (Sol et al. 2005). But, at the same time, some authors have presented a broader case for the role of behavior in evolution analogizing various types of behavioral responses in animals to responses observed in plants (West-Eberhard 1983; Huey et al. 2002). In the introduction to his edited volume, *The Role of Behavior in Evolution*, Plotkin (1988a, p. 9) points out the tension between these views:

> *…whether behavior that is a consequence of a choosing intelligence has a role in evolution that is different from the role of behavior that is not a consequence of a choosing intelligence is a question that is left open in this book.*

By 'choosing intelligence' Plotkin is differentiating between organisms that show higher-level cognitive behavior and organisms that only show stimulus-response type behavior. In the next section, we explicitly pick up where Plotkin's book left off to address the ways that behavioral mechanisms are similar and different across different taxa before turning to the implications of these similarities and differences for understanding behavior's role in evolution.

12.3.1 Organism-Level Coordination of Response to Stimulus

To mount an appropriate behavioral response to a constantly changing environment, all organisms need an effective system of internal communication. While the specific

mechanisms differ among taxa, all involve similar functional components: a sensor, a system of internal communication and integration, and an external response at the whole-organism level. These response systems can be complex even in groups which are not typically considered consciously aware. For example, like animals, plants use an array of sensory systems to distinguish between up and down, between self and non-self; to sense light, heat, moisture, and nutrient content; and to either detect objects impassible to growing roots or vibrations from potential predators (Svistoonoff et al. 2007; Bisseling and Scheres 2014; Lopez et al. 2014; Mescher and Moraes 2015; Assmann and Jegla 2016). They use these sensory systems to coordinate behavioral responses by directing movement and growth as well as upregulating defense mechanisms across the entire plant (Silvertown and Gordon 1989).

Chemical signals coordinate responses to stimuli either across cells, for multicellular organisms, or within cells but between different cellular structures, for unicellular organisms. The structures of many of these molecules are highly evolutionarily conserved, although they show a diversity of functions across clades. For instance, many hormones that are used as neurotransmitters in animals, such as glutamate (Forde and Lea 2007), dopamine (Guidotti et al. 2013), acetylcholine (Bamel et al. 2016), and GABA (Michaeli and Fromm 2015), are expressed across the diversity of life, from bacteria and fungi to plants (Kawashima et al. 2007). Thus, many of the chemical signals that we associate with eliciting behavioral responses in animals are ubiquitous and evolutionarily conserved across taxa.

For single-celled organisms, the line between physiology and organismal behavior becomes blurred, as the individual cell is also the entire organism. Despite this, behavioral responses in single-celled organisms involve the same basic components as in multicellular organisms: integration and transmission of information gathered by sensory receptors into directed movement using a motor structure. This type of system is so important it has evolved independently across multiple disparate groups, with flagella evolving in Prokaryotes, cilia in Eukaryotes, and archaella in Archaea (Albers and Jarrell 2018). The two-component signaling system in bacteria illustrates the basic principle of how these systems work. Upon detecting a stimulus, transmembrane receptors coupled with histidine-kinase proteins become methylated, which drives phosphorylation of response regulator proteins. These altered proteins then initiate the transcription of specific genes, whose products subsequently enable the components of the behavioral response for as long as the phosphorylation persists (Armitage 1992; Hazelbauer et al. 2008). This and other similar systems are responsible for variable taxis responses to light, chemical gradients, nutrient availability, and even voltage (Schweinitzer and Josenhans 2010).

Although organisms outside of Animalia lack neurons, the ability to coordinate components of behavioral responses is ubiquitous across life. This can mean chemical signaling as shown above, but interestingly also includes electrical signaling. Bioelectricity is used in many essential metabolic cellular processes (Cohen and Venkatachalam 2014) and the first action potentials and sodium and calcium ion channels likely began in Eukaryotes with the evolution of cilia (Brunet and Arendt 2016). Moreover, electrical potentials generated by a plant can propagate across its entire structure to transmit information and regulate responses (Fromm and Lautner

2006; Volkov et al. 2010). Similar to neural axons, long-distance electrical signaling is facilitated by phloem cells that preserve signal strength. It is thought that information about the specific stimulus type might even be encoded through variation in the shape, magnitude, method, and frequency of different types of electrical potentials (Canales et al. 2017). Similarly, fungi also have slow-wave electrical potentials within the hyphae and fruiting bodies (Adamatsky 2018) which change in frequency in nuanced ways in response to stimuli received by diverse sensory receptors (Xu et al. 2017). This enables the fungi to perceive changes in nutritional availability or detect dangers such as predation or fire (Olsson and Hansson 1995). Thus, the ability to transmit an electrical signal in response to a stimulus to direct a behavioral response is widespread across life even in the absence of neurons. These similarities in information acquisition across diverse taxa raise the question: What are the unique aspects of behavioral plasticity in animals?

12.3.2 Origin of Nervous Systems: Implications for Behavioral Plasticity

Beyond basic stimulus-response systems, there are multiple transitions in the complexity of mechanisms that enable higher-level behavioral responses, with the most obvious being the evolution of neurons and the nervous system in animals. Neurons emerged early in the evolution of animals with only poripherans (sponges), and placozoans (simple free-living multicellular organisms) lacking a nervous system (Figure 12.1). The earliest neural network was likely a nerve net much like that present today in cnidarians (jellyfish, corals, and sea anemones) and ctenophorans (comb jellies) (Arendt et al. 2016).

Much is still unknown about the origins of both neurons and neural organization largely because there is debate on whether ctenophores diverged before (Ryan and Chiodin 2015) or after (Moroz et al. 2014; Kristan 2016) poripherans and placozoans (Figure 12.1). Depending on where Ctenophora is placed on the evolutionary tree, it is possible that neurons either arose once and were lost in some groups or arose multiple times. Disentangling these possibilities would provide insight into the factors that lead to the evolution of neurons as the method of intercellular communication and coordination, as opposed to other mechanisms in non-neural animals, such as calcium signaling in sponges (Leys 2015) or small intercellular peptides in placozoans (Varoqueaux et al. 2018).

One of the unique contributions of the neural system to behavior is the dimensionality and complexity of behavioral response that such a system can support, but these attributes are not necessarily important in explaining its origin. Instead, it seems likely that neurons evolved primarily to coordinate motor responses across an increasingly complex organism (Bucher and Anderson 2015). Groups that do not possess a neural system either are mobile but small and simple in body form (e.g., Placozoa, Protozoa, and Archaea), or are sessile (sponges, plants, and fungi). In animals, the taxa which lack neurons also lack striated muscles (Steinmetz et al. 2012; Moroz et al. 2014). This suggests that neurons are necessary to coordinate complicated movement involving many separate muscles (Kaijzer 2015). Other organisms

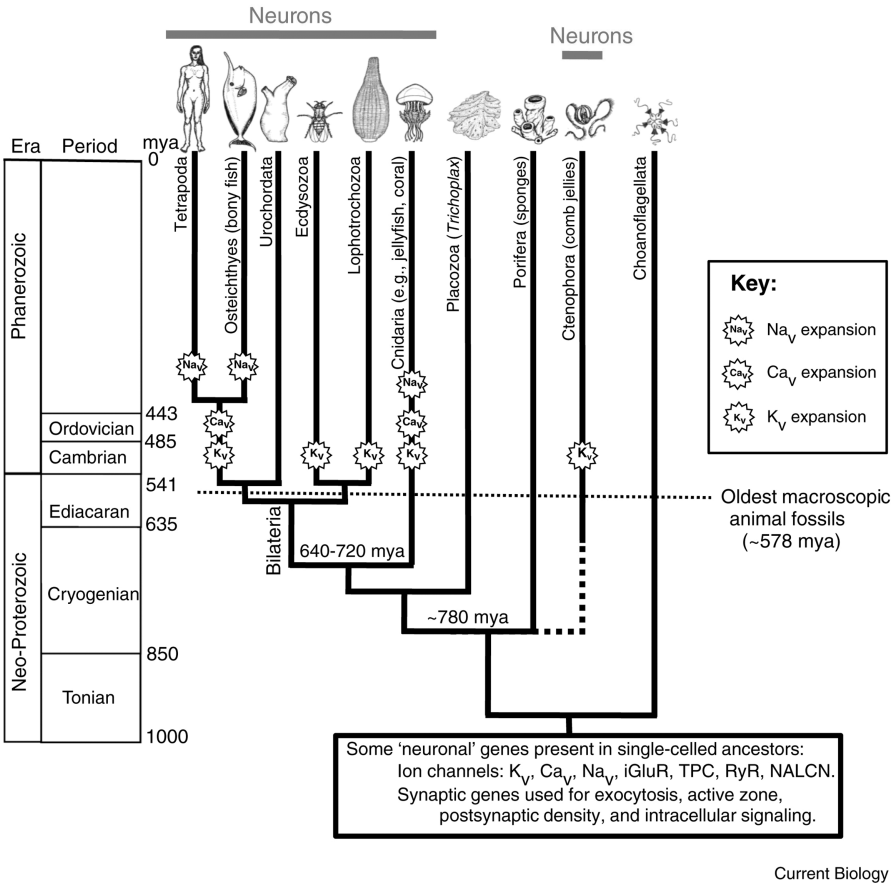

FIGURE 12.1 Phylogeny of Animalia showing approximate timing of major transitions in the evolution of neurons. Black circles with serrated edges indicate the hypothesized order of appearance of various types of voltage-gated channels. Cells with a combination of Ca_v and K_v channels could generate action potentials, which expanded intercellular communication capabilities. The addition of Na_v channels increased the speed of transmission making rapid movements possible. (Reprinted with permission from Kristan, W. B. 2016. Early evolution of neurons. *Curr. Biol.* 26:949–954.)

are capable of sophisticated movement without a nervous system, but still arguably not in the range available to neural animals.

While there is clearly an association with motor capabilities across this broad scale, once neurons evolve, if movement is lost, this does not necessarily lead to a corresponding loss of the nervous system. For instance, the adult forms of tunicates do not lose their nervous system despite metamorphosis to a sessile state. Although sections devoted to motor processes are lost, the cerebral ganglion and neural gland remain and, in fact, the adult tunicate has a larger ganglion than the mobile juvenile (Mackie and Burighel 2005). Evolutionary losses of the nervous system may be rare

or impossible because, once evolved, it becomes an essential regulator of so many fundamental bodily systems. These observations suggest that the nervous system evolved initially to coordinate complex motor behavior, but subsequently was co-opted for many more functions. Most importantly, evolution of a nervous system is often thought to underlie the extraordinary capacity of animals for 'memory' and learning, topics which we explore in the next sections.

12.3.3 Storage of Information for Later Use: Learning and Memory

The ubiquity and repeated evolution of various signaling systems across the tree of life highlight the importance of being able to mount an appropriate and coordinated response to the environment for all organisms. These signaling systems can affect a transient and reversible change in behavior, or they can catalyze longer-lasting changes. Also shared across life is the ability to consolidate information about the environment, store this information stably over long periods of time, and retrieve it later to carry out a behavioral response that differs from the original behavioral response. Storage and recall of information, and the ability to modify behavior as a result of this information, are the fundamental components of learning and memory. Although learning and memory are not necessarily behaviors themselves, they have persistent effects on behavioral flexibility as they enable organisms to reversibly modify their behavior in response to changing environmental conditions.

12.3.3.1 Evidence of Memory Mechanisms across Taxa

There are different types of memory storage and information processing across animals, some of which are analogous to information processing of non-neural organisms and some of which are much more sophisticated and complex. Information gathered by the nervous system can be used temporarily and then discarded, or consolidated into a stable form that can be drawn from repeatedly to guide adaptive behavior (Atkinson and Shriffin 1968). The most transient form of memory is sensory memory, which consists of what is currently being seen or heard and is gathered by sensory systems and then discarded within seconds (Tripathy and Öğmen 2018). The sheer quantity of sensory information is such that much of it is filtered and actively disregarded before it reaches conscious awareness (Cromwell et al. 2008). This type of transient information storage shares many parallels with mechanisms of information storage in non-neural taxa. For example, bacteria often use a nanobrain organelle that is composed of an interconnected network of the signaling and response regulator proteins, which serve as a temporary 'memory' of immediate past encounters of molecule concentrations to compare to current sensory protein inputs. These clusters of sensory receptors can result in surprisingly sophisticated behavioral responses such as changing flagella patterns in response to multiple simultaneously changing environmental conditions (Hazelbauer et al. 2008). Because of this, they have been compared to our own neural networks (Lyon 2015).

In animals, new experiences that are kept longer than sensory memories are stored as short-term memory, which can be recalled over brief periods of time. While most short-term memories are quickly forgotten, some of this new information transitions to long-term memories which are stable and can last a lifetime. Even though

single-celled organisms are not able to take advantage of the specialized interconnected cells devoted to the task of information storage that neural organisms have, they are able to store information over long time periods through other mechanisms. For example, one of the most well-known examples of such information storage is the epigenetic modification of DNA employed by bacteria as a memory of past infections: CRISPR (i.e., Clusters of Regularly Interspaced Short Palindromic Repeats). Fragments of DNA from viruses are placed between these repeats and used like an immune response to provide a memory of past infections enabling bacteria to defend against future encounters with the same virus (Barrangou et al. 2007). Despite lacking multiple cells, by using modifications to their own DNA and proteins, bacteria are able to store useful information for long periods of time and, in essence, remember natural enemies in a way that modifies their behavior toward them in subsequent encounters.

While the presence of a central nervous system can allow for the storage of more complex and information-rich memories, the CRISPR example suggests that all organisms have the basic components of long-term information storage on some level. Indeed, cellular differentiation during development involves changes in epigenetic regulatory mechanisms, which encode cellular fates and which are stably stored over time. Epigenetic mechanisms are also involved in neural memory formation (Levenson and Sweatt 2005; Heyward and Sweatt 2015; Kim and Kaang 2017). The question remains whether these types of mechanisms are used similarly across life for storage of information about past environmental experiences to influence future behavior (Thellier and Luttge 2013).

One major difference between neural and non-neural multicellular organisms (e.g., plants) is that, in the latter, there is no known central location or dedicated cell type where information is stored. Instead, in non-neural organisms, information seems likely to be recorded at a more general level in cells throughout the organism. However, one intriguing proposal, first mentioned by Darwin in *The Power of Movement in Plants*, is that actively dividing meristems and root tips might be important specific locations for information storage and retrieval, as they are places where the direction of growth is determined. Darwin (1872, p. 338) presaged current research in this area when he wrote:

> *It is hardly an exaggeration to say that the tip of the radicle thus endowed, and having the power of directing the movements of the adjoining parts, acts like the brain of one of the lower animals; the brain being seated within the anterior end of the body, receiving impressions from the sense-organs, and directing the several movements.*

Since Darwin, a number of studies have investigated the ability of plants to exhibit the capacity for learning and memory. However, research into mechanisms of learning and memory in plants has been sporadic due, in part, to a series of pseudoscientific books published in the 1970s claiming plants possessed psychic abilities (discussed in Mescher and Moraes 2015). Since then, the field has been divided between groups claiming intelligence and consciousness in plants, and those who believe that plants are incapable of any type of behavior or memory (Struik et al. 2008). The extreme views on both sides of this debate have severely stunted research in this field and empirical studies demonstrating plant mechanisms of learning and memory are few.

Despite these difficulties, evidence for a form of memory has been shown in *Bidens pilosus*, where pricking a leaf of a young plant results in directed growth of new buds days to weeks later (Thellier et al. 1982). This ability to store information has been replicated in other species of plants, has been reported to last for several weeks, and is initially sensitive to being 'overwritten' by subsequent events (Verdus et al. 2002). Like mechanisms of information storage in other groups, stored information in plants is likely facilitated through epigenetic modification of genes (Cazzonelli et al. 2014) or sustained changes in levels of signaling metabolites and transcription factors; however, the specific mechanisms and their prevalence are still unresolved (Crisp et al. 2016).

12.3.3.2 Evidence for Learning Mechanisms across Taxa

Learning can be a strong instigator of behavioral changes because it enables organisms to assess and respond to changes in the quality of an environmental stimulus. At the most basic level, it requires preservation of some information about the past state of that stimulus so a comparison can be made. Organisms then assess whether a change in stimulus is positive or negative and modify behavior accordingly. Habituation and sensitization are simple forms of learning that involve changes in the magnitude of a behavioral response due to repeated exposure to a stimulus. A stimulus that is consistently neutral can result in habituation and lack of response, whereas a stimulus that is consistently positive or negative can result in sensitization and an increased response magnitude. Sensitization and habituation seem to be a common form of learned behavior even in simple organisms. For example, despite lacking neurons, the slime mold (*Physarum polycephalum*) can become habituated to a mild adverse stimulus (caffeine) when presented with the stimulus successively over time (Boisseau et al. 2016).

One potential example of habituation can also be found in the sensitive plant (*Mimosa pudica*) which is known for folding in its leaves rapidly when triggered by vibrations or contact, an energetically expensive defense mechanism against herbivory. Repeatedly dropping the plant or otherwise triggering this response leads to habituation such that further similar stimulation no longer results in any response at all (Gagliano et al. 2014). This habituation persists even after a month, and so is independent of fatigue. Moreover, it is environmentally contingent as plants located in full sun and with access to more energy continue to exhibit the behavior despite repeated dropping, whereas shaded plants become habituated more easily (ibid).

Habituation and sensitization are types of learning in response to a stimulus currently present. Associative learning is another, more complex type that enables proactive responses in that an organism uses information about past environmental contexts and associated stimuli to change behavior in a way that maximizes benefit in a predicted future context and time. This ability is present in animals but is less well studied in other organisms because it was long thought to require a central nervous system. However, slime molds, once again, provide an example. When exposed to unfavorable conditions generated at a constant interval, they slowed down movement shortly before the next unfavorable period began, even when the unfavorable conditions were suddenly stopped (Saigusa et al. 2008). This response indicated that

slime molds not only have a mechanism to track the passage of time, but they also possess a mechanism for storage and retrieval of information of past events that influences behavior independently of present stimulus cues. So far there is only one study showing associative learning in plants. In it, pea plants (*Pisum sativum*) were conditioned to associate the presence of an unrelated stimulus (a fan) in a maze task as a cue for the presence of light. In subsequent trials, the plants grew toward the fan in the absence of light, even when the fan was moved to another location (Gagliano et al. 2016). Despite the limited examples in non-neural organisms, it seems that, if there are mechanisms for conditional information storage across life (such as epigenetic information retention) associative learning should evolve. For all organisms, the ability to adjust behavior in an anticipatory fashion based on prior experiences should have strong fitness consequences. Learning enables organisms to lessen a costly response to a stimulus that is harmless and mobilize defenses more rapidly to a repeatedly experienced harmful stimulus. However, it is currently unclear whether such learning abilities in non-neural organisms are due to true rarity or simply to a dearth of studies testing for them.

In neural organisms, centralized information storage in the brain has facilitated the development of complex neural networks and a high degree of specialization in the function of specific neurons and brain regions. In contrast, non-neural organisms lack the connected organization of a centralized nervous system that enables complex information assimilation, retrieval, and its recombination in novel ways. Essentially, non-neural organisms may have rudimentary learning and memory capabilities but lack the combinatorial power that underlies thought and reasoning. Such an ability to recombine information in novel ways is the basis for behavioral innovation and problem-solving, both of which have received much attention for their proposed role in evolution. While many of the basic mechanisms that underlie coordination of behavioral response and information acquisition are ubiquitous across taxa, evidence of problem-solving and innovation are so far limited to animals with brains. Thus, if basic elements of organismal response and information recall underlie the role of behavior in evolution then this role applies to all organisms. If, however, its role is confined to only organisms with complex cognition then its importance is limited to organisms with a brain and perhaps even to higher vertebrates. In the next section, we argue that even the most basic of behavioral responses influence evolutionary dynamics; however, we propose that characterizing how different levels of behavioral complexity affect evolutionary dynamics is the core question of the field.

12.4 BEHAVIOR AND EVOLUTIONARY DYNAMICS: A SYSTEMS APPROACH

Over the last century, there have been many proponents of the idea that behavior plays a special role in evolution (e.g., Wyles et al. 1983; Huey et al. 2003; Duckworth 2006; Munoz and Losos 2018), but there has not been agreement on the scope of this role. Discussions of behavior's role in evolution have ranged from a general intuition of its widespread importance to an emphasis on behavioral capacities that are largely taxa-specific. Researchers from Baldwin (1896) to Mayr (1963) have proposed behavior as

a causal mechanism in evolution. In general, their views reflect the idea that behavior is a 'pacemaker' of evolution because it is at the forefront of an organism's interaction with its environment and so, in large part, will determine the selective pressures an organism experiences (e.g., Duckworth 2006).

Waddington (1959), in particular, proposed that evolutionary processes were causally affected by four coequal and mutually influencing systems: the genetic system, the natural selective system, the epigenetic system, and the exploitative system (Figure 12.2). The exploitative system refers to the ability of an organism to choose and modify its habitat. Waddington (1959) stated that:

> *… [evolution] has often been envisaged as consisting of no more than a set of genotypes which are influenced, on the one hand, by a completely independent and random process of mutation and, on the other hand, by processes of natural selection which are again in no way determined by the nature of the genotypes submitted to them. Perhaps such a simplification was justified when it was a question of establishing the relevance of Mendelian genetics to evolutionary theory, but it can only lead to an impoverishment of our ideas if we are not willing to go further.…*

By going further, he advocated including both the epigenetic (developmental) and exploitative (behavioral) systems on equal footing with genetic and natural selective systems. In particular, he emphasized that, because behaviors like habitat selection were simultaneously affected by natural selection (could evolve) and also affected natural selection and development (by determining the environment), evolutionary dynamics could only be understood by incorporating feedbacks between behavioral processes, development, and evolution. Thus, Waddington was one of the earliest proponents of a systems approach to evolution—an approach which emphasizes feedbacks, nonlinear dynamics, and networks of interactions across scales (see Box 12.1; Oyama 1985; Nijhout et al. 2017; Badyaev 2019; Frank 2019).

These feedbacks largely come from the ability of organisms to choose and modify their environments because the traits involved are both determining (within-generation) and determined by (across generations) the selective environment. Such reciprocal feedbacks are not limited to animals with brains and there are many examples of 'niche constructing behavior' (behavior that alters the local environment, Box 12.1) among non-neural organisms (Odling-Smee et al. 2003) such as fungal auxin production which can change host plant growth patterns (Chanclud and Morel 2016), dopamine/L-dopa release which causes growth inhibition in rival plants, thus reducing competition (Soares et al. 2014), and bacteria that become social during stressful or low nutrient conditions (Swiecicki et al. 2014; Muñoz-Dorado et al. 2016). Plants also exhibit habitat choice through maternally influenced seed dispersal (Donohue 2003) where plants produce alternative dispersal morphs of seeds depending on the quality of the maternal environment (e.g., Larios and Venable 2015). Moreover, many plants can delay germination with consequences similar to dispersal in animals because, even though it doesn't involve movement, the seeds are essentially seeking out a better environment. Their habitat choice in this case is simply expressed across time instead of across space (Buoro and Carlson 2014). Therefore, feedbacks between habitat modifications and natural

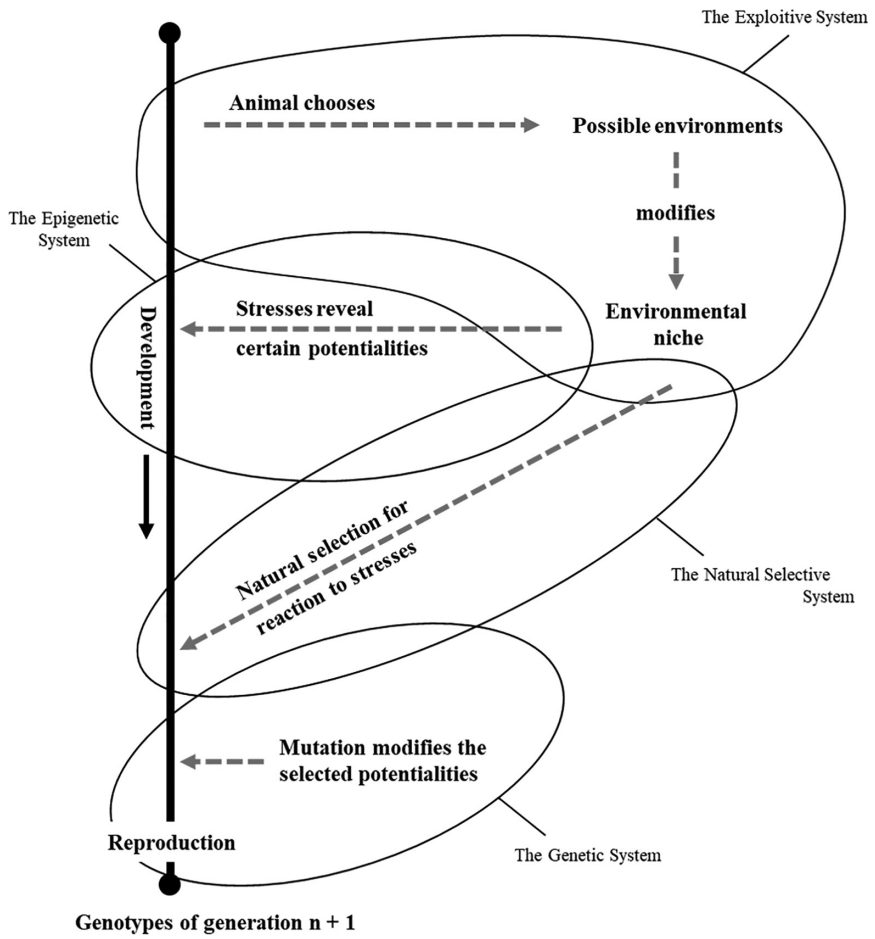

FIGURE 12.2 Waddington advocated a 'systems' approach to studying evolution. In his scheme, the exploitative system emphasizes habitat choice and modifications as a coequal force in evolutionary dynamics. See text for details. (Redrawn with permission from Waddington, C. H. 1959. Evolutionary adaptation. *Perspect. Biol. Med.* 2:379–401.)

selection are relevant to all organisms, irrespective of their mobility and expression of behavioral complexity.

However, concurrently with arguments for the role of habitat selection and niche construction, there has been a separate argument for the role of learning and behavioral innovation in evolution (Dukas 2013). Some of these studies have linked behavioral flexibility (often using relative brain size as a proxy) to either higher rates of diversification (Sol et al. 2005; Sayol et al. 2019) or higher rates of anatomical evolution (Wyles et al. 1983). Higher diversification rates could result either from the ability of species with relatively larger brains to survive in novel conditions and therefore avoid extinction, or could result from higher speciation rates (Sayol et al. 2019).

Because relatively larger brains enable both higher levels of behavioral innovation and are often associated with social behavior (e.g., Reader and Laland 2002), they simultaneously facilitate the development and transmission of novel behaviors that expose populations to new selective pressures, thus promoting evolutionary diversification. However, much of this work has focused on avian taxa and it possible that the association between brain size and diversification may be due to other factors, such as the link between diversification and song learning (Mason et al. 2017). If song evolution turns out to be more important than behavioral innovation in explaining the diversification in birds, then this may weaken the evidence for a unique role of learning in evolution. This is because song plays an important role in species recognition and sexual selection (Irwin 2000) similar to the role of floral evolution in flowering plants, another highly diverse clade (Givnish 2010). There is some evidence for this because, while songbirds are highly intelligent, their sister clade, the parrots are arguably even more intelligent; however, parrots are much less diverse and also do not have song-based species recognition and sexual selection. Thus, it may be that diversification in birds is more directly related to traits that are important in species recognition and sexual selection with brain size as an indirect correlate of these traits. Overall, this points to the need for more work on linking behavioral flexibility and diversification across a much broader variety of taxa, ideally, even including non-neural taxa.

Any associations between behavioral flexibility and diversification dynamics may also depend on the scale at which these processes are studied. One of the most convincing studies showing a link between relative brain size and diversification focused on the subspecies level. Sol et al. (2005) showed that Holarctic passerines with relatively larger brains had a greater number of subspecies compared to taxa with relatively smaller brains. They controlled for other geographic factors typically associated with higher diversification rates, such as range size and latitudinal variation as well as plumage dimorphism (a proxy for sexual selection; Barraclough et al. 1995). Yet, while this study provides convincing evidence of a link at the subspecies scale, there is a well-known bias against the survival of taxa that are longer-lived and have larger body sizes during mass extinction events (Hull 2015). During modern times, some of the most threatened taxonomic groups (e.g. non-human primates, elephants, several species of whales and parrots) are also the largest in their respective taxonomic groups, as well as some of the most cognitively sophisticated. These patterns likely reflect a trade-off between the cognitive benefits of having a large brain and the life history and dietary needs of supporting such an energetically expensive organ (Dunbar and Shultz 2007). Thus, increased cognitive abilities, while potentially contributing to diversification on short time scales, might pre-dispose taxa to heightened risk of extinction on longer evolutionary scales.

One area where greater behavioral complexity clearly has an important and distinct evolutionary role is cultural evolution. Cultural evolution requires some form of social learning such that individuals learn skills and behaviors from members of their group or community leading to changes in behavioral expression at the population level over time (Whiten et al. 1999). Such learning does not have to involve active communication as it can include the use of social information that is publicly

available and can even occur through heterospecific observations (Danchin et al. 2004). Because new patterns of behavioral expression are transmitted horizontally, cultural evolution can occur extremely rapidly. Thus, it can have important effects on genetic evolution, particularly because, by its very nature, it can influence an entire population of individuals at once (Wcislo 1989; West-Eberhard 2003).

While such rapid population-wide changes in behavior due to social learning can certainly influence evolutionary dynamics, they are not necessarily always a force in driving or accelerating evolutionary change. In fact, many have argued that learning and cultural evolution may just as likely *inhibit* genetic evolution by allowing animals to adapt plastically to environmental change without the need for evolutionary change in other traits (Price et al. 2003; Dukas 2013). Examples in humans abound. One particularly well-studied cultural change—the incorporation of cow's milk in the diets of certain human populations—has clearly led to genetic evolutionary change (Beja-Pereira et al. 2003). However, there are numerous examples of cultural changes that are either neutral in terms of genetic evolution or may actually inhibit evolutionary change. An example of the former is changes in the popularity of baby names, a cultural trait that changes through drift (Hahn and Bentley 2003) and is unlikely to have any effect on genetic evolution in a population. An example of the latter are any changes in tools, dwellings, or clothing that enable humans to maintain thermal homeostasis (Flouris 2011), functionally inhibiting genetic evolution of physiology and morphology in populations that inhabit relatively extreme climates (e.g., deserts and tundra). Thus, while cultural changes clearly have great potential to influence genetic evolution, their influences are likely to be diverse with equal occurrences of inhibiting or driving genetic change.

Interestingly, debates about whether learning (specifically) and behavior (in general) primarily drives or inhibits evolutionary change also evoke elements of a systems approach to evolution. A fundamental tenet of this approach is to understand how complex systems respond to environmental change with the idea that robust systems can maintain a steady state in the face of a wide variety of environmental challenges. Proponents of behavior as an inhibitor of evolutionary change are essentially making the argument that behavioral flexibility maintains a population at a robust or steady phenotypic state. These arguments have ranged from a focus on how behavioral changes enable organisms to maintain homeostasis (Bradshaw 1972; Huey et al. 2003; Badyaev 2005) to an emphasis on the role of learning and problem solving in enabling organisms to avoid novel selection pressures (Plotkin 1988b; Wcislo 1989; Dukas 2013).

'Robustness', while typically studied at lower biological scales, is a ubiquitous property of biological systems (Kitano 2004; Wagner 2005; Duckworth 2019). In general, to achieve stability at a higher level of organization, the lower scale entities must be in constant action—modifying their behavior and interactions in response to environmental changes. Hence, robust systems maintain a dynamic stability with built-in flexibility that tracks and responds to internal and external changes to maintain a steady output (Nijhout and Reed 2014; Badyaev and Morrison 2018). Behavioral responses and interactions at all biological scales, from molecular interactions and physiological controls to organismal and ecosystem homeostasis, are at the forefront

of how systems maintain robustness in the face of environmental change. Thus, it is not a foregone conclusion that greater behavioral complexity will necessarily drive evolutionary changes in other traits.

The systems approach also provides a framework for understanding how robust systems might change when encountering environments outside the normal range. When a complex system experiences a disturbance that is outside its normal environmental range, it can either adapt and improve or the system may fail completely. In evolutionary terms, populations, which can be thought of as complex systems, will either evolve or go extinct. Behavior is thought to be a driving force in evolution during times of major disturbance because behavioral changes are at the forefront of enabling organisms to respond rapidly and survive (West-Eberhard 2003; Losos et al. 2004). Moreover, once a novel behavioral pattern is established, it can create a consistent organism/environment interaction that stabilizes any changes in natural selection. Thus, while behavioral flexibility can enable organisms to avoid novel selection pressures under normal conditions, it also can take the lead in driving evolutionary changes during periods of disturbance by enabling organisms to persist through responses that themselves create novel selection pressures.

Consequently, from a systems perspective, the debate about behavior's role as a driver versus inhibitor of evolutionary change is a false dichotomy. Behavioral interactions and responses create a stable system of feedbacks and interactions when populations experience environmental variation within their historical norm, but unexpected perturbations can disrupt an otherwise steady dynamic equilibrium so that a population must either evolve or go extinct. It is the behavior and interaction of organisms that either maintains the stable state or directs subsequent changes in the event of a major disruption. The most important insight here is that incorporating behavior into evolution requires a shift from a static perspective, where organisms are passive recipients of mutations and natural selection, to a focus on evolutionary dynamics, where the interaction and behavior of organisms is an important co-determiner of the selective environment. This perspective requires a greater emphasis on the feedbacks and interactions across biological scales that provide the mechanistic underpinnings of evolutionary dynamics (Duckworth 2019).

Returning to our question at the beginning of this chapter, is behavior special? The answer is both 'yes' and 'no.' Behavior *is* special in the sense that it is an essential part of understanding evolutionary mechanisms because the underlying causes of patterns cannot be understood without reference to the behavior of the entities involved. But behavior is *not* special in the sense that this truism is such a basic tenet of most scientific disciplines—from molecular biology to chemistry to physics—that it does not even need to be stated. To understand how complex systems work, molecular biologists study enzyme kinetics and flux of molecular pathways; chemists study diffusion gradients, and the behavior and dynamics of how molecules interact and transform; and physicists study the movement and interaction of all matter, from the smallest subatomic particles to the largest bodies in the universe. Investigating how the behavior and interaction of entities at one scale produces patterns at another is the main object of scientific inquiry (Levin 1992).

Behavior is special in evolution simply from its absence as a core focus in evolutionary research. A static view of evolution treats the dynamics of biological systems as only relevant for studying within-generation phenotypic change and thus separates developmental dynamics, physiology, behavior, and ecological interactions from the study of genetic variation and selection. Yet, the former are processes that unfold each generation as the building blocks of longer-term evolutionary change and are key to not only understanding but also predicting evolutionary dynamics.

The implication of shifting from a static to dynamic perspective in evolution is that studying how organisms respond to their environment and how they interact is key to gaining a full picture of the causes of both evolutionary stasis and change. But, while studying dynamics is important across scientific disciplines, the behavior of living organisms is clearly different than the behavior of inanimate matter. Furthermore, the complexity of behavioral responses is also highly variable across taxa. This brings us back to Plotkin's distinction between behavior of "a choosing intelligence" and all other behavior and whether this distinction is important for understanding behavior's role in evolution. It is likely that different levels of behavioral complexity (e.g., social behavior, types of learning, levels of problem-solving) influence evolutionary dynamics in different ways. However, rather than implying that behavior's causal role in evolution is limited to a small subset of taxa, characterizing the fundamental differences in the complexity of behavior among taxa and determining their consequences are the core problem to be investigated in the field of evolutionary dynamics.

12.5 CONCLUDING REMARKS

In this chapter, we have emphasized that behavior is what biological organisms do and applies to all life, from the movement of bacteria to the growth of plants toward a source of light, from the aggressive defense of a territorial boundary to the construction of a home to live in. These interactions of organisms and their responses to the environment are what underlie the dynamics of both evolutionary stasis and change, and we suggest that the main challenge of the future is to determine how different types of behaviors may produce both similar and distinct evolutionary dynamics.

Evolutionary biology has long endured calls for a more expansive conceptual framework, from the emergence of evo-devo in the 1980s (Arthur 2002; Gilbert 2003; Love 2003) to recent arguments for an extended evolutionary synthesis (Laland et al. 2015). Integrating static and dynamic views (Box 12.1) of evolutionary theory may resolve many of these debates. A static view focuses on detecting patterns of variation in traits and genomes using more and more sophisticated statistical tools to deconstruct sources of variance and determine correlative links between the two. A dynamic view asks what are the processes that have led to these patterns. Neither of these views is better than the other; instead they complement one another. The search for pattern is essential to highlighting the important evolutionary phenomena to be explained, and the study of the developmental, behavioral, and ecological dynamics underlying these patterns is essential to determining their mechanisms. In Box 12.2, we offer some suggestions for future research.

BOX 12.2 SUGGESTIONS FOR FUTURE RESEARCH

- Investigation of the mechanisms and repertoire of behaviors in non-neural taxa. It is clear that plants, fungi, and unicellular organisms have sophisticated systems of detecting and responding to environmental variation; however, the extent that they can store and analyze information to influence future behavioral responses is less clear. We encourage additional rigorous and replicated experiments on learning and memory in a diversity of non-neural taxa.
- Determine how differences across taxa in problem-solving ability, social behavior, memory, and learning may influence evolutionary dynamics in different ways. Recent work putting together supertrees across vertebrates and other taxa means that we have more tools available to ask these questions than ever before. For example, are there really differences across taxa in their diversification rates that can be attributed to differences in cognitive behavior? Do these associations change depending on the timescale of the investigation? Comparative studies, combined with renewed research efforts on the behavioral capabilities of non-neural organisms, means that we are poised to make exciting new advances in this field.
- Integrating static and dynamic views of evolutionary theory. This requires viewing quantitative and population genetic studies as a starting point of evolutionary inquiry. These studies are essential for pointing out interesting patterns of selection and changes in genetic variation over time, but they provide an incomplete picture of causality in evolution without an understanding of how exploitative systems determine selective environments in the first place, and how epigenetic systems link genotype to phenotype.

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13 Plasticity across Generations

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CONTENTS

13.1 PLASTICITY AS A FORM OF NONGENETIC INHERITANCE

Previous chapters have outlined the classic concept of plasticity, its mechanistic basis, and its adaptive importance. Plasticity is the ability of an individual organism or genotype to respond in a consistent way to environmental conditions, either during development ('developmental plasticity') or during adulthood (sometimes called 'phenotypic flexibility,' Piersma and Drent 2003). All organisms are plastic in at least some of their traits, and some plastic responses represent evolved strategies that enhance fitness under certain conditions, while other plastic responses represent maladaptive effects of stress or pathology. Plasticity can operate through a variety of cellular, physiological, and behavioral mechanisms. Among these, epigenetic processes such as the modification of DNA methylation patterns and/or chromatin structure in certain genomic regions are believed to play a particularly important role (Duncan et al. 2014; Paksa and Rajagopal 2017). Such environment-induced epigenetic changes result in modified patterns of gene expression and thus mediate changes in phenotypic traits and fitness.

Plasticity in the classic sense involves individuals responding to environments that they experience during their lifetime ('within-generation plasticity'). For example, many organisms respond to predator cues by expressing defensive traits: plants grazed by herbivores can elevate investment in morphological and chemical

DOI: 10.1201/9780429343001-13

defenses against herbivory (War et al. 2012), while *Daphnia* hatchlings that encounter chemical cues associated with predation express defensive spines (Laforsch and Tollrian 2004). However, such plastic effects were long assumed to be lost between generations because it was believed that genes are the only factors that are transmitted to offspring and that can influence offspring development. Since genes could not be altered in consistent ways by the environment, it followed that environmental effects on bodily traits (the phenotype) could not be transmitted across generations, and 'Lamarckian' processes such as the 'inheritance of acquired traits' were not possible (for discussion of the history of these ideas see Bonduriansky 2012; Bonduriansky and Day 2018). According to this classic view, you can damage your own health by avoiding exercise, eating an unhealthy diet, or smoking, but your children and grandchildren will not be born any less healthy as a result. Rather, each individual's traits are shaped by its own unique genotype (the combination of genetic alleles determined by the random lottery of recombination) and by the environment that it experiences during its own development and lifetime. Environments experienced by ancestors matter only in so far as, by imposing natural selection on genetic variation, they have altered the frequencies of alleles in subsequent generations.

Classic theory allowed only two exceptions to this view. First, in some organisms, the mother's phenotype clearly forms an important part of the developmental environment in which the genomes of her offspring are expressed. Maternal traits involved in offspring care and provisioning were therefore assumed to shape some aspects of development, resulting in maternal effects (Bernardo 1996; Mousseau and Fox 1998). Second, in humans (and perhaps a few other cognitively complex, social animals, such as apes, monkeys, and dolphins), aspects of behavior were assumed to be shaped by culture, which could be transmitted across generations independently of genetic alleles (Mesoudi 2011).

Yet, it is now clear that many of the epigenetic and physiological mechanisms involved in plastic responses can also affect gamete formation and offspring development. Many nongenetic factors are transmitted across generations alongside genes, and variation in such nongenetic factors can have important effects on offspring phenotypes and fitness. Because many aspects of reproductive physiology are highly sensitive to environmental influence and can respond in consistent ways to particular environmental factors or stresses, some effects of ancestors' environment (including, as it turns out, effects of physical activity, diet, and smoking) can be transmitted to subsequent generations. Such effects are called transgenerational plasticity (Galloway and Etterson 2007; Bell and Hellmann 2019).

Transgenerational plasticity is a form of nongenetic inheritance that includes classic maternal effects such as effects of maternal diet (Bernardo 1996; Mousseau and Fox 1998) and can encompass effects of learning and culture in some mammals, birds, and other animals in which individuals can adjust their behavior to the environmental conditions that they encounter and then pass on those behaviors to their offspring via learning (Jesmer et al. 2018; Aplin 2019). However, the current understanding of transgenerational plasticity is much broader: transgenerational plasticity can encompass a much wider range of environmental factors and phenotypic effects than hitherto appreciated; can involve effects of environments experienced by both mothers and fathers; and, in some cases, can involve effects of environments

experienced by grandparents and even more remote ancestors. Transgenerational plasticity is therefore a phenomenon that was not recognized in classic Modern Synthesis theory, and its scope and importance have only recently come to be widely recognized by evolutionary ecologists.

Transgenerational plasticity is a young research field: many questions remain to be answered, and there is a great deal of controversy surrounding the fitness effects and evolutionary implications of this phenomenon (for a discussion of the history and current state of this controversy, see Bonduriansky and Day 2018). In this chapter, I will attempt to clarify some aspects of this controversy by considering the nature of transgenerational plasticity and its potential to influence the course of evolution. In particular, I will consider whether it is reasonable to assume that transgenerational plasticity is typically adaptive and whether transgenerational plasticity has the potential to provide variation that natural selection can act on to drive adaptive evolution. I will argue that, like classic plasticity, transgenerational plasticity need not be adaptive, and researchers should be very careful in inferring that an observed pattern of transgenerational plasticity represents an evolved, adaptive response. Yet, I will also argue that non-adaptive instances of transgenerational plasticity can be just as interesting and as important as adaptive transgenerational plasticity, and have the potential to play a substantial role in adaptive evolution.

13.2 WHAT IS TRANSGENERATIONAL PLASTICITY?

Nongenetic inheritance (or *parental effects* in the broadest sense) involves effects of ancestors on descendants that are not mediated by the transmission of genetic alleles. Transgenerational plasticity is the sub-set of nongenetic inheritance that involves nongenetic transmission of effects consistently induced by particular environmental factors (Bell and Hellmann 2019). Transgenerational plasticity excludes effects that genes expressed in parents have on offspring (*indirect genetic effects*), as well as effects of nongenetic factors that are unaffected by environment or affected in unpredictable ('random') ways (analogous to effects of mutagens on DNA sequences).

Many examples of transgenerational plasticity have been reported, affecting health, behavior, morphology, life history, and fitness (for examples see Bonduriansky and Day 2018; Bell and Hellmann 2019). For example, in some species, exposure to predator cues can induce the expression of defensive traits not only in individuals that are directly exposed to those cues but also in their offspring. Thus, plants attacked by herbivores can produce offspring with elevated defenses (Colicchio 2017), and some *Daphnia* exposed to predator cues produce offspring that express defensive spines even in the absence of predators (Agrawal et al. 1999). In such cases, the effects on offspring or grand-offspring are very similar to direct effects of environment on individual development. Such cases are clearly analogous to plasticity in the classic sense and may be mediated by the same proximate factors. For example, predator cues might induce changes in DNA methylation or chromatin structure that cause the expression of defensive traits, and those same epigenetic changes may occur in the germ-line and induce similar developmental effects (albeit not necessarily of the same magnitude) in offspring. However, in other cases, the direct effect of a given environmental factor may be quite different from its effect on offspring.

For example, many fish, including sticklebacks, respond to predator cues by adopting risk-minimizing behaviors (Wund et al. 2015), but the offspring of predator-exposed sticklebacks do the opposite, behaving in ways that increase rather than reduce their risk of being eaten (McGhee et al. 2012). Such counterintuitive effects appear to be non-adaptive and could result from the transmission of stress-induced changes in epigenetic factors such as DNA methylation. Many other environmental factors, such as toxins, diet, and social environment, can also affect offspring development (Bonduriansky and Day 2018; Bell and Hellmann 2019).

While studies at the whole-organism level have revealed many examples of transgenerational plasticity involving a great variety of environmental factors and organismal traits, the proximate mechanisms mediating such effects are still poorly understood in most systems. Transgenerational plasticity involves a complex causal chain linking an environmental exposure to changes in parental reproductive physiology, a resulting change in some factor that is transmitted from parent to offspring (such as a DNA methylation pattern, small RNA, histone, hormone, or nutrient), and a consequent cascade of developmental effects that induce a change in offspring phenotype (Figure 13.1). This process is often viewed as a signaling system whereby parents transmit 'information' or 'cues' to their offspring so as to enhance offspring

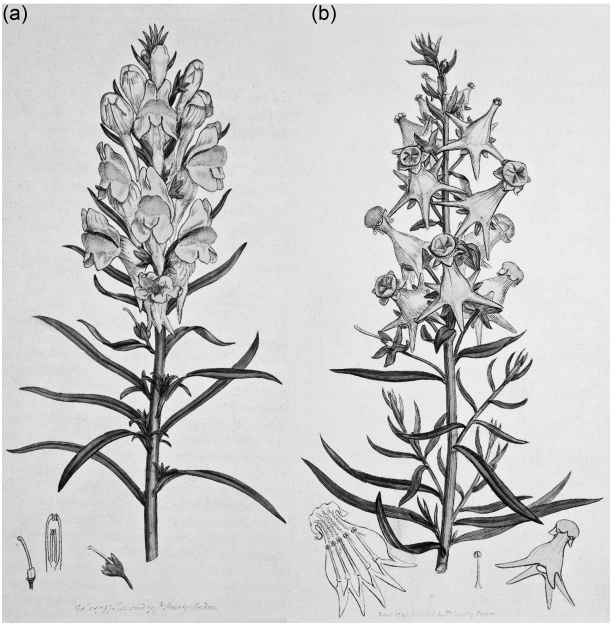

FIGURE 13.1 The normal (a) and peloric (b) flower forms of toadflax (*Linaria vulgaris*), first described by Carl Linnaeus. Peloric flower morphology is caused by reduced DNA methylation of the *Lcyc* gene—an epigenetic variant ('epiallele') that can be transmitted from parent to offspring over several generations. It is not known whether this epigenetic factor can be induced by the environment, and thus whether this famous example of nongenetic inheritance represents an instance of transgenerational plasticity. (Illustrations by James Sowerby, courtesy of the John Innes Foundation.)

fitness. Yet, many instances of transgenerational plasticity appear to involve non-adaptive or even deleterious effects, and offspring can be selected to disregard such parental effects (Bernardo 1996; Uller 2008; Bell and Hellmann 2019). To my knowledge, this causal chain has not yet been fully characterized in any organism, but studies on model organisms such as plants, mice, nematode worms, and other organisms are providing an increasingly detailed picture of parts of this chain. For example, a recent study on mice shows that psychological stress can affect the RNA content of membrane-enclosed 'extracellular vesicles' that are released by cells throughout the body and within the epididymis (where sperm undergo maturation), that these vesicles can transport their RNA cargo to developing spermatozoa, and that ovules fertilized by such spermatozoa develop into offspring with altered growth and stress responses (Chan et al. 2020). This study provides an unusually detailed picture of how stressful experiences can affect the RNA cargo of sperm. Yet, many steps in the causal chain remain to be filled in, including how psychological stress induces consistent changes in extracellular vesicle content, and how the transmission of these factors affects embryonic development so as to produce the observed phenotypic effects. Interestingly, a recent study shows that RNA can be transported from the mouse brain to the testes and then transmitted to embryos, suggesting a potential means for stress- or experience-induced changes in gene expression in the brain to lead to altered RNA content in the germ-line (O'Brien et al. 2020).

13.3 THE ROLES OF MOTHERS AND FATHERS

Maternal transgenerational plasticity (i.e., environment-induced 'maternal effects') has been recognized for a long time, although its scope and importance in ecology, evolution, and health was under-appreciated until quite recently (Kirkpatrick and Lande 1989; Bernardo 1996). Maternal effects were long treated as a nuisance factor in quantitative-genetic studies, but have now come to be seen as a fascinating and important phenomenon in their own right. Given the intimate physiological and behavioral interaction between offspring and their mothers in many animals, many examples of maternal effects are mediated by variation in maternal care. For example, in rats, variation in maternal behavior (e.g., induced by maternal stress) can induce epigenetic (DNA methylation) changes in the offspring brain, and these changes can persist throughout life and affect the adult behavior and stress responses of the offspring (Weaver et al. 2004; Champagne and Meaney 2007).

In contrast to maternal effects, paternal transgenerational plasticity was almost entirely ignored and, indeed, assumed to be virtually impossible in most systems until fairly recently. Because males do not interact with their offspring in most species (including classic model animals such as *Drosophila* and mice), it was assumed that males could not influence their offspring nongenetically. Yet, a variety of such mechanisms are now recognized (including modification of methylation and chromatin structure in sperm-borne DNA, noncoding RNA in sperm and seminal fluid, seminal proteins, and other factors), and many examples of paternal transgenerational plasticity have now come to light (Crean and Bonduriansky 2014; Soubry et al. 2014). For example, as mentioned above, male mice that experience severe stress produce offspring with altered growth and stress responses, and these effects

are mediated by molecules transmitted in the sperm (Chan et al. 2020). Paternal transgenerational plasticity may yet turn out to be as widespread and important as maternal transgenerational plasticity. Although opportunities for father-offspring influence are limited in most animals because males do not provision, nurture, or interact with their offspring (Crean and Bonduriansky 2014), the sperm epigenome may be highly susceptible to environment-induced modification and damage, potentially enabling a wide variety of epigenetically mediated effects of paternal environment to be transmitted through the male germ-line (Pembrey et al. 2014).

It is now well established that both maternal and paternal health can have profound effects on the development and health of the offspring. For example, in rats, offspring metabolism is strongly affected by paternal diet (Anderson et al. 2006; Ng et al. 2010). Paternal obesity can also affect children's health in humans via paternal influence on children's lifestyle as well as sperm-borne epigenetic factors (Donkin et al. 2016; Sharp and Lawlor 2019). Likewise, in both rodents and humans, there is abundant evidence that offspring metabolism can be strongly affected by maternal obesity (Amarger et al. 2014; Dominguez-Salas et al. 2014; Kamimae-Lanning et al. 2014). Indeed, in humans and other mammals, there is potential for complex interactions and reinforcement between effects of parental environment transmitted through the germ-line, intra-uterine effects mediated by maternal health, and postnatal effects mediated by maternal (and, in some species, also paternal) behavior (Archer 2015).

13.4 THE STABILITY OF TRANSGENERATIONAL PLASTICITY

Maternal effects were long assumed to be mediated by direct mother-offspring interaction in utero and via maternal care. However, in mammals, the germ-line develops early in embryonic development, and female embryos in the womb already contain their own eggs. These eggs could be subject to the same intra-uterine effects as the embryonic soma, resulting in grand-maternal effects (Youngson and Whitelaw 2008). While intra-uterine effects are limited to two generations, some epigenetic processes have the potential to mediate transgenerational plasticity across multiple generations (Jablonka and Raz 2009; Boskovic and Rando 2018). For example, some variable patterns of DNA methylation ('epialleles')—such as the peloric epiallele of the *Lcyc* gene that causes altered flower form in toadflax (*Linaria vulgaris*) (Figure 13.1)—appear to be stable over several generations (Cubas et al. 1999). Other non-genetic factors, such as self-regenerating gene expression loops, have the potential to persist over multiple generations as well (Jablonka and Raz 2009). Multigenerational transgenerational plasticity can occur through patrilines as well as matrilines. For example, in mice and humans, exposure to toxins can be transmitted through patrilines to grand-offspring and beyond (Chen et al. 2006; Pembrey et al. 2014; Skinner 2014). Likewise, in sticklebacks, exposure to predators induces grand-paternal effects that also differ by offspring sex (Hellmann et al. 2020). Such multigenerational examples of transgenerational plasticity could have very interesting implications for phenotypic variation and adaptive evolution (discussed below), but relatively few experimental studies have investigated the potential for transgenerational plasticity to persist over more than two generations, or for environmental effects that

act over multiple generations to accumulate and interact (Rutkowska et al. 2020). The proximate mechanisms that mediate the transmission of environmental effects across multiple generations remain poorly understood, although considerable progress in the understanding of such mechanisms has come from work on nematode worms (Greer et al. 2010; Greer et al. 2011; Klosin et al. 2017; Rechavi and Lev 2017). Many patterns of multigenerational transgenerational plasticity have been observed in empirical studies, and a wide range of proximate mechanisms might be involved in generating these patterns (Bell and Hellmann 2019).

The potential for effects of environment to be transmitted over multiple generations is of interest because such effects could provide heritable variation on which natural selection can act (Day and Bonduriansky 2011; Klironomos et al. 2013; Furrow 2014). However, even effects that fade out after a single generation could still be very important in many evolutionary contexts. For example, such effects could play an important role in allowing populations to persist in fluctuating environments (Lachmann and Jablonka 1996; Dey et al. 2016; see also Diamond and Martin 2021 in this volume). Transient effects of parental environment also have the potential to play important roles in coevolution between the sexes (Bonduriansky and Day 2013) and between hosts and parasites (Qutob et al. 2013; Gijzen et al. 2014; Bonduriansky and Day 2018), gene-culture coevolution (Feldman and Cavalli-Sforza 1989; Richerson and Boyd 2005), and even in speciation and diversification (Jablonka and Raz 2009; Pfennig and Servedio 2012). Of course, as noted above, the effects of parental environment on offspring can also have very important implications for human health. In Box 13.1, I discuss some of the terminology used to describe transgenerational plasticity.

BOX 13.1 DESCRIBING TRANSGENERATIONAL PLASTICITY

Because transgenerational plasticity has only recently become a major focus of research, terminology relating to this phenomenon is still evolving and the literature is rife with confusing jargon. The effects of parents on their offspring are sometimes called ‘intergenerational’ while effects spanning more than one generation are called ‘transgenerational.’ While this terminology is still widely used in studies on transgenerational plasticity (especially in mammals), its utility is limited. Examples of transgenerational plasticity effects spanning three, four, five, or more generations can be found in the literature but, given that most studies of transgenerational plasticity span a single generation, the terms used to describe the observed effects are often based on the number of generations over which a given effect has been investigated rather than the number of generations over which the effect can actually be transmitted. Moreover, the potential stability of a given effect over multiple generations could depend on the environment in which descendants are assayed, or even the genotype of the organisms used. To avoid confusion, researchers could simply report the number of generations over which a given effect has been studied or, in a multi-generation study in which an effect was observed to fade

out after a certain number of generations, report the number of generations over which the effect could be detected.

Likewise, factors such as DNA methylation could mediate transgenerational plasticity in diverse ways. In particular, studies on transgenerational plasticity should clearly differentiate the *transmitted factor*—that is, the environment-dependent entity (e.g., DNA methylation pattern, small RNA, hormone, nutrient, symbiont, or learned behavior) that is passed from parent to offspring through the germ-line, the intrauterine environment or post-hatching/post-partum parental care—from the consequent *developmental cascade* in the offspring (such as changes in offspring growth rate, or altered patterns of DNA methylation in the offspring brain) that brings about changes in offspring phenotype. For example, an effect mediated by the transmission of an environment-dependent pattern of DNA methylation through the germ-line can be regarded as an instance of epigenetic transmission, while an effect mediated by a parental behavior that induces changes in DNA methylation in the offspring brain can be regarded as an instance of behavioral transmission. The nature of the transmitted factor and the developmental cascade can have important implications for the environmental inducibility, stability across generations, sex-specificity, and taxonomic distribution of transgenerational effects (Bonduriansky and Day 2018).

13.5 THE EVOLUTION OF ADAPTIVE TRANSGENERATIONAL PLASTICITY

Like within-generation plasticity (see Pfennig 2021 in this volume), transgenerational plasticity can evolve as a facultative, fitness-enhancing strategy. The most widely recognized form of adaptive transgenerational plasticity is the ability of mothers to adjust the development of their offspring and thereby enhance offspring fitness in a similar environment. For example, mothers that experience predator cues can benefit by producing offspring that are primed to express anti-predator defenses (Agrawal et al. 1999), and solitary ascidians (*Styela plicata*) that develop at low density sire offspring that have enhanced performance under low-density conditions (Crean et al. 2013). Recognized at least since the 1990s (Bernardo 1996), such effects are often called 'anticipatory' parental effects (Marshall and Uller 2007). Anticipatory effects will have a net positive effect on the fitness of both mothers and their offspring when the environment experienced by parents is generally predictive of the environment experienced by offspring. According to theory, anticipatory transgenerational plasticity should therefore evolve when the environment fluctuates predictably between alternative states (e.g., predators abundant/predators rare, hot/cold, wet/dry), and the period of fluctuations is sufficiently long to generate a temporal autocorrelation across generations (Lachmann and Jablonka 1996; Proulx and Teotonio 2017). These predictions were supported by an experimental study showing that anticipatory transgenerational plasticity evolves rapidly in the nematode worm

Caenorhabditis elegans when the environment fluctuates predictably between two states (high/low oxygen), but does not evolve when environmental fluctuations are random (Dey et al. 2016). While the evolution of anticipatory transgenerational plasticity is usually considered in the context of temporal fluctuations between environmental states, the same logic applies in the case of spatial heterogeneity. For example, if individuals can exploit different host types, and parents can predict the host type on which their offspring will develop, then selection might favor anticipatory effects that enhance offspring performance on the expected host type (Fox et al. 1997). Anticipatory transgenerational plasticity is usually detected using experiments that manipulate both parental and offspring environments. The prediction is that offspring performance is enhanced when the offspring develop in the same environment as their parents (Figure 13.2a; but see Engqvist and Reinhold 2016).

Anticipatory transgenerational plasticity represents an alternative to within-generation plasticity: individuals can enhance their fitness either by responding to environmental conditions that they themselves experience, or to environmental conditions experienced by their parents. But which option is better? The broad insight from theoretical studies is that within-generation plasticity can be advantageous because it allows for a more immediate response to prevailing conditions, while anticipatory transgenerational plasticity can be favored because it can give offspring a developmental head start (Uller 2008). For example, if developmental response to predator cues takes time, offspring that encounter such cues may not be able to develop defenses quickly enough to avoid predation. Transgenerational plasticity may allow an anti-predator defense to begin developing earlier, even before offspring themselves actually encounter predator cues, or develop the sensory organs required to detect such cues. On the other hand, environmental conditions that prevailed during the previous generation can be an unreliable cue to the conditions that offspring will experience, and fitness is reduced if offspring end up developing in a mismatched environment (for example, investing in costly defenses when predators are absent). The less predictable the environment is across generations, the greater the advantage of within-generation plasticity over anticipatory transgenerational plasticity. Depending on the balance of these costs and benefits, selection could therefore favor within-generation plasticity only, transgenerational plasticity only, some combination of within-generation plasticity and transgenerational plasticity, or no plasticity at all (Leimar and McNamara 2015; McNamara et al. 2016).

The physiological, sensory, and neural mechanisms involved in detecting and assessing environmental cues are expected to impose costs, although the nature and magnitude of such costs remain poorly known (Van Buskirk and Steiner 2009; Murren et al. 2015; see also Snell-Rood and Ehlman 2021 in this volume). As noted above, plastic responses can also be prone to error when environmental cues are unreliable. The epigenetic machinery that mediates transgenerational plasticity is likely to impose metabolic costs as well, although the nature and magnitude of such costs are poorly understood (Macartney et al. 2018). If transgenerational plasticity is indeed costly, and especially if environmental conditions remain relatively constant for many generations, reducing the benefits of transgenerational plasticity, then selection could favor the replacement of transgenerational plasticity by an

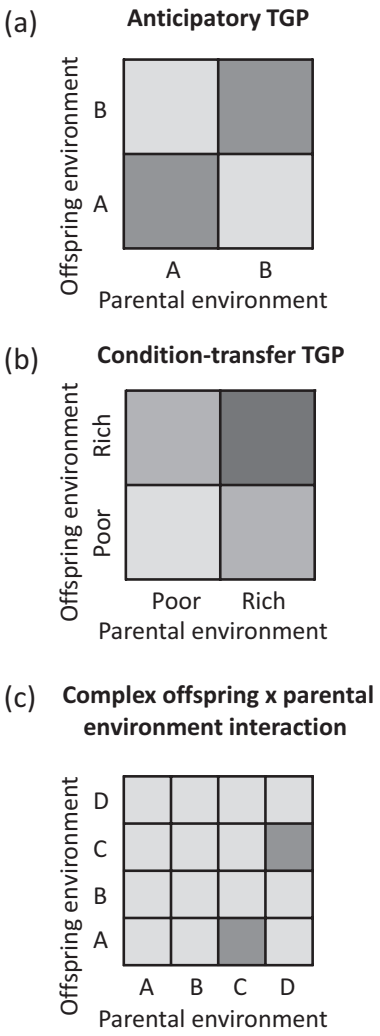

FIGURE 13.2 Potential effects of parental environment and offspring environment on offspring fitness (darker gray = higher fitness): (a) anticipatory transgenerational plasticity (TGP) is reflected in an interaction between parental and offspring environment, whereby offspring perform well when they develop in a similar environment to that experienced by their parents, but poorly when offspring environment is mismatched to the parental environment; (b) condition-transfer transgenerational plasticity is reflected in enhanced performance of offspring of high-condition parents across a broad range of environments, and perhaps especially in resource-poor environments (where, by contrast, an anticipatory effect would be expected to result in reduced performance of offspring produced by high-condition parents); (c) a complex interaction of parental and offspring environments where certain environment combinations appear to be associated with enhanced offspring performance. Such complex interactions are unlikely to represent evolved, adaptive transgenerational plasticity unless the particular environment combinations that are associated with enhanced offspring performance are commonly encountered in nature.

environment-independent developmental program—an evolutionary process called 'genetic assimilation' (see Scheiner and Levis 2021 in this volume). If a formerly fluctuating environment becomes stable, the most advantageous phenotype that formerly resulted from an epigenetic factor can therefore become genetically 'fixed' (Pál and Miklós 1999). For example, if predator abundance formerly fluctuated between high and low values but eventually stabilized at a high value, selection might favor all individuals developing anti-predator defenses without having to respond to either parental cues or the current environment.

While anticipatory effects are the most widely recognized form of adaptive transgenerational plasticity, another type of adaptive transgenerational plasticity that might be equally widespread and important is the transmission of condition-dependent nongenetic factors from parents to their offspring ('condition transfer'; Qvarnström and Price 2001; Bonduriansky and Crean 2018). Condition transfer can occur when gamete or seminal fluid production, parental investment, or other parental traits that affect offspring are condition-dependent in their expression, resulting in a correlation between parental and offspring condition. For example, Atlantic salmon (*Salmo salar*) that spend longer at sea and thereby grow to a larger size prior to spawning produce larger eggs and longer and heavier offspring (Van Leeuwen et al. 2016). Similarly, in the marine fish *Acanthochromis polyacanthus*, high-condition parents produced offspring that were longer and heavier and had enhanced viability (Donelson et al. 2009). Some degree of condition transfer is probably inevitable because reproduction is costly, and low-condition parents may be unable to provide as well for their offspring as high-condition parents can. Condition transfer is obviously disadvantageous when parents are in poor condition, and some authors have therefore argued that condition transfer should be regarded as non-adaptive. However, condition transfer is likely to enhance fitness on average. Indeed, natural selection may often drive the evolution of heightened condition-dependence of reproductive traits, for the same reasons that selection often favors heightened condition-dependence of other costly fitness-related traits, such as sexual signals. High-condition individuals leave more progeny on average than do low condition individuals, and any strategy that benefits high-condition individuals will therefore be under positive net selection. High-condition parents will benefit by transferring condition-enhancing nongenetic factors to their offspring because this will tend to enhance offspring performance across a broad range of environments, and selection is therefore expected to promote heightened condition-dependence in many reproductive traits (Bonduriansky and Crean 2018).

Detecting condition transfer involves showing that offspring of high-condition parents perform better across a broad range of environments than do offspring of low-condition parents. Like anticipatory transgenerational plasticity, condition transfer can be detected using experiments where the quality of parental and offspring environments is manipulated in a fully crossed design. However, unlike anticipatory transgenerational plasticity, condition-transfer transgenerational plasticity predicts that high-condition parents (i.e., parents reared or maintained in a resource-rich environment) will produce offspring that perform better across a range of environments, and do not suffer fitness costs when offspring environment is mismatched to parental environment. In particular, while anticipatory transgenerational plasticity predicts

that offspring of low-condition parents would outperform offspring of high-condition parents in a resource-poor environment, condition-transfer transgenerational plasticity predicts that offspring of high-condition parents would have an advantage in both resource-poor and resource-rich environments (Figure 13.2b; Donelson et al. 2009; but see Engqvist and Reinhold 2016).

13.6 WHOSE FITNESS?

Unless offspring are clones of their parents, the fitness interests of parents and their offspring are non-identical. Parents are selected to shape offspring development in a way that enhances parental fitness, and Marshall and Uller (2007) therefore defined adaptive parental effects as effects that enhance parental fitness, irrespective of effects on the fitness of individual offspring. For example, mothers in high condition might be selected to increase offspring number while reducing offspring body size, a strategy that might enhance maternal fitness while reducing the fitness of individual offspring. Such a pattern is observed in the mosquitofish (*Gambusia holbrooki*), where mothers reared at low density and abundant food produced more and smaller offspring than mothers reared at high density and limited food (O'Dea et al. 2015). Similarly, in wild chimpanzees, some mothers in high condition appear to wean offspring early in order to produce their next offspring sooner (Thompson et al. 2016). However, offspring are selected to pursue developmental strategies that maximize their own fitness, and selection should therefore favor offspring strategies that counteract parental effects (such as reduced investment per offspring) that reduce offspring fitness. In some cases, transgenerational plasticity could therefore be shaped by parent-offspring conflict (Uller 2008; Kuijper and Johnstone 2018). A full understanding of adaptive transgenerational plasticity requires taking the interests of both parents and their offspring into consideration.

Patterns of transgenerational plasticity could also be shaped by the differing interests of female and male offspring. If the sexes experience different patterns of selection, then transgenerational plasticity could be subject to sexual conflict. For example, if males and females typically experience different environmental conditions and these conditions affect the germ-line epigenome, offspring may receive conflicting epigenetic signals from their mothers and fathers, and may be selected to respond differently to these signals. If sex-specific ecology is relatively stable over generations then selection will generally favor offspring that respond to epigenetic signals received from the same-sex parent while ignoring epigenetic signals received from the opposite-sex parent. Genes that control offspring responses to epigenetic signals transmitted from their parents could therefore be subject to sexually antagonistic selection, and this could result in a range of adaptive or maladaptive patterns of transgenerational plasticity. Individual-based simulations show that, if a single locus controls offspring responses to parental signals, then adaptive transgenerational plasticity may fail to evolve because benefits to one sex will be balanced by costs to the other sex. However, if the locus is duplicated, or if sex-linked modifiers evolve, allowing offspring to respond only to epigenetic signals from their same-sex parent, then an adaptive, sexually dimorphic pattern of transgenerational plasticity may evolve (Burke et al. 2020).

13.7 WHY TRANSGENERATIONAL PLASTICITY IS OFTEN NON-ADAPTIVE

It's tempting to assume adaptive function for all or most observed patterns of transgenerational plasticity, but this assumption is unwarranted and probably reflects a lingering bias in how researchers think about nongenetic versus genetic inheritance. Biologists are accustomed to the idea that much of the variation that is transmitted genetically is non-adaptive. Genetic mutations rarely enhance fitness. All gametes carry deleterious alleles, and zygotes incorporate such alleles into the offspring genome. Biologists rarely ask why deleterious alleles (as well as transposons and other 'junk DNA') are faithfully transmitted from parents to their offspring. We simply assume that all these DNA sequences are transmitted across generations because there is no way to filter out the good from the bad.

Most instances of nongenetic inheritance should be viewed in much the same way. Non-adaptive transgenerational plasticity occurs because many aspects of reproductive physiology are sensitive to environment and, as a result, many environment-dependent factors are transferred across generations as a byproduct of the reproductive process. As with genetic alleles, there may be no way to filter out deleterious factors from advantageous ones. There is ample evidence that old, sick, or malnourished parents tend to produce offspring in poor condition, and such effects are mediated by reduced quality of the gametes, seminal fluid, intra-uterine environment, or post-partum parental care. For example, environmental stressors such as toxins and endocrine disruptors (Skinner 2014), an unbalanced diet (Ng et al. 2010; Kamimae-Lanning et al. 2014), or psychological stress (Mashoodh and Champagne 2014; Schmauss et al. 2014; Zannas et al. 2015) can cause epigenetic dysregulation. If some of these dysregulated epigenetic signatures occur in the germ-line, they could be transmitted to offspring. Moreover, if the ability to maintain the germ-line epigenome (i.e., the pattern of DNA methylation and chromatin structure across the genome) declines with age, environmental stressors may tend to have greater effects on the germ-line with advancing age (Monaghan and Metcalfe 2019). This could explain why older parents tend to produce offspring of lower condition (Wylde et al. 2019), and why the rate of decline in offspring condition with parental age can be modulated by the environment (Gribble et al. 2014). As noted above, non-adaptive or mal-adaptive transgenerational plasticity can also result from parent-offspring conflict or sexual conflict.

Many observed patterns of transgenerational plasticity are therefore probably non-adaptive or even mal-adaptive. Besides conflict between parents and offspring and between sexes (see previous section), non-adaptive patterns of transgenerational plasticity may often result from constraints on the evolution of reproductive physiology. Like within-generation plasticity, many instances of transgenerational plasticity reflect the transmission of pathology or stress and generally reduce the fitness of parents and their offspring. Recognizing the potential for non-adaptive transgenerational plasticity has implications for the interpretation of empirical results. If a seemingly adaptive effect is observed in a few treatment combinations, reflected in a complex parental × offspring environment interaction (Figure 13.2c), it's unlikely to have evolved by selection if that particular combination of ancestral and current

environments rarely occurs. As a hypothetical example, a multifactorial experiment might show that, when parents are maintained in a hot environment and at high density, their offspring show enhanced performance when provided with protein-rich food. To determine whether this pattern can be plausibly interpreted as adaptive, it is necessary to study the ecology of natural populations and establish whether or not this particular combination of parental and offspring environments happens to be common in the wild, thereby providing ample opportunity for natural selection to act on this parental effect. If information on the correlation of parental and offspring environments in the wild is lacking, the pattern of transgenerational plasticity observed in such experiments should not be assumed to represent an adaptive effect. Of course, conclusions about the fitness effects of any form of transgenerational plasticity are subject to all the complexities and caveats involved in quantifying fitness, such as environment specificity, latent effects, and trade-offs (Bell and Hellmann 2019).

13.8 CAN TRANSGENERATIONAL PLASTICITY INFLUENCE THE COURSE OF ADAPTIVE EVOLUTION?

Nineteenth-century evolutionary theories, including the progressive concept of evolution proposed by Jean Baptiste Lamarck (1809) and the theory of 'descent with modification' via natural selection on heritable variation proposed by Charles Darwin (1859, 1871, 1875), were based on the assumption that environment-induced variation could be transmitted to offspring. Darwin even suggested a detailed mechanism of heredity (called 'pangenesis') that enabled the transmission of environment-induced effects through the transfer of particles called 'gemmules' in eggs and sperm. However, with the advent of Mendelian genetics in the early 20th century, such 'inheritance of acquired traits' was deemed incompatible with the nature of genes and declared impossible. As I noted in Section 13.1, all forms of nongenetic inheritance—including transgenerational plasticity, as well as randomly mutating nongenetic factors, and indirect genetic effects—were therefore excluded from modern evolutionary theory. Although important evidence of nongenetic inheritance such as experimental evidence of the transmission of cell structure variants in isogenic lines of single-celled eukaryotes (Beisson and Sonneborn 1965; Nanney 1968), continued to accumulate, such effects were largely neglected by biologists and medical researchers until fairly recently (Sapp 1987; Jablonka and Lamb 1995; Bonduriansky and Day 2018). Nongenetic inheritance began to be rediscovered during the 1980s and 1990s through accumulating evidence of epigenetic inheritance in plants and rodents (Jablonka and Lamb 1995), reports of maternal and paternal environment effects in a variety of plant and animal systems (Bernardo 1996; Mousseau and Fox 1998), and increasing recognition of the importance of parental environment and lifestyle for children's health in humans (Pembrey et al. 2006; Gluckman et al. 2007, 2009; Pembrey et al. 2014).

While just about everyone now acknowledges that transgenerational plasticity is a real and widespread phenomenon, not everyone is convinced that transgenerational plasticity could play an important role in evolution. Some evolutionary geneticists

believe that adaptive evolution can be adequately understood through the traditional perspective of natural selection acting on genetic variation, and that plasticity—either within or across generations—is merely an evolved mechanism that should be viewed as a product of evolution rather than an independent factor that can influence the course of evolution. Proponents of this traditional gene-centric view do not deny that transgenerational plasticity exists, but they tend to downplay its scope and importance. In particular, traditionalists argue that transgenerational plasticity cannot play the same role as genetic mutation in furnishing variation on which natural selection can act because environment-induced traits tend to be less stable than genetic alleles, and because the range of variation that can be induced by environment may be limited to the presence or absence of one particular phenotype. By contrast, some researchers believe that transgenerational plasticity could play a very important role in generating heritable variation and influencing the course of evolution. They point out that examples of highly stable induced phenotypes exist, and that novel environments could induce a broad range of novel developmental responses on which natural selection could act. Moreover, as I explain below, theory suggests that even instances of transgenerational plasticity that do not persist beyond the offspring generation can still have a very substantial influence on offspring development, and thereby influence natural selection on genetic alleles.

If transgenerational plasticity is viewed as a source of heritable variation, then non-adaptive transgenerational plasticity could be just as interesting and important as adaptive transgenerational plasticity. Genetic mutation is an essentially non-adaptive process, but it plays a central role in adaptive evolution by providing a pool of variation for natural selection to act on. It has been suggested that novel forms of within-generation plasticity, expressed in novel environments that reveal cryptic genetic variation and thereby induce a range of novel phenotypes, could play a similar role (reviewed in West-Eberhard 2003; see Futuyma 2021; Levis and Pfennig 2021; and Pfennig 2021 in this volume for a discussion of the history of these ideas). If genotypes express different reaction norms across an environmental gradient, and if some reaction norms bring the phenotype closer to the adaptive peak in the new environment, then selection can favor those genotypes. Moreover, if the new conditions are stable, genetic assimilation of the optimal phenotype can occur (see Scheiner and Levis 2021 in this volume). Similarly, if a novel environment could induce novel forms of transgenerational plasticity, those that enhance fitness could be stabilized via genetic assimilation (see Pigliucci et al. 2006). In this way, forms of transgenerational plasticity (or within-generation plasticity) that were non-adaptive or even maladaptive ancestrally could provide the basis for new, adaptive evolutionary responses. Discussion continues on the role of within-generation plasticity in adaptive evolution, and a number of hypotheses have been proposed (e.g., see Levis and Pfennig 2021; Pfennig 2021 in this volume). However, while many of these ideas also apply to transgenerational plasticity, the role of transgenerational plasticity in adaptive evolution is likely to differ in some ways from that of within-generation plasticity.

First, some forms of transgenerational plasticity can have phenotypic effects that accumulate or interact over multiple generations. If the same environment is

encountered in successive generations, the magnitude of a transgenerational plasticity effect can sometimes increase over several generations, or the effect in one generation can be modulated by effects in the next (Herman et al. 2012; Wylde et al. 2019; Tariel et al. 2020). Selection can therefore act not only on the transgenerational reaction norm (i.e., the function relating a phenotypic trait in descendants to an environmental factor experienced by their ancestors), but also on the way that factors transmitted from parents, grandparents, and even earlier ancestors are integrated in development.

Second, transgenerational plasticity effects can interact with genetic alleles to bring about adaptive evolution. For example, in some insects, males that encounter abundant nutrients during the larval stage develop into large adults that express enlarged secondary sexual traits, and such males also sire larger offspring (Hunt and Simmons 2000; Bonduriansky and Head 2007). Such condition transfer from males to their offspring could select for genetically based female preferences for high-condition males: even if genetic variation in condition is lacking, females can benefit by mating with high-condition males because such males transmit their acquired condition to their offspring (Bonduriansky and Day 2013). Similar effects can occur in the context of host-parasite coevolution. If hosts acquire and transmit to their offspring some degree of resistance to infection, then this form of transgenerational plasticity will select for genetic or nongenetic parasite traits that can overcome the acquired resistance (Gomez-Diaz et al. 2012; Mukherjee et al. 2019). Likewise, if deleterious effects of an environmental stressor are more likely to be transmitted by older parents, resulting in environment-dependent parental age effects, then a change in the environment that results in increased stress might result in declining phenotypic quality and select for alleles that bring about earlier reproduction (Bonduriansky and Day 2018). In this way, a deleterious form of transgenerational plasticity can drive adaptive evolution of life history.

Importantly, such interactions between environment-induced nongenetic factors and genetic alleles can occur even when transgenerational plasticity is limited to single-generation (parent to offspring) transmission. Indeed, because transient environmental effects that are induced *de novo* by environment in every generation represent heritable variation that cannot be depleted by selection, such transient nongenetic factors have the greatest potential to influence genetic evolution (Bonduriansky and Day 2013). Theory therefore suggests that both stable and transient environment-induced effects can play important but distinct roles in adaptive evolution. Relatively stable nongenetic factors that can persist over multiple generations (such as the peloric epiallele of the *Lcyc* gene that influences flower form) could provide abundant heritable variation on which selection can act, potentially allowing for rapid adaptation to a novel environment (Day and Bonduriansky 2011; Bonduriansky et al. 2012; Klironomos et al. 2013; Furrow 2014). Transient nongenetic factors that can only be transmitted over a single generation (such as effects of acquired condition on offspring growth) could interact with more stable nongenetic factors or with genetic alleles to influence the course of evolution (Feldman and Cavalli-Sforza 1989; Richerson and Boyd 2005; Bonduriansky and Day 2013).

Understanding the evolutionary role of transgenerational plasticity will therefore require a combination of empirical and theoretical research. We need data on the potential for transgenerational plasticity effects to accumulate and interact over

multiple generations, the potential for novel environments to induce the expression of novel plastic phenotypes, and the potential for formerly deleterious transgenerational plasticity effects to enhance fitness in novel environments. At the same time, theoretical research is required to identify potential contexts in which transgenerational plasticity can interact with genetic variation to drive adaptive evolution. The available evidence is frustratingly sparse because the studies required to answer these questions have rarely been attempted (see Futuyma 2021 in this volume). Much more work is needed to test ideas and clarify the role of transgenerational plasticity in adaptive evolution. In Box 13.2, I outline some questions for future research on transgenerational plasticity.

BOX 13.2 SUGGESTIONS FOR FUTURE RESEARCH

- Under what conditions does transgenerational plasticity evolve?
- Over how many generations can environment-induced effects persist?
- What proximate mechanisms mediate adaptive transgenerational plasticity, and what are the metabolic/physiological costs of those mechanisms?
- What proximate mechanisms mediate non-adaptive transgenerational plasticity?
- What are the conditions that select for anticipatory versus condition-transfer forms of adaptive transgenerational plasticity?
- Under what circumstances does selection favor genetic assimilation of phenotypes ancestrally engendered by adaptive or non-adaptive transgenerational plasticity, and how much does this process contribute to adaptive evolution?
- Under what circumstances is transgenerational plasticity subject to parent-offspring or sexual conflicts?

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14 How Does Phenotypic Plasticity Fit into Evolutionary Theory?

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Phenotypic plasticity, in the broadest sense, has been recognized in evolutionary biology since its inception, but like several other important topics, became a major focus of inquiry and theory only in the 1960s and 1970s. The question posed in my title requires a historical approach. I will trace the history (as I understand it) of interpretation and research on phenotypic plasticity in evolutionary thought, emphasizing antecedents of today's interpretations, debates, and research, especially on the role of plasticity in trait evolution.

Darwin was very aware of the distinction between inherited variation and that caused by "the direct action of the physical conditions of life" (Darwin 1996 [1859], p. 376), and, as is well known, he acceded slightly to Lamarck's doctrine in the later editions of *The Origin of Species* (see Costa 2021 in this volume). The first substantive attention to plasticity that resonates today was the independent proposition in 1896, by James Baldwin, Lloyd Morgan, and H. Fairfield Osborn, of what Simpson (1953) would later call the 'Baldwin effect.' Simpson interpreted this to be the evolutionary phenomenon whereby "characters individually acquired by members of a group of organisms may eventually, under the influence of selection, be reinforced or replaced by similar hereditary characters" (Simpson 1953). I shall follow Simpson's use of the term 'Baldwin effect,' although other interpretations are possible (West-Eberhard 2003, p. 24; Crispo 2007).

During the earliest days of Mendelian genetics, variation attributable to genotype, environment, and their interaction became clarified. Woltereck (1909) introduced the concept of a genotype's norm of reaction, the array of phenotypes a genotype can produce under different environmental conditions—and thought it could be adaptive. Turesson (1922) recognized habitat-associated forms of many plant species that he called ecotypes, by which he meant "the product arising as a result of the genotypical response of an ecospecies or species to a particular habitat" (Turesson 1922, quoted by Stebbins 1950, p. 42). He and many later botanists, motivated largely by the need to clarify species taxonomy, developed the common-garden method of distinguishing genetic and nongenetic variation. Based on their common-garden study of several species, Clausen et al. (1940) concluded that both heredity and 'modification'

DOI: 10.1201/9780429343001-14

by the environment contribute to differences among species, 'races,' and individuals within populations. They concluded that regional 'races' are heritably distinct ecotypes, not 'modifications,' and that ecotypes generally "are unable to succeed in conditions very unlike those of their native environs." They did not address the adaptive importance of any of the specific features they measured, nor which features might underlie the ecotypes' fitness in native or foreign environs.

Turesson (1922) emphasized that 'modifications' may closely resemble heritable variations. Richard Goldschmidt (1935), now known mostly for his unfortunate ideas about macroevolution, discovered 'phenocopies:' environmental modifications of the phenotype that resembled specific gene mutations. The important conclusion is that specific developmental pathways can be affected similarly by genetic or environmental perturbations. This understanding underlies Waddington's (1942) argument that natural selection builds up 'canalization' of an adaptively important character, buffering it against both genetic and environmental alteration.

In the founding works of the Evolutionary Synthesis, phenotypic plasticity was discussed only marginally. Dobzhansky, in the first edition of *Genetics and the Origin of Species* (1937), referred to the work of Turesson, Clausen et al., and Goldschmidt, and emphasized that "what is inherited…is not this or that morphological character, but a definite norm of reaction to environmental stimuli" that differs between 'wild type' and mutants. In the third edition of his book (Dobzhansky 1951), he drew repeatedly on the views of Schmalhausen (1949), who extensively discussed norms of reaction, shaped, he wrote, by stabilizing selection. Bernhard Rensch (1947, 1959) noted (p. 26) that "Modification [by environment] and mutation often act in the same direction, giving similar phenotypic results (phenocopy)…" In *Variation and Evolution in Plants* (1950), Stebbins wrote that "…in some species natural selection has favored a high degree of phenotypic plasticity in terms of environmental modification, in spite of the low heritability and consequent inefficiency of selection which this brings about" (p. 106). This last phrase, conveying the view that plasticity is likely to reduce the response to selection, was also expressed by Wright (1931), Mayr (1963), and others.

Attention to plasticity increased in the 1950s. For example, Thoday (1953) contrasted developmental and behavioral plasticity. Genetic and nongenetic variation were important to plant and animal breeders, and many experiments with model species (*Drosophila*, *Mus*) had implications for understanding variation in both domesticated and natural populations (cf. Falconer [1981]). In a symposium on this theme, Mather (1955) emphasized that environmental variance in a character could signal either adaptive plasticity or nonadaptive instability. Perhaps most important, and most relevant to current concerns, were Waddington's experiments and ideas about canalization and genetic assimilation (Waddington 1942, 1952, 1953a,b). Waddington, like Schmalhausen, proposed that stabilizing selection for an optimal phenotype would result in the evolution of canalization, whereby phenotypic effects of environmental (or genetic) perturbations are minimized (see Scheiner and Levis 2021 in this volume). Sufficiently strong, perhaps novel environmental or genetic perturbations of development may exceed a canalization threshold and reveal phenotypic variation. If this variation has a genetic component, selection can act to

favor a different mean phenotype that, in turn, can become canalized—in which case it appears that an environmentally induced phenotype has become inherited, or 'genetically assimilated.' In one experiment, Waddington acted on his observation that a heat shock during pupal development resulted in *Drosophila* adults with an interrupted crossvein in the wing. By breeding from flies that showed the most pronounced effect of the heat shock, he eventually produced a lineage that lacked the crossvein even without heat shock. He interpreted the result to mean that many genes affect the susceptibility of vein development to this environmental stimulus.

This idea—that a phenotypic state expressed at first in response to an environmental stimulus may become more genetically determined—is much the same as Simpson's description of the 'Baldwin effect;' Simpson's Baldwin effect and Waddington's genetic assimilation are much the same hypothesis, for all practical purposes. In some of today's literature (e.g., Chevin and Hoffmann 2016; Scheiner et al. 2017), two kinds of loci are envisioned: those with a fixed effect on a trait (i.e., a flat norm of reaction) and those with a plastic effect (lending a nonzero slope to the reaction norm). Genetic assimilation is the replacement of plastic allelic effects with fixed effects.

Although Waddington's genetic assimilation is often thought to challenge the Evolutionary Synthesis, I have been impressed by how little it seems to have bothered adherents to the traditional view. Mayr (1959) accepted Waddington's interpretation of his experiment on the *crossveinless* phenotype: "We are here simply dealing with a threshold phenomenon where numerous genes contribute to a certain phenotype but where the potentiality for it will not be pushed above the visible threshold until a sufficient number of genes have accumulated in the genotype" (Mayr 1959). Dobzhansky (1970) accepted that 'canalizing selection' can result in traits that show little or no variation, but can be selected when variation is revealed by an environmental change (citing the *crossveinless* experiment) or by a genetic mutation. For example, the number of scutellar bristles (four) is highly canalized in *Drosophila* but is variable in strains with the *scute* mutation. In such stocks, the number of bristles can be altered by selection (Rendel 1959), and selection can increase or decrease the feature's temperature-dependence (Kindred 1965). In his very influential book on quantitative genetics, Falconer (1981) cited Waddington's work in his discussion of threshold characters, which had been known at least since Sewall Wright's early research on guinea pigs. These studies and others (e.g., Milkman 1964) made canalization and robustness a live topic in the 1960s, but it then largely disappeared until the late 1990s (see Wagner et al. 1997; Hermisson and Wagner 2004; de Visser et al. 2003).

Leading evolutionary biologists, then, did not doubt that genetic assimilation was compatible with the synthetic theory of evolution; the question, rather, was whether or not it has often played a role in evolution. Simpson (1953) wrote that all of the processes that underlie the Baldwin effect are known to occur, that there is no reason to doubt that they could co-occur, that "there is even some probability that they must have produced that effect sometimes," and that "the Baldwin effect is fully plausible under current theories of evolution." He noted that Julian Huxley, in *Evolution: The Modern Synthesis* (1942), accepted it and invoked it to explain

early stages of population divergence, such as host races of some herbivorous insects. Although Simpson accepted the Baldwin effect as a theoretical possibility, he questioned whether it explains any particular instances of evolutionary change, and whether it has been common and explains adaptation in general. He concluded that "the Baldwin effect [is]…well worthy of further study. It does not, however, seem to require any modification of the opinion that the directive force in adaptation, by the Baldwin effect or in any other particular way, is natural selection" (Simpson 1953, p. 116).

To the question, "how do the Baldwin effect and genetic assimilation fit into evolutionary theory?", the answer seems to be "comfortably enough that they could be tolerated, even if not welcomed as the solution of a thorny problem." If anything, they might have been considered a plausible solution in search of a problem.

Phenotypic stability, inconstancy, and plasticity became an increasing focus in field and experimental studies of plants and *Drosophila* in the 1950s and 1960s. Anthony Bradshaw (1965) magisterially reviewed and interpreted much of this work, especially in plants, citing evidence that plasticity varies among characters and among related species, populations, and genotypes; that character stability can be decreased or increased by artificial selection, "perhaps most elegantly demonstrated by Waddington's genetic assimilation studies" (cf. also Reeve 1960, Prout 1962); and that adaptively important characters are developmentally more stable than less important ones. (For example, he cited Mather's [1953] finding in *Drosophila* that the coefficient of variation was ten times greater for bilateral asymmetry in the number of sternopleural bristles than for asymmetry of wing length.) He reviewed cases of clearly adaptive plasticity, as well as evidence that under stabilizing selection, plasticity can result in phenotypic uniformity that masks genetic variation. (For example, he cited a study of *Plantago maritima*, which was phenotypically uniform in the field but variable in an environmentally more uniform experimental garden [Gregor 1956].) Bradshaw identified some 'open problems,' including the mechanistic basis of discrete versus continuous plasticity, the mechanisms by which different plastic traits covary, and especially, how much genetic variation for plasticity exists in natural populations, and how responsive it is to natural selection.

Phenotypic plasticity attracted more research effort in the 1980s. In a review of "the evolution of phenotypic plasticity in plants," Schlichting (1986) argued that a character and its plasticity may be under somewhat separate genetic control and could therefore evolve independently. In that case, phenotypic plasticity might not inhibit character evolution. He cited evidence (e.g., Scheiner and Goodnight [1984] in the grass *Danthonia*; Stearns [1983] in *Gambusia* mosquitofish) that "plastic traits do not necessarily evolve less." Since then, genome-based analyses have shown that, at least in *Drosophila*, largely different genes control the value of a trait and its plasticity (Ørsted et al. 2017; LaFuente et al. 2018, 2019).

The 1980s saw a profusion of relevant theory, studies of the quantitative genetics of reaction norms, and focus on plasticity as a common adaptation to temporal or spatial variation in environment (reviewed by Scheiner 1993). Optimality models were developed especially for life history traits (e.g., Stearns and Koella 1986; Moran 1992). Quantitative genetic models by Lynch and Gabriel (1987), Gavrilets

and Scheiner (1993), and others showed that plasticity is likely to be favored if it has low fitness cost and if different environmental states are equally frequent, impose strong selection, and are reliably cued. Via and Lande (1985) introduced an influential quantitative genetic model, in which phenotypes expressed in different environments are treated as genetically correlated traits. This seemed to call into question of whether plasticity should be viewed as a genetically variable character in itself that may be the direct target of selection (Schlichting and Piglucci 1998). The recent evidence that genetic variation in plasticity and in the trait mean can be independent suggests that plasticity can be considered a character if that is useful for questions about the evolution of reaction norms.

Since the 1980s, considerable research has confirmed that many plastic responses are adaptive. In many cases, as in the foraging behavior of birds or the growth patterns of plants in sunlight and shade, the adaptive value of plasticity is almost self-evident. In some cases, traits vary as predicted by theory, such as paedomorphosis in *Ambystoma* salamanders (Semlitsch et al. 1990), offspring sex ratio in parasitoid *Nasonia* wasps (Orzack 1986), and heterophylly in aquatic versus terrestrial *Ranunculus* buttercups (Cook and Johnson 1968). Nevertheless, patterns of plasticity do not always meet our perhaps naïve expectations. For example, variation among *Drosophila* species in phenotypic plasticity for heat tolerance is only weakly correlated with the thermal regimes they experience in different geographic regions (Overgaard et al. 2011).

The 1980s were also a period in which the evolution of ecological specialization and generalization became a research focus. Phenotypic plasticity, of one kind or another, would be expected to underlie greater niche breadth, whereas habitat or diet specialists might be less plastic (Futuyma and Moreno 1988; van Tienderen 1991). Futuyma and Moreno (1988) noted that populations with broader niches usually are composed of broad-niched, flexible individuals, rather than sets of specialized morphs: the 'between-phenotype' component of niche width (Roughgarden 1979; Taper and Case 1985) is usually small. They found that ecological specialists sometimes, but not always, display narrower physiological tolerances or efficiency of resource use.

Having become a focus of research programs in the 1980s, phenotypic plasticity has since enjoyed a large, diverse literature. A fundamental question is whether or not it increases fitness. Despite many examples like those I have cited, formal selection analyses have been more equivocal. Van Buskirk and Steiner (2009) and Arnold et al. (2019) reviewed studies that estimated selection on plasticity and found that positive and negative selection gradients were both common. A critical question is how to determine if an instance of phenotypic plasticity is an adaptation that has been built by natural selection in the environment in which it is manifested, or evolved in a very different context, or is a 'side effect' of development that only incidentally happens to increase fitness in a certain environment (Fox et al. 2019).

The nature and consequences of costs and limits of plasticity have also been an important topic (DeWitt et al. 1988; see also Snell-Rood and Ehlman 2021 in this volume). It is important to distinguish between the fitness cost of a particular phenotypic state and the cost of maintaining the underlying ability to produce that

phenotype or others: the cost of plasticity as such. Van Buskirk and Steiner (2009) and Murren et al. (2015) reviewed relevant studies, found rather little evidence of plasticity costs, and concluded that they are hard to demonstrate. In contrast, Snell-Rood et al. (2018) describe a wide array of costs and argue that plastic responses are costly, especially as manifested by cases of 'developmental selection' (trial-and-error exploration, as by plant roots that may find water or nutrients in some places but not others). Some causes of a plasticity cost, such as maintenance of a potential developmental trajectory, might be hard to detect; others, such as developing an inappropriate phenotype because of an undependable environmental cue, might be easier.

Much recent research and discussion pertains to the evolutionary consequences of plasticity. I will mention four possible consequences: the effect of plasticity on responses to selection, the consequences of maladaptive plastic responses, the role of plasticity in averting extinction, and its role in the evolution of new or modified phenotypic traits.

An important question is, does plasticity reduce the genetic response to directional selection? A contrary view is that plasticity allows the increase of cryptic genetic variation that might be expressed when exposed by environmental stress (Paaby and Rockman 2014). That is, plasticity might serve as a 'genetic capacitor' (Rutherford and Lindquist 1998; see also Schlichting 2008). In a genetic network model, Draghi and Whitlock (2012) described a trait that evolves plasticity, accumulates greater genetic variance, and enhances evolution along the axis of plastic phenotypic variation. Based on a meta-analysis of relevant studies, Noble et al. (2019) concluded that plastic responses to different environments are, indeed, fairly well aligned with phenotypic dimensions that are highly genetically variable. They note, however, that this makes it difficult to distinguish a 'plasticity-led' evolved difference between populations from genetic constraint: evolution along the genetic line of least resistance (Schluter 1996).

Perversely, genetic evolution can also be provoked by *maladaptive* plasticity, as illustrated by instances of countergradient variation, in which the direction of genetic differences between populations is opposite to environmental effects on the phenotype (Conover and Schultz 1995). Grether (2005) termed such evolution 'genetic compensation.' For example, Ghalambor et al. (2015) found that guppy populations transplanted from streams with cichlid predators to cichlid-free streams evolved changes in the expression of many genes in the brain—and that most of these showed the opposite plastic change when fishes in the source population were reared in the absence of predators. Likewise, plastic changes in gene expression that occurred in experimental populations of yeast and *Escherichia* coli were mostly maladaptive, since they were generally counteracted by genetic changes that reversed the expression level back toward its original state (Ho and Zhang 2019). Maladaptive plastic changes may deserve considerable further study.

A third current question concerns the role of phenotypic plasticity in rescuing populations endangered by environmental change, such as anthropogenic climate change (see Diamond and Martin 2021 in this volume). In the first of several papers, Lande (2009) modeled adaptation to a sudden drastic environmental change in a

population that has a genetically variable reaction norm, but with little expressed $G \times E$ variation, due to stabilizing selection. The environmental change reduces mean fitness which, however, rapidly increases due to plasticity, up to some limit. In the new environment, plasticity evolves so that the mean phenotype reaches a new optimum, which is slowly fixed by genetic assimilation due to stabilizing selection. (Note that Lande, a strong adherent to the Evolutionary Synthesis, accepted genetic assimilation as an evolutionary process.) Chevin et al. (2010) followed with a model of evolution in a gradually changing environment and found that although plasticity reduces the rate of genetic evolution by weakening natural selection, "this is more than compensated by the plastic change that brings the phenotype closer to the optimum" (p. 5). They noted, though, that extreme, stressful environments may disrupt the phenotypic response, as such environments will have been rarely encountered in the past, and so will have exerted little selection (cf. Hoffmann and Parsons 1991). Chevin and Hoffmann (2017) emphasized that plasticity is likely to rescue populations under new extreme conditions only if the phenotype expressed in the extreme environment (e.g., very hot) is genetically correlated with the expression in the ancestral environment (warm or moderately hot). There are few data on such genetic correlations.

Research on the role of plasticity in averting population extinction is increasingly important, as we recognize how drastic the effects of human activity are. For example, as spring has shifted earlier with global climate change, many temperate-zone bird species have shifted their nesting and egg-laying dates, and so have tracked resource peaks and avoided excessively high temperatures (Phillimore et al. 2016; Socolar et al. 2017). However, species that breed in the temperate zone but overwinter in the tropics have no information on the beginning of suitable nesting conditions, so plastic responses are of no avail. Their arrival and nesting are mistimed, and some such species have suffered population declines (Both et al. 2006; Mayor et al. 2017). Plasticity is not always an adaptive option.

Surely the most prominent and controversial current question about phenotypic plasticity concerns its role in evolutionary change of traits. (This is often expressed as the evolution of 'novel' traits, but I will restrict 'novel,' as does Wagner [2014], to the origin of a new character, a trait that is not simply a quantitative change in a preexisting trait.) This topic was greatly enlivened by the publication of West-Eberhard's extraordinarily comprehensive *tour de force*, *Developmental Plasticity and Evolution* (2003), which expanded on and sharpened her earlier treatments of the topic (e.g., West-Eberhard 1986, 1989). West-Eberhard proposed that many fixed, species-typical features represent part of a plastic ancestral reaction norm that has been 'genetically accommodated.' 'Genetic accommodation' was broadly defined and can include genetic assimilation, modification of the expressed trait, or evolutionary changes in other traits that interact with the focal trait. Most subsequent discussion has concerned that aspect of genetic accommodation that is tantamount to genetic assimilation. This is now an active topic, which I treat very briefly.

West-Eberhard, like earlier authors such as Goldschmidt and Waddington, noted that the development of a trait may react similarly to mutational or environmental

perturbations. Recurrence of the perturbation produces a subpopulation of individuals that express the trait, affording the opportunity for gene-frequency change due to selection on its expression (p. 140). Although recognizing that either genetic or environmental perturbation initiates this evolutionary process, West-Eberhard devoted much of the book to her view that environmental initiation is more important by far, and drew on an extraordinary range of information to develop her argument. The critical reactions of some traditional evolutionary biologists (e.g., de Jong and Crozier 2003) may have been influenced by her colorful, sometimes challenging, statements like this often-quoted passage: "…most phenotypic evolution begins with environmentally initiated phenotypic change…Gene-frequency change follows, as a response to the developmental change. In this framework, most adaptive evolution is accommodation of developmental-phenotypic change. Genes are followers, not leaders, in evolution" (pp. 157–158).

My own reactions to Mary Jane's thesis were cautious. (I should say that I had the good fortune of being a fellow graduate student with her, then as now an awesomely creative biologist, outstanding naturalist, and friend.) Recall Simpson's (1953) judgment of the Baldwin effect: he said it is fully compatible with modern evolutionary theory, but asked for evidence that it occurs in natural populations, or that "it is a frequent and important element in adaptation." So did I.

West-Eberhard's examples of possible cases of evolution by genetic accommodation of environment-induced phenotypes could easily be matched or exceeded by straightforward examples of a genetic basis for characters that distinguish populations and closely related species. And in writing several editions of an evolution textbook, I had sought in vain clear examples of genetic assimilation in nature. (Carl Schlichting and I discussed this point at an evolution meeting, about 15 years ago. Neither of us knew of a convincing example at that time.) What was necessary, I felt, was historical evidence, perhaps from phylogeny, that a relatively invariant trait in one population had evolved from a broader norm of reaction, a more plastic ancestral trait as seen in another population. West-Eberhard (p. 204) discussed the importance of establishing the polarity of evolutionary change, but most of the examples in her book did not provide the historical element. de Jong and Crozier (2003) wrote that West-Eberhard had failed to show that developmental plasticity is "the initiating factor of adaptive novelty, preceding genetic change."

More recently, a number of phylogenetically supported cases of evolution by genetic assimilation and abbreviation of ancestral phenotypic plasticity have been described (see Scheiner and Levis 2021 in this volume). In an early review, Schwander and Leimar (2011) cited several cases in which conditional alternative phenotypes have been lost, as in one of the worker castes of certain ants and the development of horns in some species of *Onthophagus* dung beetles (Moczek et al. 2006 and later papers).

Some studies have provided more detailed analyses of evolutionary changes by genetic assimilation of an ancestrally plastic condition. A carefully documented example is the fixation of an amelanic phenotype, which ancestrally develops only under low ultraviolet light, in populations of *Daphnia* that experienced increased

exposure to predatory fish (Scoville and Pfrender 2010). In a rather similar case, a population of the side-blotched lizard (*Uta stansburiana*) on a lava flow is much darker than other populations that live on sand. Individuals of both forms become darker if kept on sand for a year, but lava-population lizards become darker than sand-population individuals. The difference was attributed to recently derived, positively selected variants in two genes that regulate the melanogenesis pathway (Corl et al. 2018). The authors noted its conformity to the Baldwin effect. In female *Drosophila melanogaster*, temperature-dependent development of abdominal pigmentation is due largely to modulation of histone marks in a promoter of the *tan* gene, and a loss-of-function mutation in this enhancer appears to underlie the fixed expression of pale coloration in the related species *D. santomea* (Gilbert 2017).

All of these cases represent not the origin of a feature, but instead an extension or an abbreviation of a broader ancestral reaction norm. Among the several examples that support the 'plasticity-first' view of evolution (see Levis and Pfennig 2021), few purport to explain the origin and evolution of what I consider novel traits, such as multi-cusped teeth (in ancestors of mammals), branched body hairs (of bees), or tubular corollas (of many plants). To be sure, the genetic and developmental origins of most novel traits are poorly understood; see Wagner (2014). And the distinction between 'novel' and 'highly modified' traits is fuzzy. In the great adaptive radiations of African cichlids, for example, some of the trophic variation among species is attributable to functionally important differences in the form of the pharyngeal jaws and the teeth they bear. As West-Eberhard (2003) describes, some of these differences arise among conspecific individuals that are reared on different diets—phenotypic plasticity that might have played a role in the evolution of adaptive trophic diversity.

Another possible example of a role for plasticity in macroevolutionary novelty concerns the origin of tetrapod vertebrates (see also Lister 2021). Bony fishes are divided into two clades, the diverse ray-finned fishes (Actinopterygii) and the lobe-finned fishes (Sarcopterygii), from which tetrapods evolved. Bichirs (*Polypterus*) are a basal clade of ray-finned fishes that are semiterrestrial, using their pectoral fins to crawl on land, as long as they can stay wet and breathe. Compared to individuals that developed in water, individuals that were raised on 'land' used their fins more effectively and showed modest alterations of the pectoral girdle that parallel those seen in some Devonian stem tetrapods (Standen et al. 2014). Whether or not this is evidence of a role for plasticity in tetrapod origins is hard to say. Because environmental and genetic perturbations of a developmental pathway can have similar effects (Noble et al. 2019), the bichir experiment may illustrate coincidence of similar developmental responses, rather than bearing on the actual historical origin of tetrapod features. This seems especially likely because bichirs are not closely related to tetrapods, but in general, satisfying de Jong and Crozier's criterion—showing that there has been a historical shift from a plastic reaction norm to a fixed character state—is a challenge.

This challenge is most likely to be met by information on closely related populations or species (cf. Levis and Pfennig 2016). In well-documented cases, a key question is how the ancestral reaction norm originated. Ghalambor et al. (2007)

and others have pointed out that plasticity is most likely to play a role in adaptive evolution if the ancestral reaction norm happens to be directed more or less toward the new phenotypic optimum. Plasticity is less likely to enhance genetic adaptation if it is nonadaptive (as in developmental instability) or if it is maladaptive (as in the common phenomenon of countergradient variation) (Conover and Schultz 1995; Grether 2005; Storz and Scott 2019). The plastic ancestral reaction norm is likely to be aligned with the direction of selection in a new environment that is not entirely novel but is more or less an extension of the ancestral environment (Pigliucci 2010; Snell-Rood et al. 2018), and if the phenotypic states expressed in ancestral and new environments are genetically correlated, as Chevin and Hoffmann (2016) emphasized. A somewhat higher temperature is more likely to be met by a suitable plastic response than a novel chemical such as DDT or thalidomide. (This was a drug prescribed for pregnant women in the 1950s, that resulted in thousands of children's being born with shortened limbs and other deformities.)

In many postulated cases of 'plasticity-led evolution,' the phylogenetically derived state appears to be a modification of adaptive rather than non- or maladaptive plasticity. This is clearly true of cases in which the ancestral reaction norm has been simply abbreviated, reduced to expression of only one of the ancestrally possible expressions (e.g., neoteny in salamanders, fixation of the less melanized state in *Daphnia* and in *Drosophila santomea*, loss of horns in *Onthophagus*). The evolutionary scenario is one in which: (1) natural selection acting on genetic variation in reaction norms has shaped adaptive phenotypic plasticity; (2) later, selection on genetic variation in this adaptive reaction norm shapes a phenotype that is less plastic and more genetically canalized. Adaptive evolution is still ultimately a matter of natural selection and genetic variation, the central model in the Evolutionary Synthesis. But genetic assimilation of an advantageous plastic phenotype is by no means certain. Scheiner et al. (2017) simulated evolution when there exists genetic variation for both fixed and plastic effects on a phenotype. Adaptation to a new environment can occur by increased plasticity if the cost of plasticity is low, but is more likely to evolve by the increase of fixed-effect alleles if plasticity is costly or has only small phenotypic effects. If adaptation does occur by plasticity, genetic assimilation—replacement of plasticity alleles by fixed-effect alleles—occurs only if plasticity is costly, but even then, it occurs very slowly (as also found by Chevin et al. 2010; see also discussion in Scheiner and Levis 2021).

Because there is hardly any challenge to standard theory when derived characters are a fixed state of an advantageous ancestral reaction norm, I find most interesting several cases in which the ancestral state seems *not* to have been an adaptive reaction norm. Aubret and Shine (2009) showed that greater head size of juvenile tiger snakes (*Notechis scutatus*) is advantageous in island populations, where prey are large. In populations on recently colonized islands, juveniles develop larger heads if they are fed larger prey, but this plasticity is lower in older island populations. The authors attribute this to genetic assimilation. But the peculiar feature of this study is that the mainland source population does not exhibit plasticity in head size; it appears to have

evolved rapidly, *de novo*, in island populations. The authors noted that "this aspect is not predicted by current evolutionary models."

Another curious case is afforded by the 'carnivore morph' of larval spadefoot toads in the genus *Spea*, described by Pfennig and his collaborators (Ledón-Rettig et al. 2010; Levis et al. 2018), which displays several features that are induced by feeding on animal prey. The more common 'omnivore' morph, which feeds on detritus, resembles in diet and form the larvae of the sister genus, *Scaphiopus*, as well as more distantly related genera, in which detritus-feeding is the ancestral habit (Ledón-Rettig et al. 2010). Surprisingly, *Scaphiopus* larvae that were fed shrimp developed some features of the carnivore morph, such as a shorter gut, despite no suspected history of having experienced selection for this developmental response. The developmental response seems not to be an adaptation, even though it can have an advantageous effect.

Are cases such as these odd, rare 'accidents' of development, rare enough to count for little, or are such instances common and in need of explanation? Much of the argument for the evolutionary role of phenotypic plasticity in trait evolution describes evolutionary modifications of supposedly ancestral plastic phenotypes, yet seems not to burrow into the origin of those ancestral reaction norms themselves. If development and phenotypic plasticity are to play the truly fundamental, creative role that some adherents to the 'extended evolutionary synthesis' claim (Laland et al. 2015), it will be in cases that are not ascribable simply to selection on genetically variable plasticity that is itself a result of natural selection.

As I emphasized, major figures in the Evolutionary Synthesis and since found evolution by genetic assimilation and canalization quite compatible with their theory of evolution; they questioned only how common it might be. It seems that it has occurred more frequently than we knew and poses a range of questions that can't fail to enhance our understanding of evolution. Many of these questions will require understanding processes of development, and how genes are regulated and exert their developmental effects. Some of these processes will undoubtedly be surprising and will call for evolutionary explanation, just as some molecular processes did (e.g., transposable elements, alternative splicing).

One such process is transgenerational phenotypic plasticity, whereby a parent's environment affects the phenotype, and often the fitness, of the offspring (see discussion in Bonduriansky 2021 and Pfennig 2021 in this volume). This has emerged as a second point of current controversy about phenotypic plasticity, even though environmental maternal effects have long been known, are often adaptive, and have been treated in evolutionary models (e.g., Mousseau and Fox 1998; Wolf et al. 1998; Kirkpatrick and Lande 1989; Lande and Kirkpatrick 1990). Nongenetic paternal effects have also been described in diverse species (e.g., host-plant responses in leaf beetles, Futuyma et al. 1993). They can affect diverse traits, and in some cases, they increase offspring fitness (Crean et al. 2013; Schmid and Dolt 1994). Imprinting and other epigenetic mechanisms seem likely to underlie many cases of transgenerational plasticity and other instances of inheritance that are not based on DNA sequence variation (Lawson et al. 2013; Bonduriansky and Day 2009, 2018). This form of 'extended heredity' does depart substantially from the heredity known

during and for several decades after evolutionary synthesis, and undoubtedly will add significantly to evolutionary theory.

As nongenetic inheritance and phenotypic plasticity are prominent planks in the platform of the 'extended evolutionary synthesis' (Pigliucci and Müller 2010; Laland et al. 2015), I end with some thoughts on how they may fit into the history of evolutionary biology (see also Futuyma 2015, 2017). The core theory of the evolutionary synthesis, framed in terms of the effects of mutation, gene flow, genetic drift, and especially natural selection on the frequencies of alleles and genotypes, has been a grandly successful theory because it is so general, encompassing any trait you might think of. It is silent about what the trait might be: a morphological character, a life history feature, an enzyme, a doubling of the genome. During and ever since the Synthesis, theoreticians, naturalists, and experimentalists have been amplifying and particularizing the theory to describe the evolution of real traits: life histories; cooperation, conflict, mate choice and other behaviors; genes and then genomes; physiological tolerances; ecological specialization; geographic range limits; and much more. The evolutionary theory developed for these classes of traits requires knowledge of their function and, ideally, mechanistic development. For some of these classes of traits, other biological disciplines have described previously unknown mechanisms and processes that have become *explananda* for evolutionary biology; many have been successfully explained; and the union of biological mechanism with evolutionary explanation, the union of proximate and ultimate causes, has expanded evolutionary biology. (Evolutionary genomics is perhaps the most striking example.) Phenotypic plasticity and extended heredity follow this historical path; they are currently among the subjects that both look for further explanation and contribute to the continuing expansion of evolutionary theory.

Perhaps it need not be said, but it remains important to eschew any hint of vitalism. There is no reason to suppose that organisms are endowed with an inherent ability to react advantageously to perturbations and stresses. It remains true that most mutations that affect fitness are deleterious, as are many or most novel changes in environment. (The vast majority of species are extinct.) Plastic compensatory responses of individual organisms, that mitigate harmful effects, are not inherent in living things; they have evolved (Kirschner and Gerhart 2005). So, if a phenotypically plastic capacity provides a foundation for an advantageous, genetically accommodated character, that plasticity itself requires explanation—and the explanation will often, or usually, be our old friends, genetic variation and natural selection. Many nonbiologists may welcome theories such as Lamarckism instead of natural selection, for, as the historian of science Peter Bowler (1989, p. 258) wrote, "Lamarckism allows life itself to be seen as purposeful and creative. …Life becomes an active force in nature, no longer merely responding in a passive manner to environmental pressures." As scientists, we do not succumb to that seduction. Of course, the evolved characters of a species—including various forms of plasticity, even human cognition that conceives of purpose—do influence its effective environment and selective pressures. But it's still genes and selection, all the way down.

BOX 14.1 SUGGESTIONS FOR FUTURE RESEARCH

- Do ecologically generalized species have greater plasticity in key traits than related specialists? And does this make generalist lineages more 'evolvable' than specialists? For example, there is evidence that gene families associated with chemoreception, detoxification, and digestion are larger in generalist than specialist herbivorous insects (Calla et al. 2017; Pearce et al. 2017; Cheng et al. 2017). Do generalists have a greater capacity than specialists to evolve new host-plant associations (Janz and Nylin 2008; Hardy 2017; Nosil and Mooers 2005)? How do these questions apply in other aspects of niche evolution?
- Waddington's classic experiments on genetic assimilation were based on unusual stresses, such as heat shock, rather than an accentuation of an environmental change that had exerted selection for phenotypic plasticity. Are there any examples in which adaptive evolution based on phenotypic plasticity can be ascribed to novel stresses of this kind? (cf. Hoffmann and Parsons 1991.)
- Compared to populations that experience less variable environments, do related populations in more variable environments display both greater phenotypic plasticity and greater additive genetic variance for relevant traits?
- Noble et al. (2019) note that it can be difficult to distinguish plasticity-led evolution from genetically constrained evolution if plasticity and genetic variation are aligned in 'phenotype space.' Are they often aligned? If so, are there solutions to this dilemma? Might molecular characterization of the relevant genes and their regulation answer these questions?
- If we compare an array of characters in related populations and species, do divergent characters display greater plasticity than characters that have not diverged? Is the plasticity, if present, aligned with the direction of divergence? Is there a difference between multidimensional characters (perhaps coloration) and unidimensional traits (perhaps measures of body size)?
- How diverse are the features for which 'plasticity-led evolution' might be suspected? How about, in angiosperms, flower structure, the form and density of trichomes, leaf shape, margin, and venation, drought tolerance? Sexual and social display characters in insects, fishes, birds? Lepidopteran wing patterns, gastropod shell geometry, fly chaetotaxy, fin shape and ray number in fishes, scale counts in squamate reptiles? Parasites' behavioral responses to hosts? Possible examples of many of these have been described (West-Eberhard 2003), but these and countless other taxonomically and/or ecologically important features have been little studied.

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15 Plasticity and Evolutionary Theory: Where We Are and Where We Should Be Going

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CONTENTS

15.1 INTRODUCTION

Although the evolutionary importance of adaptive phenotypic plasticity as a mechanism of adaptation to spatial and temporal environmental variation is acknowledged as part of the mainstream of evolutionary theory, arguments for a broader importance of phenotypic plasticity are contentious, mainly hinging on its potential role as a facilitator of evolutionary change (see Futuyma 2021; Levis and Pfennig 2021; Pfennig 2021 in this volume). Some proponents of an enhanced role for plasticity have called for its inclusion as an important component of an 'extended evolutionary synthesis' (trait-specific changes to offspring phenotypes induced by parental environmental conditions; Pigliucci 2007; Laland et al. 2015).

In this chapter, I will show that plasticity has important ramifications for many aspects of evolutionary theory. I will highlight current research on several of these topics as a means of demonstrating the impacts of plasticity on a range of evolutionary processes. Subsequently, I will address the broader issue of how responses to 'environmental' variation can be more fully incorporated into our understanding of the evolution of phenotypes.

DOI: 10.1201/9780429343001-15

15.2 THE CONTEXT-DEPENDENCE OF GENE EXPRESSION

Although we tend to forget this, gene expression is always context-dependent (Nijhout 1990). Genes require an environmental cue to be expressed whether that cue is an external environment or the product of some other gene, and phenotypes can vary as a function of differences in those cues (see examples in Sultan 2021 in this volume). So, while one can readily assign a fitness to a DNA sequence in a computer model, in the real world, it is that sequence's expressed phenotype that has a fitness. One consequence of this 'forgetting' is that we neglect the fact that patterns of gene expression leading to 'normal' development have evolved in the context of specific ranges and frequencies of various environmental parameters (Pei et al. 2020). When organisms are placed in environmental contexts beyond the scope of their evolutionary memory, their development may no longer be predictable.

Normal developmental sequences are often considered to be 'canalized' or 'robust' to genetic or environmental changes. Within the normal range of environments, novelties in canalized developmental pathways are most likely to be produced via mutation. However, outside of those environments where canalization has evolved, reaction norms are inherently plastic and thus also inherently prone to produce phenotypic novelty (hidden reaction norms: Schlichting and Pigliucci 1998; Schlichting 2003; Ghalambor et al. 2007; Schlichting 2008). 'Hidden reaction norms' (Figure 15.1) represent plastic responses to novel or infrequently experienced conditions for which there has been no selection for either canalization of a particular phenotype or an adaptive plastic response. Collectively, the hidden reaction norms of different genotypes express 'cryptic genetic variation' (Figure 15.1). Such cryptic genetic variation has been suggested to represent a store of variability that can be revealed in novel environments or genetic backgrounds (Gibson and Dworkin 2004; Le Rouzic and Carlborg 2008; Moczek 2008; Schlichting 2008; Paaby and Rockman 2014; Schneider and Meyer 2017; Donnelly et al. 2018; Zheng et al. 2019; Pilakouta et al. 2020).

Zan and Carlborg (2020) investigated the organization of 130 yeast growth regulation genes in response to 20 different growth media. They discovered that epistatic gene interactions were significantly reorganized across environments, with a few individual loci involved in epistatic interactions on as many as 16 different growth media, but the majority were expressed in fewer than four. Thus, there were different patterns of hidden and revealed growth-regulating loci contributing to plasticity and robustness across the different growth conditions.

Adaptive phenotypic plasticity represents a type of buffering at a different scale—canalization of *responses* of different genotypes to environmental shifts (e.g., the set of parallel horizontal reaction norms for flowering in Timberline and Mather; Figure 15.1). Convergence of responses on an adaptive reaction norm will also shield unexpressed genetic variants among those genotypes from selection (Wright 1931; Schwab et al. 2019). Signor (2020) documents the canalization of reaction norms in a comparison of ethanol tolerance in *Drosophila simulans*—possessing the ancestral non-tolerant phenotype—and *D. melanogaster*—with an evolved tolerance to ethanol. She finds substantial genetic variation in the reaction norms of gene expression to ethanol of *D. simulans* but no such variation in *D. melanogaster*, a lack of variation expected if an ethanol tolerant reaction norm had been strongly selected.

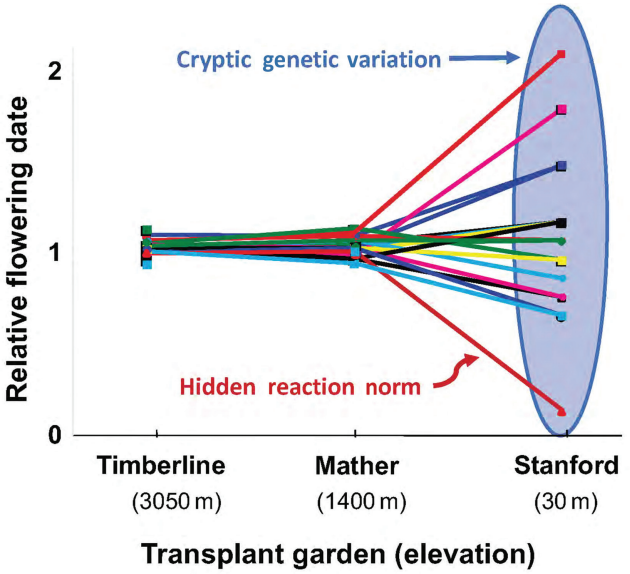

FIGURE 15.1 Graph of variation in relative flowering times of *Potentilla glandulosa* clones grown at three transplant gardens. Little variation is observed at the elevations where populations are normally found (i.e., at Timberline and Mather), and the reaction norm is itself canalized between these sites. However, at the sea level garden at Stanford, clones express hidden reaction norms resulting in the expression of significant cryptic genetic variation in this population. (Data from Clausen et al. [1940]; figure modified, with permission from The New York Academy of Sciences, John Wiley & Sons, Inc., from Schlichting [2008].) A modern example of hidden reaction norms and cryptic genetic variation can be seen in Hintze et al. (2020, Figure 5A) for stem cell proliferation in *C. elegans*.

15.3 PLASTICITY MAY BE ANCESTRALLY ANCESTRAL

Futuyma (2021) raises the important question of how trait plasticity arises. Most models of the evolution of development postulate that new genotypes are initially sensitive (plastic) to environmental change, with robustness (loss of plasticity) as a subsequent evolutionary feature (Schlichting and Pigliucci 1998; Newman and Müller 2000; Nijhout 2003; Schlichting 2003; Nijhout et al. 2017; Lafuente and Beldade 2019). Evidence for sensitivity of new mutants goes back to the classic work of Dun and Fraser (1959)—who showed that the *tabby* mutation in mice increased whisker number variability—and is supported by numerous other studies showing that new mutations are relatively uncanalized (Rendel 1967; Baer 2008; Hallgrimsson et al. 2019).

Non-plasticity (i.e., canalization, robustness) is thus arguably a derived state in most biological systems (Nijhout 2003). If plasticity is the ancestral state, then the equally intriguing question for evolutionary biologists is; How does trait stability evolve? Dun and Fraser's (1959) study also showed that variation in whisker number could be decreased via selection (see Hallgrimsson 2019 for further references on

selection for canalization). There has been a resurgence of interest in how buffering of developmental processes has evolved as evidenced by the plethora of recent reviews on canalization and robustness that document the growing empirical work in this area (Bateson and Gluckman 2011; Félix and Barkoulas 2015; Geiler-Samerotte et al. 2019; Hallgrimsson et al. 2019; Klingenberg 2019; Nijhout et al. 2019). In fact, systems that have evolved genetic and environmental robustness may be more prone to produce truly novel features when these buffering mechanisms, that suppress expression of genetic variants, no longer function (Wagner 2005).

Evolutionarily fine-tuned systems of development (i.e., well buffered against environmental or genetic perturbations) likely arise via functional diversification of duplicate copies of genes. Al Asafen et al. (2020) show that the evolved robustness of dorsal-ventral patterning in the *Drosophila* embryo requires at least three mechanisms of feedback in the *Dorsal* signaling module. Ghosh et al. (2019) have shown that selection for increased canalization of development time in *Drosophila melanogaster* is effective for some environmental perturbations (density) but not for others (temperature). Green et al. (2017) examined robustness by manipulating the dosage of a critical regulator of vertebrate development, the fibroblast growth factor signaling molecule (Fgf8). They found that phenotypic variation in face and skull measurements of mice embryos is not related linearly to the variation in *Fgf8* expression, and that observed differences in levels of robustness among genotypes are predicted by this non-linear 'genotype-phenotype mapping'.

Such buffered systems may subsequently be further altered for cue-specific plasticity, again by deployment of duplicate receptor and signal transduction genes modified to control new plastic responses (Smith 1990; Schlichting and Smith 2002). Ancestral patterns of plastic response may be maintained, modified, or eliminated through processes jointly referred to as 'genetic accommodation' (West-Eberhard 2003). Bhardwaj et al. (2020) have examined the evolution of plasticity in the classic case of seasonal 'polyphenism' of wing eyespots (larger in the warmer dry season) in the butterfly *Bicyclus anynana*. In *Bicyclus*, warmer temperatures induce higher production of the hormone 20E and a corresponding increase in eyespot size. Examining 12 other species of butterfly, Bhardwaj et al. (2020) found that the plasticity of hormone production to temperature is an ancestral feature. However, although receptors for 20E in the eyespot region are found in several species, only *B. anynana* shows an increase in eyespot size in response to temperature. Their results suggest that the plastic response of increasing eyespot size results from a modification of the ancestral response via derived genetic changes linking eyespot size and hormone production.

BOX 15.1 TERMS AND DEFINITIONS

Canalization or robustness. Stability of phenotypes during development following perturbation. Genetic robustness typically refers to the reduction of developmental noise, and environmental robustness refers to the reduction of plasticity.

Cryptic genetic variation. Genetic variation revealed by environmental or genetic perturbations (Gibson and Dworkin 2004).

Developmental bias. The disproportionally higher production of certain variants following environmental or genetic perturbation (Uller et al. 2018).

Developmental noise. Variation in a phenotype of a trait of a particular genotype under constant environmental conditions.

Ecological developmental biology (eco-devo). The study of how organisms' responses to their environment influence the expression of genetic and developmental programs.

Epigenetic. Originally intended to indicate organism features that arise as a consequence of developmental processes (*sensu* Waddington; see 'Genotype-phenotype mapping' below); now the term is most often used to refer to whether a particular DNA sequence or histone protein is methylated (an epigenetic mark).

Evolutionary developmental biology (evo-devo). The study of developmental processes in an evolutionary context; e.g., to infer developmental changes leading to phenotypic changes in ancestral-descendant or phylogenetic relationships.

Evolvability. The tendency of a genotype or lineage to generate genetic variability and produce or maintain phenotypic variation over evolutionary time, enabling it to pursue diverse evolutionary trajectories (Schlichting and Murren 2004).

Extended evolutionary synthesis (EES). Driven by a perception that standard evolutionary theory provides an incomplete theoretical scope, the EES proposes to include topics such as evo-devo, evolvability, niche construction, and non-genetic inheritance, and to take into account reciprocal causation (i.e., where identities of causes and effects are fluid; Svensson 2018) and context-dependence (see Müller 2007; Pigliucci 2007; Laland et al. 2015; Fábregas-Tejeda and Vergara-Silva 2018).

Genetic accommodation. A process by which phenotypic variants that are initially strictly environmentally induced are selected to become genetically determined (i.e., heritable) (West-Eberhard 2005; Schlichting and Wund 2014; Ledon-Rettig and Ragsdale 2021). Plasticity may be enhanced or refined (i.e., 'plasticity-led evolution': Levis and Pfennig 2021), or even eliminated (genetic assimilation: Scheiner and Levis 2021).

Genotype-phenotype mapping. Describes the processes that convert the genetic instructions into the visible characteristics of an organism. Explicitly considers effects of gene-gene interactions (pleiotropy and epistasis) and gene-environment interactions that lead to changes in gene expression. In the case of phenotypic plasticity, there is a one-to-many mapping of a single genotype to the alternate phenotypes possible in different environments.

Hidden reaction norms. Unpredictable extensions of reaction norms in environments outside normally encountered ranges.

Modern synthesis. Stemming from population genetics theory developed by R.A. Fisher, S. Wright, and J.B.S. Haldane, the modern synthesis melded

concepts of genetics (Mendelian and quantitative) with natural selection to illuminate evolution in natural populations of organisms. The modern synthesis was advanced during the late 1930s and 1940s by T. Dobzhansky, E. Mayr, and others.

Niche construction. The modification of environmental conditions by the actions of an organism; e.g., edifices: beaver dams, burrows, and nests; soil or water alterations: actions of lichens and earthworms, decomposers; allelochemicals of plants.

Non-genetic inheritance. Trait-specific changes to offspring phenotypes induced by parental conditions (Bonduriansky 2021). Also referred to as epigenetic or transgenerational inheritance, and maternal effects (Rossiter 1996).

Phenotypic plasticity. Any change in phenotype by a genotype in response to changes in environmental conditions. A 'reaction norm' is a plot of the response across a set of environments (Sultan 2021).

Polyphenism. The formation of two (or more) distinct phenotypes in response to changes in environmental factors. Classic examples include the castes of social insects, seasonal differences in eyespot size in butterflies, host plant-related larval coloration in caterpillars, and light/dark winter/summer fur or plumage in birds and mammals.

Standard evolutionary theory (SET). The set of generally accepted principles encompassed by the 'modern synthesis' and its post-DNA expansion. Futuyma and Kirkpatrick (2017; Chapter 1: Box 1A) provide a concise listing.

Transcriptome. Whereas a genome records the DNA sequence of an organism revealing its complement of genes, 'transcriptomes' record the expression levels of those genes. They are often used to contrast gene expression levels in different environments, different tissues, or at different life stages (e.g., juvenile versus adult).

15.4 PLASTICITY AS AN ORIGINATOR OF NOVELTY

Several authors have pointed out the potential role of plasticity as an originator of new phenotypes for the evolution of behavior (Wcislo 1989; West-Eberhard 1989) and development (Schlichting and Pigliucci 1998; Newman and Müller 2000; Schlichting 2003; West-Eberhard 2003; Schlichting 2008; Gavrilets 2010; Moczek et al. 2011; Wagner et al. 2019). The development of multicellular organisms is itself a continuous set of plastic responses to internally produced stimuli driving changes in gene expression (Sachs 2002). We (Schlichting and Pigliucci 1998; Schlichting 2003) have proposed that multicellularity and differentiation may have arisen through genetic assimilation of sequential plastic responses to changes in internal environments, with novel developmental phenotypes initiated plastically and subsequently canalized so that adaptive developmental sequences are repeatable under broad conditions. In this scenario, the evolution of multicellular development is a recursive process alternating between plasticity (sensitivity of the phenotype to stimuli) and the evolution of robustness (see also Nijhout 2003; Bateson 2017;

Salazar-Ciudad et al. 2019; Schwab et al. 2019). This is an area of active investigation (Ratcliff et al. 2015; Wolinsky and Libby 2016; Grochau-Wright et al. 2017; Ratcliff et al. 2017; Herron et al. 2018; Herron et al. 2019; Laundon et al. 2019; Rivera-Yoshida et al. 2019; Davison and Michod 2021).

In addition to its potential primal role in multicellular evolution, plasticity has also been implicated in the origin of several major evolutionary features. The social insects, perhaps the most successful group of organisms (Hölldobler and Wilson 2009), owe their success to features that are due to a plastic response: the production of castes and divisions of labor. Berens et al. (2014) compare 'transcriptomes' of fire ants (*Solenopsis*), honey bees (*Apis*), and paper wasps (*Polistes metricus*) to look for commonalities in the plasticity of gene expression across lineages. Although they found little evidence for shared responses at the specific gene level, responses were similar at the level of metabolic pathways, suggesting that these different lineages are employing a shared 'toolkit' for generating convergent plastic responses to nutritional signals (e.g., in the insulin/insulin-like growth signaling pathway, Corona et al. 2016). Sun et al. (2019) investigated transcriptional changes in five castes of the higher termite *Macrotermes barneyi*, documenting extensive changes between castes in both gene expression and alternative splicing.

Kapheim et al. (2020) have investigated patterns of gene expression in the facultatively eusocial sweat bee *Megalopta genalis*. This species is an ideal model because females within populations vary in their tendencies towards sociality—some are solitary and others are social. Kapheim et al. designed a comprehensive suite of analyses using an annotated genome, a set of life-stage and sex-specific transcriptomes, and a population genomic comparison of solitary and social females. They discovered very strong associations of genes that have plastic expression during development (i.e., during sexual differentiation and metamorphosis) and genes with expression differences associated with divergence among castes. This suggests that genes with plastic expression patterns were later co-opted/redeployed during the evolution of caste specification. In addition, a broader comparison among species finds that this set of genes shows signs of strong positive selection in many independently derived eusocial insects (Jones and Robinson 2018; Kapheim et al. 2020).

Several research groups have taken advantage of deviations from the canonical insect caste system to examine the origins of plasticity. The ant *Diacamma* sp. has lost morphological castes, but still has divisions of labor based on social dominance. Okada et al (2017) demonstrated rapid changes in expression levels of genes involved in nutrition (e.g., insulin signaling) that accompany changes in rank. Several studies have focused on the solitary queen species *Pogonomyrmex barbatus* to examine the plasticity of divisions of labor when social interactions are experimentally imposed and multiple queens are grouped at nest establishment. Fewell and Page (1999) found that phenotypic specialization for nest excavation arose spontaneously in nests populated with multiple queens. Subsequently, Cahan and Gardner-Morse (2013) further showed a nascent division of reproductive labor: although average productivity per queen was maintained, one queen tended to take on a larger fraction of the reproduction.

The nematode genus *Pristionchus* has delivered several examples of plasticity-based novelty. The best studied is the feeding polyphenism of *Pristionchus pacificus*—individuals with a narrow 'mouth' and one 'tooth' are bacterivores, and those with a

wider two-toothed mouth are predators on fungi and other nematodes (Bento et al. 2010). The two-toothed form is induced under low-food conditions, and two genes, the sulfatase gene *eud-1* and the sulfotransferase *seud-1*, have been identified as antagonistic controllers of this plasticity-induced novelty (Ragsdale and Ivers 2016; Namdeo et al. 2018). These genes are both duplicate members of larger gene families that apparently evolved functional specialization to control the mouth polyphenism (Biddle and Ragsdale 2020). Further analyses have identified more details of the regulatory network involved in the switch (Kieninger et al. 2016; Sommer et al. 2017; Sieriebriennikov and Sommer 2018; Bui and Ragsdale 2019): intriguingly loss-of-function *eud-1* mutants result in all one-toothed individuals, while loss-of-function *nhr-40* mutants result in all two-toothed individuals, identifying these as plasticity-specific genes. Susoy et al. (2016) identified three new species of *Pristionchus* postulated to have feeding polyphenisms; each of these species has five different mouth shape morphologies!

Griffith et al. (2017) present evidence suggesting that the decidua, a distinctive cell layer forming the maternal portion of the placenta in eutherian mammals, arose as a stress response to implantation of the embryo. They demonstrate that features of the attenuated pregnancy of marsupials, such as the inflammation response when the fetal placenta contacts the maternal endometrium, are homologous to events in eutherian pregnancy. The authors propose that this plastic inflammation response is the evolutionary precursor enabling the origin of the anti-inflammatory phase that defines eutherian pregnancy (see also Wagner et al. 2019).

15.5 GENETIC ACCOMMODATION AND PLASTICITY-LED EVOLUTION

As mentioned previously, the expansion and refinement of plastic responses represent important evolutionary changes modifying phenotypes of organisms. The general topic of genetic accommodation has been reviewed fairly extensively (Schlichting and Wund 2014; Levis and Pfennig 2017; Schneider and Meyer 2017; Jones and Robinson 2018; Velotta and Cheviron 2018; Kelly 2019), and the specific topic of plasticity-led evolution is covered by Levis and Pfennig (2021), so I will highlight just a few exemplary recent studies.

Plastic responses to low oxygen levels, both short-term (acclimation) and long-term (adaptation), are well-studied, and some short-term changes that accentuate blood flow (hypertrophy of the ventricles) may be maladaptive in the long-term (right ventricular hypertrophy can result in lung disease and heart failure). Velotta et al. (2018) compared two species of deer mice (*Peromyscus*) that occupy different elevational ranges, comparing heart muscles at normal and reduced oxygen levels (hypoxia). The species had similar right ventricle sizes in normal oxygen levels, but high elevation *P. maniculatus* had much larger left ventricles. Exposed to low oxygen levels, *P. maniculatus* did not significantly change the size of either ventricle, while the low elevation species *P. leucopus* greatly increased the size of both: under low oxygen *P. leucopus*'*s* left ventricle was about the same size as *P. maniculatus*, but the right ventricle was now 30% larger than that of *P. maniculatus*. Velotta et al. (2018) suggest that this represents genetic accommodation in *P. maniculatus* via an

increase in the size of the left ventricle relative to that of *P. leucopus* and a reduction of the plasticity of both ventricles in response to changes in oxygen levels.

The work of Armin Moczek and colleagues on the plasticity of horn size in dung beetles of the genus *Onthophagus* represents some of the most in-depth examination of the process and mechanisms of genetic accommodation. Males of different species of *Onthophagus* show varying degrees of nutrition-related plasticity, but in general enhanced nutrition leads to the production of significantly larger horns (Emlen 1994; Moczek 2006). Casasa et al. (2020) examined transcriptomes of adult beetles of three species: *Digitonthophagus gazella* with a small nutritional response representing the ancestral state; *Onthophagus taurus* with a derived large nutritional response; and *O. sagittarius* with a derived lack of nutritional response (Emlen et al. 2005). Comparing transcriptomes of orthologous genes across species, Casasa et al. (2020) found only 8 differentially expressed (DE) genes between small and large males of the nutritionally non-responsive *O. sagittarius*, 946 DE genes for the moderately responsive *Digitonthophagus gazella*, and 1685 DE genes for the highly responsive *O. taurus*, revealing the expected correlation between the plasticity of gene expression and morphological plasticity. Eight hundred and fifty-nine genes that were strongly up-regulated in *O. taurus* were not differentially expressed in either of the other species, indicating that many genes have evolved increased nutritional sensitivity in the polyphenic *O. taurus*. The gene expression evidence also indicates that the secondary loss of plasticity in *O. sagittarius* results from the loss of nutritional sensitivity of many genes. These results indicate widespread genetic accommodation of plastic responses, both to enhance and reduce plasticity in different species. Evidence for evolutionary changes in plasticity have also been documented among different populations of *O. taurus* (Moczek et al. 2002; Rohner and Moczek 2020).

Corl et al. (2018) examined color dimorphism among populations of side-blotched lizards (*Uta stansburiana*) occupying different substrates in southern California. Populations of typical gray and tan mottled lizards from sandy substrates and dark lizards from the Pisgah lava flow (22,500 years ago; Figure 15.2a) were established in the laboratory on both lava rock and sand. Skin reflectance was measured after 1 year for each of the four groups: sand-sand (Figure 15.2b), lava-lava (Figure 15.2c), lava-sand (Figure 15.2d), and sand-lava. Lizards from both sand and lava substrates exhibited substantial phenotypic plasticity in coloration: both groups that were switched adopted coloration more similar to the new substrate (Figure 15.2e). Lizards from the lava flow were significantly darker on both substrates, and Corl et al. identified genetic differences between the sand and lava populations. Allele frequencies of two candidate genes regulating melanin production were found to differ strongly between populations: sand populations were monomorphic for the ancestral allele, but the lava population had derived alleles at each locus with frequencies >20%. Hatchling lizards carrying the derived alleles were darker. The combined results imply a scenario where plasticity present in the initial population provided an adaptive advantage to lizards living on the new lava substrate; subsequently, new alleles arose that increased the constitutive darkness of lava lizards—demographic simulations suggest that these alleles arose about 1000 generations ago and both are under positive selection (Corl et al. 2018).

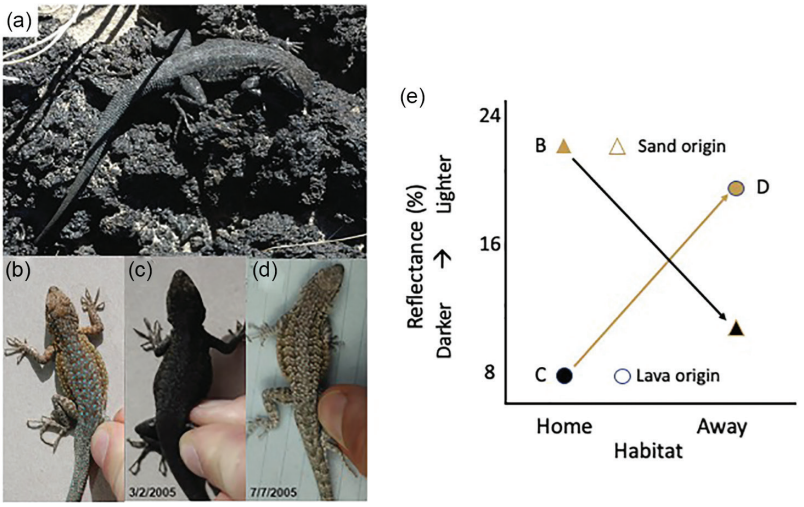

FIGURE 15.2 (a) The side-blotched lizard *Uta stansburiana* on lava rock. Individuals were collected from populations on sand and lava rock substrates. In the lab, they were placed in habitats with either sand or lava gravel. After 1 year, their reflectance was measured. (b) Sand origin after 1 year on sand. (c) Lava origin after 1 year on lava. (d) The same Lava origin lizard after 4 months on sand. (e) Lizards switched both from sand to lava and lava to sand responded plastically to produce coloration more similar to the resident populations. (Panels [a], [c], and [d] are reprinted from *Current Biology* 28:18, Corl et al., The genetic basis of adaptation following plastic changes in coloration in a novel environment, pp. 2970–2977, Copyright [2018], with permission from Elsevier; [b] courtesy of Ammon Corl.)

15.6 PLASTICITY AND SPECIES DIVERGENCE

Plastic responses to environmental variation may initiate reproductive isolation between adjacent populations. This isolation can take various forms—via alterations of phenology (Rajakaruna et al. 2003; Buckley et al. 2015; Dittmar and Schemske 2017; Taylor and Friesen 2017; Osborne et al. 2019), diet or host (Smith et al. 2013; Moser et al. 2015; Silva-Brandão et al. 2017), signaling (Iglesias et al. 2018; Otte et al. 2018), or even morphology (Garduno-Paz et al. 2020). Schlichting (2004), West-Eberhard (2005), and Pfennig et al. (2010) reviewed many examples, and subsequent authors have elaborated (Levin 2009; Fitzpatrick 2012; Schneider and Meyer 2017; Otte et al. 2018).

Treehoppers (Homoptera: Membracidae) of the *Enchenopa binotata* species complex are remarkable for host-plant associated speciation across a broad diversity of host trees (from eight different plant orders). *Enchenopa* communicate via vibrational signals through the substrate (i.e., stems, leaves, and petioles), and the comparison of signals of males from 11 *Enchenopa* species (each from a different tree species) revealed extensive differentiation in all measured aspects of the signals (e.g., frequency, amplitude, and duration; Cocroft et al. 2010). The key driver of speciation appears to be the species' philopatry—females show strong preferences for laying their eggs on the tree species upon which they developed (Wood and Guttman 1982;

Stearns et al. 2013). However, signal propagation changes plastically on different plant species (Sattman and Cocroft 2003; McNett and Cocroft 2008), and Rodriguez et al. (2008) have shown not only differences in signaling of individuals on different host species, but also genetic variation in signaling on a novel host—i.e., the raw material for precipitating genetic changes.

Etges and colleagues have studied populations of the cactophilic *Drosophila mojavensis* from Baja California (ancestral) and Sonora (derived) adapted to different cactus species, *Stenocereus gummosus* (pitaya agria) and *S. thurberi* (organ pipe), respectively (Etges et al. 2010). Cuticular hydrocarbons (CHCs) are important pheromones involved in sexual discrimination among males by females, and the differences in CHC mixtures between Baja and Sonora populations are important for premating isolation between them (Etges and Ahrens 2001). Although growth on the different cactus species significantly alters CHC profiles of flies (Stennett and Etges 1997; Etges and de Oliveira 2014), a direct link between plastic changes in CHC composition and mating preferences has not been made.

Sorenson et al. (2003) documented a remarkable example of behavioral plasticity initiating components of an adaptive radiation of 19 bird species (the indigobirds, genus *Vidua*) as brood parasites on grassfinches (Estrildidae). Rapid speciation is possible because *Vidua* species acquire mating traits through imprinting on their hosts—i.e., males learn their songs from their foster parent male and females adopt preferences both for those songs and for nests of the host species. This system has recently been revisited by Jamie et al. (2020), who find that additional components of the parasitic *Vidua*'s phenotypes also mimic those of the host species, enhancing reproductive isolation among *Vidua* species. They document several putative adaptations: mimicry of nestling behavior (matching of the presence/absence of head rotation or tongue movement while begging), mimicry of the parasites' calls to those of the host species (rather than to calls of other local grassfinch species), and mimicry of hatchling mouth coloration used as signals to parents while begging (more similar to markings of host than to markings of other local grassfinch species). Such further adaptations become more likely following reproductive isolation established by the initial plastic imprinting.

Garduno-Paz et al. (2020) started with a single population of stickleback and then induced plastic benthic (shoreline) and pelagic (open-water) feeding morphologies via diet modifications. Body morphology after 10 months on each diet showed the typical differences—benthic fish with comparatively shorter heads and jaw bones and deeper bodies (each fish was given a multivariate pelagic-benthic score based on morphology relative to a benthic-pelagic continuum). 'Benthic' and 'pelagic' experimental females were then simultaneously shown size-matched mates of each morphology group. Pelagic females were significantly more likely to mate with males that were similar in their shape score, i.e., males with similar morphology. They note that coupling the sharing of feeding locations with mating preference for similar shapes could make this plastic response a 'magic' trait—i.e., a simultaneous change in the morphology and a preference for it.

Plasticity may also play an important role in character displacement with plastic responses increasing differences between sympatric populations of species (Pfennig and Pfennig 2012; Robinson and Pfennig 2013 specifically examine the case for

character displacement following competition). An example of character displacement by means of the evolution of plastic responses is seen in the competing toad species *Spea bombifrons* and *S. multiplicata* (Pfennig et al. 2007). Both species are detritivores that can plastically produce a carnivorous tadpole morph when shrimp are present—this morph is larger, with larger jaws and a shorter gut. Remarkably, in sympatry with *S. multiplicata*, *S. bombifrons* does not produce the detritivore morph. Levis et al. (2017) pinpoint some of the genetic correlates of the loss of plasticity, identifying two genes in particular that show patterns of expression that differ between diets (detritus versus shrimp) and *S. bombifrons* populations (sympatric with *S. multiplicata* versus allopatric). The protein-coding peptidase gene *Pm20d2* had higher expression in the shrimp diet, but relatively reduced expression in *S. bombifrons* populations sympatric with *S. multiplicata*. The transcription factor *Btf3* also has higher expression in the shrimp diet, but only in allopatric *S. bombifrons*; gene expression plasticity has been lost in sympatric populations. Thus, these two genes show the evolution of expression patterns; their particular functions in morph production are yet unknown.

15.7 PLASTICITY PRODUCES DISTINCTIVE EVOLUTIONARY DYNAMICS

One significant consequence of plasticity for evolutionary dynamics was pointed out by Sewall Wright—plasticity can hide genetic variation (Wright 1931). A plastic response can move organisms to a different adaptive peak, even if allelic variation is available that might otherwise facilitate adaptive evolution. If the new environment is stable, we then have adaptation via plasticity but evolutionary stasis, a fundamentally different dynamic than adaptive evolution by allelic substitution.

Another fundamental role of plasticity in altering evolutionary dynamics arises from the changes in gene expression that altered environmental conditions produce (see Goldstein and Ehrenreich 2021 in this volume). Numerous studies have demonstrated that the genetic architecture (i.e., pleiotropy, epistasis, and genetic correlations) of a population may itself be strongly modified by the environment (Draghi and Whitlock 2012; Wood and Brodie III 2015; Parsons et al. 2016; Rowinski and Rogell 2017; Gibert et al. 2019; Schou et al. 2019). Thus, if one were to raise *genetically identical populations* in alternate environments (e.g., different temperatures, water availability, pH, and predators), their divergent genetic architectures can cause them to exhibit distinctive evolutionary responses to subsequent evolutionary challenges (e.g., herbivory, predation; Schlichting 1989).

Pespeni et al. (2017) found that, in *Onthophagus* beetles, genes that are conditionally expressed, i.e., only in specific environments, showed much stronger signals of previous natural selection. This pattern is predicted because selection on such alleles is relaxed in non-eliciting environments (Snell-Rood et al. 2010). Along these lines, a meta-analysis by Noble et al. (2019) indicated that the directions of plastic responses to novel environments are aligned with those aspects of the phenotype that also have significant heritability, suggesting that integrated phenotypes respond similarly to genetic and environmental perturbations.

Thus, the dynamics of both population genetic and quantitative genetic models can be altered by plastic responses or forms of ‘non-genetic inheritance’ (changes to

offspring phenotypes induced by parental environmental conditions; Danchin et al. 2011; Bonduriansky 2021). Early models demonstrated that non-genetic modes of inheritance can significantly alter the evolutionary dynamics of populations relative to predictions of standard genetic models (Hinton and Nowlan 1987; Laland et al. 1999). These have been augmented by more general models incorporating plasticity or non-genetic inheritance (Bonduriansky and Day 2009; Day and Bonduriansky 2011; Frank 2011a,b; Klironomos et al. 2013; Bonduriansky and Day 2018), showing lags, accelerations, bumps, and plateaus in evolutionary change.

Recent models have extended findings for plasticity. Coulson et al. (2017) develop a series of integral projection models that incorporate development (i.e., genotype-phenotype mapping) and mechanisms of inheritance, and contrasted how different instantiations of phenotypic plasticity (e.g., no phenotypic plasticity, adaptive phenotypic plasticity, and non-adaptive phenotypic plasticity) can influence evolutionary responses to environmental change. Populations with adaptive plasticity, despite suffering initial declines, show the most rapid rate of adaptive evolution to new environments. Their models also suggest that some reported examples of rapid evolution are more likely to be due to initial plastic responses than to genetic evolution.

Jeremy Draghi has developed models that examine the interplay of developmental noise and plasticity. Draghi (2019) examined a model in which developmental noise is controlled by the genotype and thus can evolve. In this model, low developmental noise (i.e., canalization) is favored in constant environments but tends to impede further evolution of plasticity via the reduction in phenotypic variability. This is particularly true when the suppression of developmental noise arises through negative feedbacks (e.g., antagonistic pleiotropic effects of different genes). However, replicates that evolved canalization without pleiotropy were not as constrained, indicating that how variability is controlled will influence the evolution of the system. Draghi (2020) took a different approach, allowing developmental noise to be induced by the environment, but the results are similar in that plasticity is again more likely to evolve when environmental noise is higher because the developmental noise creates more phenotypic variability and thus more opportunities to achieve phenotypes that increase fitness.

Several recent studies have also experimentally evaluated the effects of non-genetic inheritance. Dey et al. (2016) found that *C. elegans* subjected to predictable (alternating) normal versus low oxygen environments evolved plastic anticipatory maternal effects, such that mothers increased or decreased glycogen provisioning 'anticipating' next-generation conditions. They modeled evolution in predictably fluctuating versus randomly fluctuating environments and found that populations that evolved anticipatory maternal effects had increased adaptability (i.e., higher fitnesses) in subsequent random environmental sequences. Samani and Bell (2016) experimentally examined the dynamics of evolutionary rescue of wild yeast populations (*Saccharomyces paradoxus*) in relation to their prior evolutionary histories. They first selected lines for 4 weeks on fructose (non-stressful) and 11 other sugars that reduced population growth rates (starvation-adapted), then subjected all lines to four novel environmental factors: high temperature, high pH, alcohol, and salt. Intriguingly, the starvation-adapted lines had *reduced* adaptive plasticity relative to the fructose lines, but a higher subsequent likelihood of adapting to the novel

challenges: the more versatile fructose lines did not adapt to any of the novel challenges. Their results suggest that initial favorable plasticity actually inhibited subsequent evolutionary rescue. The diverse results of such experimental and model studies are fueling new hypotheses about how plasticity can evolve in heterogeneous environments (Bono et al. 2020).

15.8 PLASTICITY AND EVOLUTIONARY THEORY

The core structure of the modern synthesis, population genetics, is a rigorous and successful set of models of how evolutionary forces can alter allele frequencies. Likewise, quantitative genetics is a proven model for projecting statistical changes in distributions of polygenic traits over spans of a few generations. Neither population genetics nor quantitative genetics, however, inform us about how phenotypes are produced, how development proceeds, or how environment alters gene expression (Gawne et al. 2018; Hallgrimsson et al. 2019). Because selection operates on phenotypes, and only indirectly on the alleles that produce them, it is of paramount importance to understand mechanisms by which variation in phenotypes arises and increases, whether these variants are behavioral, physiological, or developmental. Phenotypes result from 'epigenetic' processes (in the Waddingtonian sense) that act to produce developmental trajectories (Schmalhausen 1949; Waddington 1953). Traits of organisms and their responses to variation in local environments (both external and internal) are central to understanding how evolutionary processes of selection and drift impact trajectories of phenotypic evolution.

To answer questions about phenotypes and traits, we need disciplines including 'evolutionary developmental biology' (evo devo), 'ecological developmental biology' (eco devo; Sultan 2015), and molecular ecology and genetics to investigate phenomena such as 'cryptic genetic variation' (Gibson and Dworkin 2004; Ledón-Rettig et al. 2014; Zheng et al. 2019), 'evolvability' (Wagner and Altenberg 1996; Schlichting and Murren 2004; Payne and Wagner 2019), canalization and robustness (Flatt 2005; Nijhout et al. 2019), 'developmental bias' (Uller et al. 2018), 'niche construction' (Laland et al. 2019), non-genetic inheritance (Danchin et al. 2011), and of course phenotypic plasticity. These fields and topics represent a broad research initiative focused on understanding the processes and mechanisms underlying the production, evolution, and importance of phenotypic diversity (e.g., Rollo 1994; Schlichting and Pigliucci 1998; West-Eberhard 2003; Pfennig et al. 2010; Bolnick et al. 2011; Moczek et al. 2011; Laland et al. 2015; Sultan 2015, 2017; van Gestel and Weissing 2016; Peichel and Marques 2017; Des Roches et al. 2018; Duclos et al. 2019; Glastad et al. 2019; Müller 2019; Herrel et al. 2020).

In this essay, I have highlighted evidence that plasticity occupies a conceptual nexus with links throughout evolutionary biology, impacting all hierarchical levels of evolution—macroevolution (origin of novelties), mesoevolution (speciation), and microevolution (adaptation). As Futuyma (2021) points out, plasticity has been identified as a key concept in an array of topics that some authors have suggested raise questions for evolutionary theory, and thus merit an 'extended evolutionary synthesis' (EES; Müller 2007; Pigliucci 2007). The ensuing arguments and counterarguments between proponents of EES and 'standard evolutionary theory' (SET) have been

predictably bloody in the academic fashion, with defenders of SET asserting that it can easily encompass all these topics (Laland et al. 1999; Wray et al. 2014).

The philosophy of biology community has welcomed the controversy between the EES and SET camps as a testing ground for ideas about research programs and paradigm shifts (Fábregas-Tejeda and Vergara-Silva 2018; Lewens 2019), and as with the debates among evolutionists, there are a diversity of opinions. In both fields, many arguments revolve around 'virtue-based' disputes (Buskell 2020): are plasticity's effects 'likely' enough, or 'significant' or 'strong' enough (Kovaka 2019; Baedke et al. 2020). Such arguments are ultimately obfuscatory, calling for decisions about whether some arbitrarily defined hurdle has been surmounted.

Rather than focusing on polarizing differences of opinion, it is probably most interesting for our purposes to be curious about these external views for their prescriptions on strategies for informing the debate. Baedke et al. (2020) call for an approach based on 'explanatory' power and applying explanatory standards (*precision*, *proportionality*, *sensitivity*, and *idealization*): "explanatory power can be assessed by comparing the range of inferences to potentially new counterfactual situations and, accordingly, of answers to *w-questions* ['what-if-things-had-been-different'] that alternative explanations make possible. For example, an explanation of a particular population dynamic that includes developmental factors might be able to answer more questions on what would happen to this population if it was changed" (Baedke et al. 2020 pp. 7–8, see also Uller et al. 2020).

Kovaka (2019) advocates a related approach, employing evidentiary standards of 'discrimination' and 'significance-relevant.' She suggests that, at least for the debate about plasticity-led evolution, "researchers need a richer middle-range theory" (i.e., one describing the relationship between plasticity-led evolution and observable evidence). This middle-range should be focused on evidentiary support to identify the 'traces' that characterize plasticity-led evolution. Kovaka proposes that, although direct evidence from natural populations is important, identifying signatures of plasticity-led evolution that distinguish it from SET models may be accomplished via, for example, experimental evolution, genomic comparisons, and modeling of plasticity-led versus genotype-led evolutionary dynamics.

Uller et al. (2020) propose that it may be the representation of plasticity itself as a reaction norm that is creating a block to appreciating its 'transformational' role in evolution. Because reaction norms have been conceptualized as manifestations of specific genotypes, they can easily be binned as yet another example of genetic variation to be collapsed into relative fitnesses on which selection may operate. Uller et al. (2020) argue that it is this "genetic idealization of evolution by natural selection" that allows unique details of development, physiology, or behavior to be ignored because they are unnecessary to explain a pattern of evolutionary change. They suggest that adopting alternative idealizations of evolutionary dynamics may produce more satisfactory explanations of patterns of phenotypic evolution.

15.9 WHERE SHOULD WE BE GOING?

As documented in this chapter and volume, phenotypic plasticity has multifarious connections with evolutionary biology and can have striking non-canonical effects

on evolutionary processes and their outcomes. I have previously avoided joining the arguments about EES versus SET, expecting that, after the initial volleys, both sides would continue profitable work in their specialties, with eventual *rapprochement*. However, I have become frustrated as defenders of the omniscient nature of SET continually expand the list of concepts that it can accommodate—such claims appear increasingly Procrustean. There *are* areas of evolutionary biology on which the SET is silent—the origin of novelties; biases in mutation or development; evolvability; evolution of the genotype to phenotype map; and the unfolding of development. Should we be content leaving these issues to the side, settling for a theory that seems adequate, or do we want to build theories with increased explanatory power?

The SET is in no danger of being eclipsed—it is still clearly fundamental for understanding the evolution of populations subject to selection and drift, but it just as clearly is not an encompassing theory of evolutionary processes. Futuyma (2021) argues that understanding the evolutionary importance of plasticity and related concepts just requires details of "the effects of mutation, gene flow, genetic drift, and especially natural selection on the frequencies of alleles and genotypes, … it's still genes and selection, all the way down." I counter that this distinctly gene-centric view of evolution, with its explicit perspective of phenotypes as byproducts, is myopic. The standard view of a genotype mapping to a single phenotype with a particular fitness is misleading at best, and likely inaccurate for most genes: via plasticity, a single genotype can produce multiple phenotypes, and each of those phenotypes will have its own environment-dependent *fitness*.

I propose that a view of evolution as a recursive process involving both the generation and sorting of variation is a more accurate and flexible perspective. Many of the topics embraced by supporters of an EES are related to the generation of variation. The SET, on the other hand, encompasses a wealth of theory about the sorting of variation. A full view sees new phenotypes produced via new mutation or exposure to new 'environments' (including new genetic backgrounds, new developmental milieus, and new external conditions), followed by processes that sort such variation—selection, drift, and gene flow (see e.g. Fábregas-Tejeda and Vergara-Silva 2018).

Thus, new empirical and modeling efforts to identify the roles of plasticity in evolution must explicitly recognize the complementarity of the processes involved in generating and sorting variation. Advances are already being made to construct more realistic models of evolution that incorporate separate terms for both genetic and environmental components of phenotypic variation. Such models can incorporate either of the benchmark equations of trait evolution (Breeder's or Price's equations) into population dynamic models for forecasting evolutionary trajectories (Coulson et al. 2020; Helanterä and Uller 2020; Rice 2020).

There are many avenues to explore on our way to a more complete understanding of how plasticity evolves. This will require building upon the recent surge of excellent work in ecological and evolutionary developmental biology and molecular ecology and genetics to get a clear picture of the genes and signal transduction pathways that control plastic responses. A better phylogenetic understanding of patterns and processes of the evolution of variation in plasticity is also needed. Results from work in these areas can then be viewed through the lens of robust models of evolution in populations that account for both genetic and environmental sources of phenotypic variation. Box 15.2 lists some suggestions for future research.

BOX 15.2 SUGGESTIONS FOR FUTURE RESEARCH

- Cryptic genetic variation and hidden reaction norms. Theory suggests that novel or rarely encountered environments should reveal cryptic genetic variation. This is an empirical question that can be addressed with experiments that expose organisms to a range of environments that span normal and unusual conditions.
- Plasticity in a phylogenetic context. Despite well-developed theory about when plasticity *should* evolve, most evidence of evolutionary change consists of comparisons of pairs of species or populations (Murren et al. 2014). There are actually very few mappings of divergence in plastic responses across phylogenies (Relyea et al. 2018). These should be produced via controlled environment studies examining responses of a group of species (e.g., congeneric taxa) to a set of environmental conditions. Such studies can answer questions about magnitude, direction, and rate of evolutionary change of plastic responses.
- Distinguishing adaptive from non- or maladaptive plasticity is experimentally difficult due to both the lack of appropriate genetic variants (highly adaptive plastic responses are likely to be fixed) and the scope of experimental designs. We need to employ the power of genomic and transcriptomic studies to uncover signatures of selection on genes and pathways related to plastic responses.
- Integration of plastic responses of phenotypes. How are plastic responses of different traits coordinated? (Schlichting 1989). There have been investigations examining pieces of this puzzle from the trait side (e.g., Buehler et al. 2012; Michimae and Emura 2012; Ellers and Liefting 2015; Rusman et al. 2018; Wright et al. 2018; Parsons et al. 2020) and many studies from the gene expression side (e.g., Mäkinen et al. 2017; Mark et al. 2019). Although examples are beginning to accumulate that link trait values and gene expression patterns (e.g., Casasa et al. 2020; Jacobs et al. 2020), there is a need for coordination of the concepts and literatures on the integration of trait plasticities and plasticity/modularity of gene expression.
- Following from Kovaka's (2019) prescriptions for detecting evolutionary signatures:
  - Contrast evolutionary dynamics of plastic versus non-plastic lineages in selection experiments. Such experiments could contrast outcomes for the evolution of plasticity from (1) experiments that select for a new trait mean versus (2) experiments that select on the plastic responses themselves. Both real and *in silico* populations could be followed (see e.g. Sikkink et al. 2019).
  - Examine the evolution of patterns of gene expression (transcriptomics) among recently diverged populations to discover characteristic patterns of change related to the evolution of plasticity (see discussion of work by Pfennig and colleagues above).

ACKNOWLEDGMENTS

Thanks to Doug Futuyma, David Pfennig, and an anonymous reviewer for their cogent comments on a draft of this chapter. Their observations and requests for clarity have made this a much better piece.

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