Phenotypic Plasticity: From Theory and Genetics to Current and Future Challenges

Ralf J. Sommer[1]
Max Planck Institute for Developmental Biology, Department for Integrative Evolutionary Biology, 72076 Tübingen, Germany
ORCID ID: 0000-0003-1503-7749 (R.J.S.)

ABSTRACT Phenotypic plasticity is defined as the property of organisms to produce distinct phenotypes in response to environmental variation. While for more than a century, biologists have proposed this organismal feature to play an important role in evolution and the origin of novelty, the idea has remained contentious. Plasticity is found in all domains of life, but only recently has there been an increase in empirical studies. This contribution is intended as a fresh view and will discuss current and future challenges of plasticity research, and the need to identify associated molecular mechanisms. After a brief summary of conceptual, theoretical, and historical aspects, some of which were responsible for confusion and contention, I will formulate three major research directions and predictions for the role of plasticity as a facilitator of novelty. These predictions result in a four-step model that, when properly filled with molecular mechanisms, will reveal plasticity as a major factor of evolution. Such mechanistic insight must be complemented with comparative investigations to show that plasticity has indeed created novelty and innovation. Together, such studies will help develop a true developmental *evolutionary* biology.

KEYWORDS phenotypic plasticity; polyphenisms; switch genes; plasticity first evolution; canalization; genetic assimilation; genetic accommodation; *Pristionchus*; *Spea*; *Ontophagus*; *Manduca*

PHENOTYPIC plasticity is the ability of a genotype to produce different phenotypes in response to distinct environmental conditions (Schlichting and Pigliucci 1998; Pigliucci 2001; West-Eberhard 2003; deWitt and Scheiner 2004; Whitman and Ananthakrishnan 2009; Moczek *et al.* 2011). Intrinsically, phenotypic plasticity refers to all kinds of environmentally induced phenotypic variation and it can affect morphological, physiological, and behavioral aspects of an organism's phenotype, but also its life history. Plasticity is a universal property of living things, because all organisms respond to genes and the environment alike; thus, plasticity is found throughout all domains of life. While botanists have long appreciated the environmental influence on plant morphology, plasticity was less valued in animal systems, although it is as widespread in animals as in plants (West-Eberhard 1989). In addition, plasticity is known from bacteria, and even phage λ and other bacteriophages with their lytic (virulent) *vs.* lysogenic (temperate) life cycles. It is an interesting oddity of the history of biology that the first molecular process that was ever elucidated to near completion, the regulation of the lytic cycle in phage λ, represents an example of plasticity, even though it is rarely discussed as such (Ptashne 2004).

Plasticity is pervasive, as demonstrated by the many examples currently studied in laboratories around the world. In the interest of space, I will not provide an overview of these study systems as there are simply too many. Instead, I refer the reader to the many review articles that have been published in recent years and that provide excellent overviews (Abouheif *et al.* 2014; Lande 2014; Laland *et al.* 2014, 2015; Moczek *et al.* 2015; Nalepa 2015; Nijhout 2015; Brisson and Davis 2016; Phillips 2016; Susoy and Sommer 2016; Tandonnet and Pires-daSilva 2016; Gibert 2017; Noble *et al.* 2017; Projecto-Garcia *et al.* 2017; Reuter *et al.* 2017; Schneider and Meyer 2017; Serobyan and Sommer 2017; Gilbert 2018; Jones and Robinson 2018; Josephs 2018; Oettler *et al.* 2018; Sanger and Rajakumar 2018; Sieriebriennikov and Sommer 2018; Uller *et al.* 2018; Lafuente and Beldade 2019; Levis and Pfennig 2019). While this list contains only

doi: https://doi.org/10.1534/genetics.120.303163
Manuscript received November 29, 2019; accepted for publication March 9, 2020; published Early Online March 10, 2020.
Available freely online through the author-supported open access option.
[1]Address for correspondence: Max Planck Institute for Developmental Biology, Max-Planck Ring 9, 72076 Tübingen, Germany. E-mail: ralf.sommer@tuebingen.mpg.de

Box 1

A: Historical skepticism against phenotypic plasticity and its significance for evolution
1. Empirical evidence for plasticity?
2. Can environmental responsiveness promote evolution?
3. Molecular mechanisms of environmental influence?
How should environmental effects be targeted by selection?

B: Developmental plasticity and evolution - West-Eberhard and four unique contributions for plasticity as a mechanism of evolution
1. A giant collection of alternative phenotypes
2. Alternative phenotypes as functionally independent targets of selection
3. A general critique of Neo-Darwinism and its inconsistencies and gaps
4. Plasticity as a facilitator of novelty (The facilitator hypothesis)

C: Three predictions for contemporary research to test the facilitator hypothesis
1. The origin of novelty starts with environmentally responsive and developmentally plastic organisms
2. Environmental responsiveness requires developmental switch genes to allow developmental reprograming
3. Pulses of plasticity end by environmental influences becoming genetically encoded – genetic accommodation and genetic assimilation

those reviews published since 2014 and is likely still incomplete, it demonstrates the growing awareness about plasticity and its evolutionary significance.

This growing awareness is in strong contrast to a long phase of neo-Darwinism that neglected the importance of development and the significance of the organism's responsiveness to the environment for evolution. For example, Williams objected that plasticity guarantees a dead end for the underlying traits, arguing that plasticity hinders evolution (Williams 1966). Similar arguments were made by Charlesworth *et al.* (1982), disputing the importance of development and the environment in favor of selection as the "*main guiding force of phenotypic evolution*" (Charlesworth *et al.* 1982, p. 474). Such skepticism remained for decades and largely centered around three major reservations (Box 1A) (Wund 2012). First, is there sufficient empirical evidence for plasticity in general, and for plasticity as a driver of evolutionary change? Second, does plasticity act to promote or hinder evolution? Finally, what could be the molecular mechanisms of the environmental influence on phenotypes, how do such mechanisms become genetically encoded, and how do they become a target of selection? These reservations highlighted the fundamental challenges for research on phenotypic plasticity, but they also provided a road map for novel investigations.

Eventually, only the identification of the molecular mechanisms enabling plastic responses of the organism to the environment will pave the way for full acceptance of plasticity in evolution and its significance for evolutionary change. Importantly, such investigations must contain a strong comparative perspective involving multiple species in a phylogenetic context to delineate plasticity as a potential originator of evolutionary novelty. Such studies must also reveal that plasticity is subject to selection, ultimately resulting in adaptive phenotypes. After a very short summary of theoretical and historical aspects of phenotypic plasticity, this contribution will formulate the three major research directions that plasticity research must take to provide mechanistic insight. Such mechanistic insight from selected model systems can provide the necessary empirical support for recent theoretical attempts to incorporate plasticity into an extended evolutionary synthesis (Uller *et al.* 2018). Finally, I will present a four-step model for the role of plasticity in evolution, which can help develop a true developmental *evolutionary* biology.

Three Independent, Conceptual Features of Phenotypic Plasticity

Three conceptual features of plasticity are important to properly evaluate the significance of plasticity for evolution. First, the phenotypic variation of plastic traits can be continuous or discrete, the latter resulting in alternative phenotypes. While continuous plasticity is more common in nature, it carries the inherent difficulty of properly distinguishing if the observed phenotypic variation indeed results from plasticity in response to the environment or, instead, from genetic polymorphisms. In contrast, alternative phenotypes, such as seasonal polyphenisms in butterfly wing patterns and the discrete defense phenotypes of clonally propagating rotifers that are preyed upon by various invertebrates (Brakefield *et al.* 1996; Gilbert 2018), exhibit a well-defined environmental response element. Therefore, discrete plasticity and alternative phenotypes have been crucial for advancing the theory of phenotypic plasticity, as will be discussed in more detail below. Additionally, alternative phenotypes have a number of advantages for experimental analysis given their binary readout. This is important for many contemporary case studies of plasticity in both animals and plants.

Second, phenotypically plastic traits can originally be adaptive or nonadaptive. Most researchers studying plasticity would argue that only the former can contribute to evolution when organisms are faced with a new environment. In

contrast, nonadaptive plasticity in response to extreme and stressful environments is likely to result in mal-adaptive traits with little evolutionary significance. However, some authors have recently argued the importance of such nonadaptive plasticity and its potential for rapid evolution that ultimately might become adaptive (Ghalambor *et al.* 2015). While the study design used in these experiments is itself contentious (Mallard *et al.* 2018; van Gestel and Weissing 2018), the general idea of nonadaptive phenotypic plasticity for contemporary adaptation to new environments (Ghalambor *et al.* 2007) might become an important research topic, and particularly so in light of climate change.

Finally, the threshold response of plasticity might be regulated in a conditional or a stochastic manner. While the former is more common, the potential role of stochastic factors is well known in microbes. For example, persister cell formation in *Staphylococcus aureus* and spore formation in *Bacillus subtilis* are examples of what, in microbial terminology, is often referred to as phenotypic heterogeneity or bistability (Dubnau and Losick 2006; de Jong *et al.* 2011; Ackermann 2015). However, stochastic factors are also increasingly recognized in examples of plasticity in multicellular organisms and conditional *vs.* stochastic regulation of plasticity are not mutually exclusive (Susoy and Sommer 2016). Together, continuous *vs.* discrete, adaptive *vs.* nonadaptive plasticity, and conditional *vs.* stochastic regulation represent important distinctions for the evaluation and significance of plastic traits in evolution.

The History of a Concept

From Baldwin to Bradshaw

Some of the controversy and contention around phenotypic plasticity has a historic basis. In this short section, I will only briefly summarize some of the major contributions that have influenced the perception of plasticity. For a full account, I refer the reader to an extended analysis of the history of phenotypic plasticity by Nicoglou (2015). The first example of plasticity was the so-called "Baldwin effect" published by James Baldwin in 1896, which did not even mention the word plasticity. The Baldwin effect describes the influence of learned behavior on evolution, suggesting that the organism's ability to learn a new behavior (for example in response to a new stressor) might affect fitness and therefore influence natural selection (Baldwin 1896). Similar ideas have been proposed multiple times independently and Gilbert Gottlieb has provided the most recent summary of plasticity, learned novel behaviors, and psychology (Gottlieb 1992).

Much of the controversies around plasticity are due to the vocabulary used in the premolecular era. As pointed out by Canfield and Greene (2009), this vocabulary is diverse, has changed over time, and, most importantly, has often been used inconsistently. This began more than a century ago when Richard Woltereck carried out the first experiments on plastic characters. In 1909, he used the water flea *Daphnia* to describe the relationship between the expressions of phenotypes across a range of different environments and coined the term "reaction norm" (Schlichting and Pigliucci 1998). The full significance of the phenomenon remained elusive because Woltereck had missed the opportunity to properly define phenotypes. Indeed, it was Johannsen who, in 1911, first distinguished between genotype and phenotype, and introduced the concept of genotype–environmental interaction (Nicoglou 2015).

Three decades later, Schmalhausen in Russia and Waddington in Great Britain further developed the concept of phenotypic plasticity. Schmalhausen developed a theory of "stabilizing selection," arguing that environmentally induced plastic traits, when adaptive, can become genetically fixed (Schmalhausen 1949). We will return later to this form of "genetic assimilation." Waddington, by using environmental perturbation of development, provided important conceptual contributions based on his work with the bithorax and crossveinless phenotypes in *Drosophila*. However, Waddington was inconsistent in his nomenclature, as can be seen from his late monograph *The Evolution of an Evolutionist* (Waddington 1975). While he clearly introduced the concept of genetic assimilation, and discussed the importance of developmental switches and epigenetic processes, he did not consistently use the same nomenclature and terminology. For example, in different publications on his selection experiments in *Drosophila*, he would sometimes omit the term genetic assimilation or "developmental switch," while in others the whole arguments centered around these terms. In parts, this was based on the missing genetic and molecular foundation of developmental biology in the 1940s. As a result, Waddington's argument for genetic assimilation to allow environmental responses to be incorporated into the developmental program of the organism was controversially discussed but found little support among neo-Darwinists (Amundson 2005).

In 1963, Ernst Mayr pointed toward another inconsistency in nomenclature. At that time, the term polymorphism was used to describe any kind of phenotypic variation independent of the underlying causes. Mayr suggested that the term polymorphism should be used only for variation that was genetically based. Simultaneously, he introduced the term "polyphenism" for nongenetic variation of the phenotype. He wrote:

> Polyphenism is discontinuous when definite castes are present or definite stages in the life cycle or definite seasonal forms. Polyphenism may be continuous, as on the cyclomorphosis of fresh-water organisms and some other seasonal variation (Mayr 1963, p. 150).

The distinction between genetic polymorphism and environmentally induced polyphenism is an important one, even if it has not been followed by all scholars in a consistent manner (Canfield and Greene 2009).

A short 2 years later, an even more important conceptual breakthrough was achieved when Anthony Bradshaw proposed that phenotypic plasticity and the ability to express

alternative phenotypes must be genetically controlled. Bradshaw developed this idea in 1965 based on the analysis of plants that develop alternative phenotypes in response to extreme environmental conditions. Bradshaw realized that the plasticity of a trait could differ between close relatives of the same genus, independently of the trait itself (Bradshaw 1965). For example, the degree of heterophylly can differ remarkably between closely related water plants, such as *Ranunculus peltatus* and *R. hederaceus*, or *Potamogeton natans* and *Po. lucens*. Similarly, marked differences in plasticity are known from varieties within certain crop species. Bradshaw concluded that "*such differences are difficult to explain unless it is assumed that the plasticity of a character is an independent property of that character and is under its own specific genetic control*" (Bradshaw 1965, p. 118). Together with Mayr's separation of polymorphism and polyphenism, Bradshaw's remarkable conclusion represents the key foundation for modern studies of plasticity.

West-Eberhard and alternative phenotypes

While Mayr's and Bradshaw's contributions were important, neither of them resulted in the acceptance of plasticity as a significant factor in evolution. As already indicated above, skepticism remained for decades, building on the reservations described in Box 1A. Therefore, the most important challenge was to provide empirical evidence for the widespread occurrence of plasticity and to simultaneously develop a theoretical concept that would support its significance for inducing evolutionary innovations. In 1989, Mary Jane West-Eberhard published a review article entitled *Phenotypic plasticity and the origin of diversity* that made the first convincing argument for plasticity to act as a diversifying agent in evolution. This idea was further developed and exhaustively expanded in her 2003 monograph *Developmental Plasticity and Evolution*. This monograph represented the most significant turning point in considering the role of plasticity in evolution by offering four unique contributions (Box 1B). First, it displayed an immense collection of polyphenisms. It showed "*once and for all*" (West-Eberhard 2003, p. vii) how pervasive alternative phenotypes and plasticity are in nature. This observation resulted in the important conclusion that organisms are universally responsive to the environment, similar to their responsiveness to genes. As such, environmental influences on organisms and their phenotypes must not be ignored in evolutionary theory. Second, West-Eberhard argued that alternative phenotypes become developmentally and functionally independent subjects of selection. The independent expression in different individuals and populations can therefore lead to evolutionary novelty and adaptation. Both of these conclusions, the universal interdependence between the environment and organisms, as well as selection independently targeting the alternative phenotypes of plasticity, were made possible by West-Eberhard's liberate restriction on alternative phenotypes.

She also pointed toward existing gaps and inconsistencies in mainstream evolutionary theory, which represented the third major contribution of her monograph. She clearly delineated how neo-Darwinian thinking sidelines organismal responses to the environment as being of little significance for evolution, although they are so tremendously widespread. Similarly, mainstream theory pays little attention to development as a proximal mechanistic principle that delineates all phenotypes. Instead, it relies on genes and mutations as the originator of new phenotypes, which results in important contractions and inconsistencies because selection cannot directly act on genes (except for those acting directly on germ cells). This critical review allowed West-Eberhard to propose plasticity as a mechanism that can fill existing gaps; by definition, phenotypic plasticity demonstrates the importance of the environment and development for the generation of phenotypic traits. Therefore, the final contribution of the book was to propose plasticity as a major facilitator of novelty: "*alternative (phenotypes) permit the elaboration of a new trait without eliminating an established one, thereby facilitating the evolution of new adaptive specializations*" (West-Eberhard 2003, p.377). This idea resulted in the hypothesis that novelty in evolution is often associated with plasticity. Plasticity, with its inherent consideration of environmental and developmental influences on phenotypes, is therefore a logical extension to evolutionary theory that can overcome existing inconsistencies. In summary, *Developmental Plasticity and Evolution* represented a *critique* on evolutionary thought that simultaneously provided new hypotheses, which can be empirically tested. As will be discussed below, the confirmation of the facilitator hypothesis and the identification of associated molecular mechanisms are the most critical challenges for current and future plasticity research.

Three Predictions for the Role of Plasticity in Evolution

Three predictions have to be fulfilled to support the role of plasticity as facilitator for evolutionary novelty and diversity (Box 1C). First, the origin of novelty often starts with environmentally responsive and developmentally plastic traits. This proposal is also referred to as "plasticity first evolution" or the "flexible stem hypothesis" (Gibert 2017; Levis and Pfennig 2019). Second, environmental responsiveness requires developmental reprogramming in the form of developmental switch genes. And third, pulses of plasticity are restricted in evolutionary time. Ultimately, plasticity, and with it environmental responsiveness, becomes genetically encoded, a phenomenon that is also known as "canalization," "genetic accommodation," or genetic assimilation with slightly different meanings, which will be discussed below. All three of these predictions need empirical support to show the role of plasticity in evolution. Ultimately, only case studies identifying associated molecular mechanisms can provide the necessary insight that will allow a general acceptance of plasticity as major factor of evolution. Therefore, in the second part of this contribution, I will provide three selected examples of plasticity from vertebrates, nematodes, and insects, respectively, resulting in a four-step model for the role of plasticity in evolution.

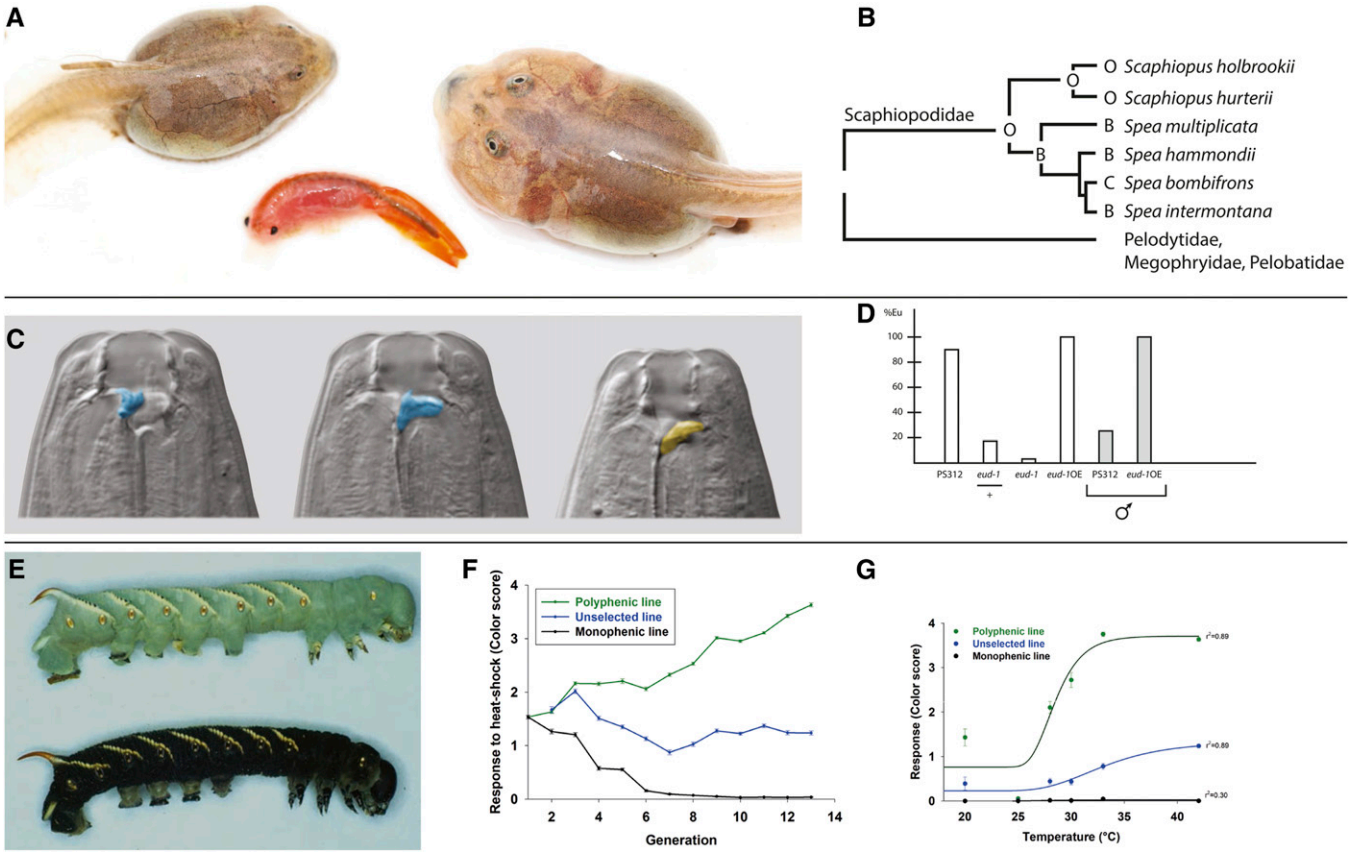

Figure 1 Three predictions for the role of plasticity in evolution. Prediction 1, novelty relies on plasticity (A and B); prediction 2, developmental switch genes and the molecular basis of plasticity (C and D); and prediction 3, regimes of canalization (E–G). (A) Spadefoot toads in the genus *Spea* produce alternative, environmentally induced tadpole morphs: a slower developing omnivore morph (left) and a more rapidly developing carnivore morph (right), which is induced by, and specializes on, animal prey, such as fairy shrimp (center). Photo: David Pfennig. (B) Plasticity first evolution. A phylogenetic comparison between different *Spea* species and the outgroup *Scaliphiopus* reveals that the novel carnivorous morph evolved through a phase of phenotypic plasticity. *Scaliphiopus* displays only the omnivorous morph. In contrast, *S. bombifrons*, a species that only exhibits the carnivorous morph, is secondarily derived, representing a secondary character loss consistent with plasticity first evolution (O, strict omnivore; B, both morphs; and C, strict carnivore). (C) Mouth-form plasticity in the nematode *P. pacificus*. The predatory eurystomatous (Eu) mouth form (left and center) exhibits a dorsal tooth (colored blue in left picture) and a subventral tooth (blue in central picture). In contrast, the bacterivorous stenostomatous (St) morph has only a dorsal tooth (not visible in this focal plane), whereas the subventral tooth is reduced to a ridge (yellow in picture to the right). Photo: Tobias Theska. (D). Mouth-form plasticity is controlled by a developmental switch gene. The PS312 wild-type strain is predominantly Eu. Animals heterozygous for a mutation in the switch gene *eud-1* are already predominantly St, whereas homozygous mutants are all-St. Overexpression (OE) of *eud-1* reverts the phenotype to all-Eu, suggesting that the activity of *eud-1* is dose-dependent. This is further supported by the fact that *eud-1* is located on the *X* chromosome and males, carrying a single *X* chromosome, are preferentially St. However, overexpression of *eud-1* from a transgene converts the phenotype to all-Eu. [redrawn and modified from Ragsdale *et al.* (2013)]. (E) The tobacco hornworm *M. sexta* develops green larvae, but black mutants exist that recapitulate the evolutionary ancestral state. This state, as shown for *M. quinquemaculata*, exhibits a color dimorphism with black larvae when cultured at 20° and green larvae when cultured at 28°. Photo: Fred Nijhout. (F) Selection results in genetic accommodation. Changes in the mean coloration of heat-shocked larvae in response to selection for increased (green) and decreased (black) color response to heat-shock treatment. The blue line represents the color score of an unselected control line [redrawn with permission from Suzuki and Nijhout (2006)]. (G) Reaction norm after 13 generations of selection for polyphenic or monophenic lines. Culturing at constant temperatures between 20° and 40° reveals that only the polyphenic line shows a strong temperature response in coloration, indicating that genetic accommodation can be selected for in only 13 generations. In contrast, no or little coloration differences in response to different culture temperatures were seen in the monophenic and unselected lines, respectively [redrawn with permission from Suzuki and Nijhout (2006)].

Prediction 1: novelty relies on plasticity

West-Eberhard proposed that the origin of novelty often starts with environmentally responsive and developmentally plastic traits (West-Eberhard 2003). Testing this prediction requires comparative studies in a strict phylogenetic context to determine the direction of change and, thus, polarity. In the past, such studies have been difficult because potentially novel (apomorphic), plastic characters have themselves been used for phylogenetic reconstruction. This resulted in problems for phylogenetic reconstruction if the plasticity and potential canalization of characters were not properly considered. Now, molecular sequence analyses provide robust phylogenies independent of morphological characters. Such phylogenetic frameworks allow the proper use of the comparative

method (Harvey and Pagel 1991), and indeed, various case studies confirmed the prediction that plasticity correlates with phenotypic novelty.

One example is the origin of predatory morphs in spadefoot toad tadpoles [for recent review, see Levis and Pfennig (2019)]. The North American toad genus *Spea* has invaded an unexplored ecological niche consisting of rapidly drying ponds that usually exist only for a short period of time (Figure 1, A and B). Tadpoles of most anurans are omnivores, such as species of *Scaliphiopus*, a group that is closely related to *Spea*. Such omnivore tadpoles have small jaw muscles, smooth mouthparts, and a long gut, and they eat detritus, algae, and small crustaceans. In contrast, tadpoles of *Spea* have evolved an extreme case of polyphenism, exhibiting an alternative, carnivorous morph. Carnivore tadpoles have large jaw muscles, notched mouthparts, and a short gut, and they preferentially eat larger shrimps and even other tadpoles. These tadpoles are an evolutionary novelty because they are only known from species of *Spea*. Interestingly, most *Spea* species display the described polyphenism through diet-induced plasticity (Figure 1A). For example, *S. multiplicate* exhibits plasticity in muscles, mouthparts, gut length, and body size. However, another species, *S. bombifrons*, only forms the carnivorous but not the omnivorous morph. The phylogenetic relationship of the described species is consistent with the plasticity first hypothesis: species of *Scaliphiopus*—the outgroup to *Spea*—only form omnivore tadpoles, representing the ancestral pattern. Then, the origin of plasticity resulted in the alternative carnivorous morph (Figure 1B). Finally, the absence of the omnivorous form in *S. bombifrons* represents a secondary loss.

In a recent study, Levis and co-workers extended their previous analysis, and tested to which extent alternative diet (detritus *vs.* shrimp) could induce plasticity and change-associated morphologies (Levis *et al.* 2018). In *Scaliphiopus*, diet-induced morphological changes were observed in several traits, but only some of them were adaptive. In contrast, the phenotypically plastic *S. multiplicate* exhibited adaptive diet-induced plasticity in all investigated traits. Finally, the canalized *S. bombifrons* showed an even greater refinement of characters in response to a shrimp diet. These studies strongly support the role of plasticity for the evolution of the carnivorous morph as a novel trait and suggest the following scenario. First, environmental changes trigger and induce phenotypic variation through phenotypic plasticity. Second, different genotypes and populations differ in the type and abundance of their response patterns. Third, natural selection can act on these response patterns and can finally result in a canalized phenotype that is itself still subject to selection. Therefore, such an evolutionary scenario creates both morphological novelty and diversification, as seen in *S. bombifrons*.

Increased comparative research activities provide strong evidence for the plasticity first hypothesis. One other example in the context of feeding plasticity is the evolution of predatory *vs.* nonpredatory mouth forms in the nematode *Pristionchus pacificus*. The investigation of $\sim$100 species of $>$ 20 genera of the same family of nematodes, the Diplogastridae, revealed that the evolutionary novelty, the formation of teeth-like denticles enabling predation, was also associated with plasticity (Nijhout 2015; Susoy *et al.* 2015). Mouth-form plasticity in *P. pacificus* will be the subject of the next paragraph, testing the second major prediction of the facilitator hypothesis. Indeed, nematodes are a prime target for the study of plasticity for multiple reasons. First, $\sim$80% of animal species on Earth are believed to be nematodes (Smythe *et al.* 2019; van den Hoogen *et al.* 2019). Second, besides their abundance they display enormous diversity, in particular with regard to feeding structures and feeding strategies. Finally, several selected model systems can be cultured under laboratory conditions, providing the necessary tools to obtain molecular and mechanistic insights. This includes *Caenorhabditis elegans* and the aforementioned *P. pacificus*, which will be described in more detail below.

Prediction 2: developmental switch genes and the molecular basis of plasticity

Nonplastic developmental processes are hardwired against environmental fluctuations, whereas plastic processes are characterized by being able to sense and respond to environmental information. It has been proposed that developmental switch genes fulfill this function by first, sensing the environment and second, controlling alternative phenotypes. However, the identity of such developmental switch genes remained completely elusive for a long time. In developmental biology, so-called "genetic switch genes" are well known from various developmental pathways. For example, the proto-oncogene RAS in EGF/EGFR signal transduction, when permanently activated through a gain-of-function mutation, results in a conformational change that leads to the overexpression of certain cell fates (Han and Sternberg 1990). As part of more complex gene regulatory networks (GRNs), such signal transduction pathways control gene expression via individual or groups of transcription factors (Davidson 2006). However, it was unclear if genetic switch genes were part of "environmentally induced developmental switches" in the context of phenotypic plasticity. Similarly, the GRNs that control plastic phenotypes have not yet been identified. Therefore, testing this prediction, and identifying developmental switch genes and associated GRNs, were essential to confirm the significance of plasticity for evolution. Such endeavors require a model system approach with molecular and genetic investigations providing mechanistic insight.

One such system is the nematode *P. pacificus*, which exhibits a feeding dimorphism in form of a bacterial feeding morph (stenostomatous, St) and a facultative predatory (eurystomatous, Eu) morph (Bento *et al.* 2010) (Figure 1C). *P. pacificus* is a self-fertilizing nematode that usually propagates as hermaphrodites with the rare occurrence of males. Self-fertilization in this species results in a unique advantage for the study of phenotypic plasticity. All the progeny of an individual worm, and usually even all members of a population, are clonal and thus genetically identical. *P. pacificus* has been established as a genetic system with forward and reverse

genetic tools (Sommer 2015), allowing unbiased genetic studies of mouth-form plasticity. Also, mouth-form plasticity in *P. pacificus* shows strong conditional regulation. When grown on standard nematode agar plates with *Escherichia coli* as a food source, worms are preferentially predatory. In contrast, when grown in liquid culture, worms are nearly exclusively nonpredatory (Werner *et al.* 2017). Thus, *P. pacificus* allows fast genetic and environmental manipulation of a plastic trait under laboratory conditions.

Strikingly, the first genetic investigations of mouth-form plasticity indeed identified a developmental switch gene. Ragsdale and co-workers performed unbiased screens for mutants that would alter the mouth-form ratio of *P. pacificus* on agar plates and identified a gene that, when mutated, would result in all-St worms (Figure 1D) (Ragsdale *et al.* 2013). This gene was named *eud-1*, for *eu*rystomatous-form -*d*efective; it encodes a sulfatase, and mutations in *eud-1* show a number of unusual characteristics. First, mutants are dominant so that hermaphrodites with a single mutant copy are already preferentially St. Second, these *eud-1* mutants are loss-of-function, a rare genetic phenomenon. For example, in *C. elegans*, only one dominant loss-of-function mutant is known after 50 years of genetic studies, whereas dominant gain-of-function mutants are frequent. In *P. pacificus*, the dominant *eud-1* phenotype already indicates the role of this gene as a developmental switch. Indeed, further studies revealed that *eud-1* is extremely dose-sensitive and overexpression of *eud-1* in a *eud-1* mutant background can completely revert the all-St mutant phenotype into an all-Eu phenotype (Figure 1D) (Ragsdale *et al.* 2013). Thus, *eud-1* represents a classical genetic switch gene, as described from developmental genetic studies in multiple model organisms. These findings suggest that environmentally induced developmental switch genes share, at least in part, characteristics of genetic switches. However, the situation is more complicated, as indicated below.

Subsequent investigations showed that *eud-1* represents a complex genetic locus. It contains an antisense transcript that acts positively on *eud-1* expression (Serobyan *et al.* 2016). Furthermore, *eud-1* is part of a multigene locus that contains two pairs of duplicated genes in a tandem inverted configuration (Sieriebriennikov *et al.* 2018). These genes, *nag-1* and *nag-2*, encode for N-acetyl-glucosaminidases and mutants in these genes have an opposite phenotype to *eud-1*, resulting in all-Eu animals under all culture conditions. *eud-1*, *nag-1*, and *nag-2* are expressed in different sensory neurons, further supporting the notion that they are involved in sensing the environment. Thus, the environmentally induced mouth-form switch is a network with modular organization, the full complement of which is still to be investigated. Such a switch network cannot act alone and it has been anticipated that it must function in concert with a "phenotypic execution network," which most likely represents a typical GRN (Sieriebriennikov and Sommer 2018).

Indeed, more recent genetic studies identified large parts of the GRN of mouth-form plasticity in *P. pacificus* (Bui and Ragsdale 2019; Sieriebriennikov *et al.* 2020 *preprint*). At the center, two nuclear hormone receptors, *nhr-1* and *nhr-40*, are involved in transmitting environmental information to mouth-form decision-making processes. Interestingly, *nhr-40* also shows characteristics of a genetic switch, as gain-of-function mutations result in all-Eu phenotypes, whereas loss-of-function mutations of *nhr-40* are all-St (Sieriebriennikov *et al.* 2020). These findings clearly indicate that both the switch network and the GRN of mouth-form plasticity are complex entities, and that genetic switches are only parts of larger regulatory networks. When Sieriebtriennikov and co-workers tried to identify the downstream targets of the nuclear hormone receptors NHR-40 and NHR-1, they found that they have a small number of common targets. However, surprisingly, all of these common targets are fast-evolving genes that have no 1:1 orthologs in *C. elegans*. This is in strong contrast to *nhr-1* and *nhr-40* themselves, which are 1:1 orthologous between *P. pacificus* and C. *elegans*; although, in general, nuclear hormone receptors evolve extremely rapidly (Sieriebriennikov *et al.* 2020). Thus, the evolution of a novel feeding behavior by a novel morphological structure depends on rapidly evolving genes, the latter of which are primary subjects of recent investigations (Rödelsperger *et al.* 2019).

Taken together, these studies strongly support the second prediction of the facilitator hypothesis: plasticity requires developmental reprogramming in the form of developmental switches that can incorporate environmental information. However, the associated molecular mechanisms are complicated, involving complex loci, such as *eud-1*, that function as switches and GRNs. While still early, it is likely that switch genes point to a general principle of plasticity because other examples of plasticity also involve complex switch mechanisms. For example, the nematode dauer stage, which represents a second example of phenotypic plasticity in nematodes, is also regulated in a complex manner and involves the nuclear hormone receptor *daf-12*, which acts as a switch (Antebi 2015). In addition, the regulation of the lytic cycle in bacteriophages identified a switch mechanism that required a complex genetic locus, and relied on genetic and epigenetic mechanisms (Ptashne 2004). Finally, the regulation of flowering in *Arabidopsis thaliana* through the flowering locus C is another example of a complex genetic locus that involves genetic and epigenetic mechanisms to regulate a phenotypically plastic trait (Costa and Dean 2019). In all these systems thresholds exist, above or below which responses to the environment result in different phenotypic outcomes. Therefore, it is very possible that future studies on other examples of plasticity will reveal similar principles involving switch mechanisms.

Prediction 3: regimes of canalization, from genetic accommodation to assimilation

Important challenges remain to prove the full significance of plasticity as a major mechanism of evolution. Are plastic traits subject to external selection pressure and what are the

mechanisms that will result in the fixation of the originally plastic traits? Many case studies in the last two decades have shown that plastic traits are indeed subject to selection, resulting in rapid evolution of the associated traits, including the *Spea* tadpoles discussed above. Another example comes from beetle horn development in the dung beetle *Ontophagus taurus*. Males of this species express two alternative phenotypes. If they grow under favorable conditions, they have a large body size and in response, develop a pair of horns that allows them to fight against other males to obtain a chance of mating with females. In contrast, males that grow under unfavorable conditions will remain small, and as a consequence, stay hornless and will not get involved in fights with conspecific males. Thus, a body weight threshold will induce the formation of horns resulting in a polyphenism associated with different mating behaviors. While *O. taurus* is originally from the Mediterranean region and is common in many southern European countries, it was introduced into several Australian states as part of a release program between 1969 and 1983. In the same time period, it was accidentally also introduced into the eastern United States with the first reports from Florida in 1971. The comparison of the material from Australia and the United States after 40 years of independent evolution revealed massive divergence in the threshold body size that induces the formation of horns. Common garden experiments showed that this divergence is genetically encoded because it is fully maintained under laboratory conditions for multiple generations (Moczek and Nijhout 2002). In a follow-up study between native (Mediterranean) and exotic (Australian and United States) populations, it was shown that threshold divergence has indeed evolved in the 40 years of independent evolution (Moczek and Nijhout 2003). These studies clearly indicated that polyphenisms are subject to selection, which will result in adaptive changes.

Experimental studies under laboratory conditions can provide direct evidence that polyphenisms might evolve through genetic stabilization of an originally environmental signal. Selection experiments have been extremely powerful in butterflies, such as for eyespot patterns in *Bicyclus anynana* and larval color variation in the tobacco hornworm *Manduca sexta* (Brakefield *et al.* 1996; Suzuki and Nijhout 2006). *M. sexta* is monomorphic with regard to its larval color with only green larvae (Figure 1, E–G). In contrast, the close relative *M. quinquemaculata* exhibits a color dimorphism, developing a black phenotype at 20° and a green morph at 28° (Figure 1E). Suzuki and Nijhout performed selection experiments with a *black* mutant of *M. sexta* that shows reduced juvenile hormone secretion resulting in a black morph, similar to the one known from *M. quinquemaculata* (Figure 1F). Heat-shock experiments at 42° in the sensitive period allowed a range of color morphs to be generated that can be selected for in experimental evolution settings. Within 13 generations, polyphenic and monophenic lines were selected that resulted in green and black morphs, respectively (Figure 1F). When cultured under constant conditions between 20° and 33°, only the polyphenic line exhibited a strong threshold response with temperatures $> 30°$, showing a similar response to the heat-shock condition of 42° (Figure 1G). Physiological studies revealed that the polyphenic line had higher juvenile hormone titers at higher temperatures, suggesting that changes in hormonal regulation may underlie the evolution of color polyphenism (Suzuki and Nijhout 2006). This study is a striking example of what was originally designated as genetic accommodation (West-Eberhard 2003). During genetic accommodation, a novel phenotype caused by mutation or environmental change becomes ultimately manifested as an adaptive phenotype through quantitative genetic changes. Together, the study of various insect systems by Nijhout and colleagues, as well as others, clearly indicates that plastic traits are subject to selection leading to phenotypic divergence through accumulated genetic accommodation.

It is important to note that genetic accommodation, as described above, is clearly distinct from genetic assimilation and canalization. Genetic accommodation is a mechanism by which a phenotypic variation, originally induced by a mutation or an environmental change, becomes adaptive. In contrast, genetic assimilation results in the genetic fixation (canalization) of the novel trait, thereby eliminating its environmental responsiveness altogether (Suzuki and Nijhout 2006; Jones and Robinson 2018). Both processes are important for the role of plasticity in evolution; ultimately, associated molecular mechanisms must be identified, like in the case of environmentally induced developmental switches. Indeed, the identification of the associated molecular mechanisms represents a major remaining challenge for a detailed understanding of phenotypic plasticity.

Challenges and Opportunities for Current and Future Research

The identification of environmentally induced developmental switches and the indication that plastic traits are indeed subject to selection leave one major challenge unanswered: what are the mechanisms that will result in the genetic assimilation of a trait in the final step of plasticity evolution? How do environmental influences on phenotypes become genetically encoded? Do these phenomena involve transgenerational effects? Is epigenetic information involved in these processes? The identification of the molecular mechanisms that enable the transition from an "environmentally induced" to a "genetically encoded" state is thus a prime area of future research.

Fortunately, a rich range of literature is currently accumulating that studies transgenerational and epigenetic effects. In particular, the nematode *C. elegans*—with its clonal reproduction, rapid growth in the laboratory, and dietary simplicity—exhibits a number of examples of such "noncanonical" inheritance. While these studies are not primarily concerned with phenotypic plasticity, they offer important insight into the mechanisms that are potentially associated with genetic assimilation and canalization. Below, I provide a brief overview

about transgenerational inheritance in *C. elegans* and will finally discuss its potential power for plasticity research.

Transgenerational epigenetic inheritance was first documented in plants, where germ cells often derive from somatic tissues and are exposed to the environment (Heard and Martienssen 2014). In contrast, in animals, transgenerational effects have been more difficult to identify. It was early work in *C. elegans* on germline immortality that revealed reprogramming of epigenetic memory (Xu *et al.* 2001). Strome and co-workers identified in forward genetic screens the "*m*aternal *e*ffect *s*terile" (*mes*) genes that are maternally required to contribute to germline immortality. If MES proteins are not provided by the mother, germ cells will die and the animal will be sterile. Subsequent studies have shown that MES proteins repress the epigenetic memory of the *X* chromosome on H3K27me (Gaydos *et al.* 2014). At the same time, a number of related findings were made. For example, mutants in the H3K4me2 demethylase, called *spr-5* in *C. elegans*, exhibit progressive sterility over 20 generations, while original mutant cultures are fertile (Katz *et al.* 2009). Similarly, transgenerational epigenetic inheritance of life span was shown to require H3K4me3 (Greer *et al.* 2011). Other studies revealed that foreign DNA also induces transgenerational responses in worms, largely by acting through different forms of epigenetic inheritance. Various small RNAs are involved in gene silencing and are transmitted for multiple generations in a non-Mendelian manner (Rechavi *et al.* 2011; Shirmayama *et al.* 2012). In this context, the genetic cofactors required for gene silencing were found to involve various Argonaute proteins, which are encoded by a gene family that is massively expanded in worms (Ashe *et al.* 2012; Conine *et al.* 2013; Seth *et al.* 2013; Wedeles *et al.* 2013). More recently, starvation- and temperature-induced transgenerational effects were shown to also require small RNAs (Rechavi *et al.* 2014; Jobson *et al.* 2015; Klosin *et al.* 2017; Belicard *et al.* 2018). Thus, a substantial body of evidence has accumulated in *C. elegans* that bridges the gap between transgenerational effects and epigenetic memory, and identifies associated mechanisms (Lim and Brunet 2013; Klosin and Lehner 2016).

Genetic assimilation might be initiated by transgenerational effects that build upon epigenetic processes. For example, heritable chromatin marks are known to be deposited at RNA interference-targeted loci in *C. elegans* and they might affect the biogenesis of heritable small RNAs (Rechavi and Lev 2017). However, it is unlikely that traces of these processes can still be found in the already canalized phenotypes discussed above. Instead, one has to search for such mechanisms in experimental evolution settings. The increasing evidence that plastic traits can evolve rapidly makes them amenable to experimental evolution studies, in particular in insects and nematodes, but also other rapidly propagating species. Unbiased searches for associated epigenetic mechanisms will be necessary to create the ultimate link between environmentally induced epigenetic processes and properly inherited genetic changes. A true challenge for the next decade.

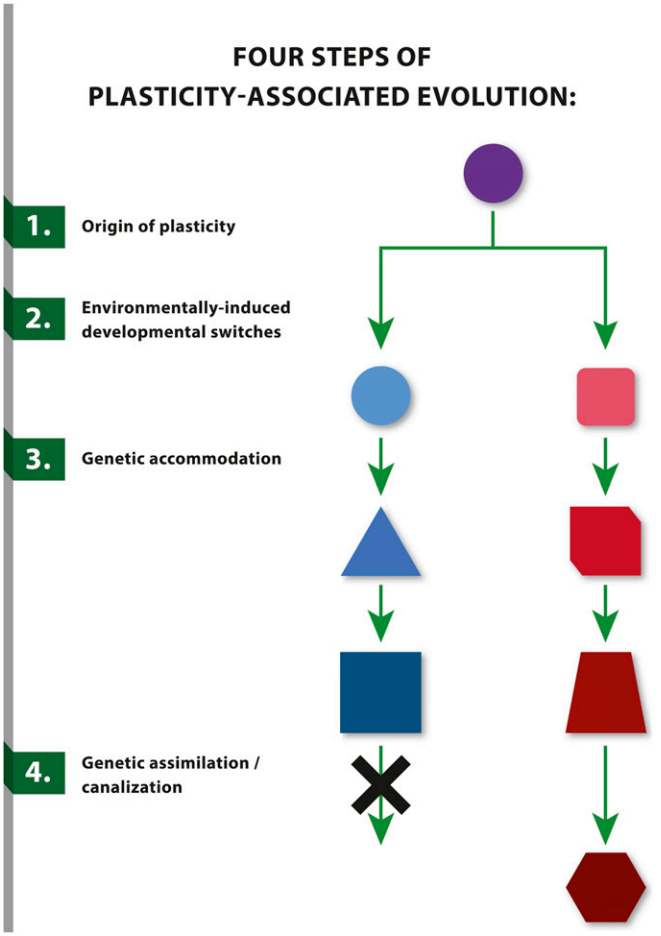

Figure 2 A four-step model for the role of phenotypic plasticity in evolution. First, the evolution of novelty (light-blue circle and light-red square) starts as an environmentally sensitive and phenotypically plastic trait from a previously hardwired monomorphic phenotype (purple circle). The origin of plasticity might be caused by environmental change and/or genetic mutations. Second, environmentally induced developmental switches regulate the expression of alternative phenotypes after sensing environmental variations. Alternative plastic traits are independently expressed in different individuals and populations. They can be the target of selection because they are functionally and developmentally independent. Therefore, selection will result in adaptation and further phenotypic diversification, a phenomenon referred to as genetic accommodation (indicated as shape variations in both phenotypes). Note that the color difference of both shape groups indicates that morphological and physiological traits, and their evolutionary variation, might influence the interaction of such organisms, *i.e.*, their behavior. In the final, fourth step, a phase of plasticity is terminated in a process called genetic assimilation or canalization (X). The associated molecular mechanisms that will give rise to canalized phenotypes have yet to be identified, similar to those associated with genetic accommodation. This represents the major challenge for plasticity research in the decade to come.

A Four-Step Model for the Role of Plasticity and the Origin of Novelty

Plasticity first evolution in spadefoot toad tadpoles, environmentally induced developmental switch genes in predatory nematodes, and genetic accommodation and assimilation in dung beetles and tobacco hornworms not only confirm the

major predictions of the facilitator hypothesis, but they also represent the different phases of phenotypic plasticity. I propose a four-step model to visualize the different phases of plasticity as an evolutionary trajectory and as a framework for associated phenotypic transitions (Figure 2). In the first step, environmental changes and/or genetic mutations result in the occurrence of plastic traits (Figure 2, step 1). While several examples of such plasticity first evolution have already been documented based on careful comparative and phylogenetic investigations, the associated molecular mechanisms await future analysis. Second, the conditional regulation of development requires developmental switches, the identification of which moved the analysis of plasticity from the phenotypic and phylogenetic levels to molecular mechanisms. However, the nematode example of feeding structure plasticity revealed that the genetic control of plasticity is complex, and requires sophisticated genetic and genomic tools (Figure 2, step 2). In the third step, the independent expression of plastic traits in different individuals and distinct populations results in local adaptation through independent selection. Associated changes in the molecular network controlling plasticity will result in genetic accommodation and, thus, further diversification (Figure 2, step 3). However, the identification of such changes is only possible once the regulatory developmental switches and associated GRNs have been identified. Ultimately, in the fourth step, one of the diversified traits might become genetically encoded and thereby independent of environmental influence. Such genetic assimilation or canalization will fix the trait, and will end the evolutionary pulse of plasticity (Flatt 2005) (Figure 2, step 4).

It is important to note that phenotypic innovation is not restricted to the origin of plasticity and genetic accommodation. Instead, the study by Levis and co-workers described above indicated that the canalized *S. bombifrons* showed the strongest character divergence upon diet induction (Levis *et al.* 2018). Similarly, studies in nematodes indicated that the loss of polyphenism of feeding structures was followed by an even stronger rate of subsequent evolution of new phenotypes (Susoy *et al.* 2015). Thus, the loss of plasticity can also be associated with increased evolvability. Stabilizing mechanisms, which originally buffer the alternative phenotypes of the organism against genetic changes, might represent an explanation for this increased evolvability (Nijhout 2015). Such buffering mechanisms will result in the gradual accumulation of mutations that would otherwise affect the alternative phenotypes. When the polyphenism is lost, the need for stabilization disappears and some of the accumulated genetic variation is no longer buffered. This might cause the canalized phenotype to vary even more, and through selection can result in diverse adaptations (Nijhout 2015). Thus, all phases of plasticity are involved in creating novelty and diversity, making phenotypic plasticity a rich source of phenotypic innovation in evolution.

Conclusions

Phenotypic plasticity was, for a long time, an underappreciated and in large parts neglected mechanism and concept of evolution. This perspective is changing with new theoretical and empirical studies that point toward the significance of plasticity for facilitating the novelty and diversity of morphological, physiological, behavioral, and life history traits. Four features are necessary to reveal the importance of plasticity for the evolution of a given trait. First, comparative phylogenetic studies must show that plasticity indeed coincides with a novel trait. Second, molecular mechanisms must provide insight into how environmental information is perceived and how switch genes regulate alternative phenotypes. Third, the role of selection driving the adaptation of plastic traits has to be investigated in a phylogenetic context, resulting in genetic accommodation and assimilation. However, the true challenge for the decade to come will be to show the molecular mechanisms of genetic assimilation and, thus, how environmental information becomes genetically encoded during character canalization.

Acknowledgments

I thank M. Dardiry, T. Renahan, and Dr. M. Werner for critically reading this manuscript; M. Voetsch for the artwork; and members of my laboratory, and the many international colleagues and friends, for stimulating discussions about phenotypic plasticity and its role in evolution over the years. The author also wants to apologize to important contributions in the field that could not be cited because of space restrictions.

Literature Cited

Abouheif, E., M.-J. Favé, A. S. Ibarrarán-Viniegra, M. P. Lesoway, A. M. Rafiqi *et al.*, 2014 Eco-evo-devo: the time has come. *Adv. Exp. Med. Biol.* 781: 107–125. https://doi.org/10.1007/978-94-007-7347-9_6

Ackermann, M., 2015 A functional perspective on phenotypic heterogeneity in microorganisms. Nat. Rev. Microbiol. 13: 497–508. https://doi.org/10.1038/nrmicro3491

Amundson, R., 2005 *The Changing Role of the Embryo in Evolutionary Thought*. Cambridge University Press, Cambridge. https://doi.org/10.1017/CBO9781139164856

Antebi, A., 2015 Nuclear receptor signal transduction in *C. elegans* (June 9, 2015), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.64.2, http://www.wormbook.org. https://doi.org/10.1895/wormbook.1.64.2

Ashe, A., A. Sapetschnig, E. M. Weick, J. Mitchell, M. P. Bagijn *et al.*, 2012 piRNAs can trigger a multigenerational epigenetic memory in the germline of *C. elegans*. Cell 150: 88–99. https://doi.org/10.1016/j.cell.2012.06.018

Baldwin, J. M., 1896 A new factor in evolution. Am. Nat. 30: 441–451. https://doi.org/10.1086/276408

Belicard, T., P. Jaresettasin, and P. Sarkies, 2018 The piRNA pathway responds to environmental signals to establish intergenerational adaptation to stress. BMC Biol. 16: 103. https://doi.org/10.1186/s12915-018-0571-y

Bento, G., A. Ogawa, and R. J. Sommer, 2010 Co-option of the hormone-signalling module dafachronic acid-DAF-12 in nematode evolution. Nature 466: 494–497. https://doi.org/10.1038/nature09164

Bradshaw, A. D., 1965 Evolutionary significance of phenotypic plasticity in plants. Adv. Genet. 13: 115–155. https://doi.org/10.1016/S0065-2660(08)60048-6

Brakefield, P. M., J. Gates, D. Keys, F. Kesbeke, P. J. Wijngaarden *et al*., 1996 Development, plasticity and evolution of butterfly eyespot patterns. Nature 384: 236–242. https://doi.org/10.1038/384236a0

Brisson, J. A., and G. K. Davis, 2016 The right tools for the job: regulating polyphenic morph development in insects. Curr. Opin. Insect Sci. 13: 1–6. https://doi.org/10.1016/j.cois.2015.09.011

Bui, L. T., and E. J. Ragsdale, 2019 Multiple plasticity regulators reveal targets specifying an induced predatory form in nematodes. Mol. Biol. Evol. 36: 2387–2399. https://doi.org/10.1093/molbev/msz171

Canfield, M., and E. Greene, 2009 Phenotypic plasticity and the semantics of polyphenism: a historial review and current perspectives, pp. 65–80 in *Phenotypic Plasticity of Insects: Mechanisms and Consequences*, edited by D. W. Whitman and T. N. Ananthakrishnan. Science Publishers, Jersey. https://doi.org/10.1201/b10201-3

Charlesworth, B., R. Lande, and M. Slatkin, 1982 A neo-Darwinian commentary on macroevolution. Evolution 36: 474–498. https://doi.org/10.1111/j.1558-5646.1982.tb05068.x

Conine, C. C., J. J. Moresco, W. Gu, M. Shirayama, D. Conte *et al*., 2013 Argonautes promote male fertility and provide a paternal memory of germline gene expression in *C. elegans*. Cell 155: 1532–1544. https://doi.org/10.1016/j.cell.2013.11.032

Costa, S., and C. Dean, 2019 Storing memories: the distinct phases of Polycomb-mediated silencing of Arabidopsis FLC. Biochem. Soc. Trans. 47: 1187–1196. https://doi.org/10.1042/BST20190255

Davidson, E. H., 2006 *The Regulatory Genome*. Academic Press, San Diego.

de Jong, I. G., P. Haccou, and O. P. Kuipers, 2011 Bet hedging or not? A guide to proper classification of microbial survival strategies. Bioessays 33: 215–223. https://doi.org/10.1002/bies.201000127

deWitt, T. J., and S. M. Scheiner, 2004 *Phenotypic Plasticity*. Oxford University Press, Oxford.

Dubnau, D., and R. Losick, 2006 Bistability in bacteria. Mol. Microbiol. 61: 564–572. https://doi.org/10.1111/j.1365-2958.2006.05249.x

Flatt, T., 2005 The evolutionary genetics of canalization. The Quaternary Review of Biology 80: 287–316. https://doi.org/10.1086/432265

Gaydos, L. J., W. Wang, and S. Strome, 2014 H3K27me and PRC2 transmit a memory of repression across generations and during development. Science 345: 1515–1518. https://doi.org/10.1126/science.1255023

Ghalambor, C. K., J. K. McKay, S. P. Carroll, and D. N. Reznick, 2007 Adaptive *vs.* non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct. Ecol. 21: 394–407. https://doi.org/10.1111/j.1365-2435.2007.01283.x

Ghalambor, C. K., K. L. Hoke, E. W. Ruell, E. K. Fischer, D. N. Reznick *et al*., 2015 Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. Nature 525: 372–375 [corrigenda: Nature 555: 688 (2018)]. https://doi.org/10.1038/nature15256

Gilbert, J. J., 2018 Morphological variation and its significance in a polymorphic rotifer: environmental, endogenous and genetic controls. Bioscience 68: 169–181. https://doi.org/10.1093/biosci/bix162

Gibert, J. M., 2017 The flexible stem hypothesis: evidence from genetic data. Dev. Genes Evol. 227: 297–307. https://doi.org/10.1007/s00427-017-0589-0

Gottlieb, G., 1992 *Individual Development and Evolution: The Genesis of Novel Behavior*. Oxford University Press, Oxford.

Greer, E. L., T. J. Maures, D. Ucar, A. G. Hauswirth, E. Mancini *et al*., 2011 Transgenerational epigenetic inheritance of longevity in *Caenorhabditis elegans*. Nature 479: 365–371. https://doi.org/10.1038/nature10572

Han, M., and P. W. Sternberg, 1990 *let-60*, a gene that specifies cell fates during *C. elegans* vulval induction, encodes a Ra protein. Cell 63: 921–931. https://doi.org/10.1016/0092-8674(90)90495-Z

Harvey, P. H., and M. D. Pagel, 1991 *The Comparative Method in Evolutionary Biology*. Oxford University Press, Oxford.

Heard, E., and R. A. Martienssen, 2014 Transgenerational epigenetic inheritance: myths and mechanisms. Cell 157: 95–109. https://doi.org/10.1016/j.cell.2014.02.045

Jobson, M. A., J. M. Jordan, M. A. Sandrof, J. D. Hibshman, A. L. Lennox *et al*., 2015 Transgenerational effects of early life starvation on growth, reproduction, and stress resistance in *Caenorhabditis elegans*. Genetics 201: 201–212. https://doi.org/10.1534/genetics.115.178699

Jones, B. M., and G. E. Robinson, 2018 Genetic accommodation and the role of ancestral plasticity in the evolution of insect eusociality. J. Exp. Biol. 221: jeb153163. https://doi.org/10.1242/jeb.153163

Josephs, E. B., 2018 Determining the evolutionary forces shaping GxE. New Phytol. 219: 31–36. https://doi.org/10.1111/nph.15103

Katz, D. J., M. Edwards, V. Reinke, and W. G. Kelly, 2009 A *C. elegans* LSD1 demethylase contributes to germline immortality by reprogramming epigenetic memory. Cell 137: 308–320. https://doi.org/10.1016/j.cell.2009.02.015

Klosin, A., and B. Lehner, 2016 Mechanisms, timescales and principles of trans-generational epigenetic inheritance in animals. Curr. Opin. Genet. Dev. 36: 41–49. https://doi.org/10.1016/j.gde.2016.04.001

Klosin, A., E. Casas, C. Hidalgo-Carcedo, T. Vavouri, and B. Lehner, 2017 Transgenerational transmission of environmental information in *C. elegans*. Science 356: 320–323. https://doi.org/10.1126/science.aah6412

Lafuente, E., and P. Beldade, 2019 Genomics of developmental plasticity in animals. Front. Genet. 10: 720. https://doi.org/10.3389/fgene.2019.00720

Laland, K., T. Uller, M. Feldman, K. Sterelny, G. B. Müller *et al*., 2014 Does evolutionary theory need a rethink? Nature 514: 161–164. https://doi.org/10.1038/514161a

Laland, K. N., T. Uller, M. W. Feldman, K. Sterelny, G. B. Müller *et al*., 2015 The extended evolutionary synthesis: its structure, assumptions and predictions. Proc. Biol. Sci. 282: 20151019. https://doi.org/10.1098/rspb.2015.1019

Lande, R., 2014 Evolution of phenotypic plasticity and environmental tolerance of a labile quantitative character in a fluctuating environment. J. Evol. Biol. 27: 866–875. https://doi.org/10.1111/jeb.12360

Levis, N. A., and D. W. Pfennig, 2019 Plasticity-led evolution: evaluating the key prediction of frequency-dependent adaptation. Proc. Biol. Sci. 286: 20182754. https://doi.org/10.1098/rspb.2018.2754

Levis, N. A., A. J. Isdaner, and D. W. Pfennig, 2018 Morphological novelty emerges from pre-existing phenotypic plasticity. Nat. Ecol. Evol. 2: 1289–1297. https://doi.org/10.1038/s41559-018-0601-8

Lim, J. P., and A. Brunet, 2013 Bridging the transgenerational gap with epigenetic memory. Trends Genet. 29: 176–186. https://doi.org/10.1016/j.tig.2012.12.008

Mallard, F., A. M. Jakšić, and C. Schlötterer, 2018 Contesting the evidence for non-adaptive plasticity. Nature 555: E21–E22. https://doi.org/10.1038/nature25496

Mayr, E., 1963 *Animal Species and Evolution*. Harvard University Press, Cambridge. https://doi.org/10.4159/harvard.9780674865327

Moczek, A. P., and H. F. Nijhout, 2002 Developmental mechanisms of threshold evolution in a polyphenic beetle. Evol. Dev. 4: 252–264. https://doi.org/10.1046/j.1525-142X.2002.02014.x

Moczek, A. P., and H. F. Nijhout, 2003 Rapid evolution of a polyphenic threshold. Evol. Dev. 5: 259–268. https://doi.org/10.1046/j.1525-142X.2003.03033.x

Moczek, A. P., S. Sultan, S. Foster, C. Ledon-Rettig, I. Dworkin *et al*., 2011 The role of developmental plasticity in evolutionary innovation. Proc. Biol. Sci. 278: 2705–2713. https://doi.org/10.1098/rspb.2011.0971

Moczek, A. P., K. E. Sears, A. Stollewerk, P. J. Wittkopp, P. Diggle *et al*., 2015 The significance and scope of evolutionary developmental biology: a vision for the 21st century. Evol. Dev. 17: 198–219. https://doi.org/10.1111/ede.12125

Nalepa, C. A., 2015 Origin of termite eusociality: trophallaxis integrates the social, Nutritional, and microbial environments. Ecol. Entomol. 40: 323–335. https://doi.org/10.1111/een.12197

Nicoglou, A., 2015 Phenotypic plasticity: from microevolution to macroevolution, pp. 285–318 in *Handbook of Evolutionary Thinking in the Sciences*, edited by T. Heams, P. Huneman, G. Lecointre, and M. Silberstein. Springer, Heidelberg.

Nijhout, H. F., 2014 A developmental-physiological perspective on the development and evolution of phenotypic plasticity, pp. 147–173. in *Conceptual Change in Biology.* Springer-Verlag, Heidelberg.

Nijhout, H. F., 2015 To plasticity and back again. Elife 4: e06995. https://doi.org/10.7554/eLife.06995

Noble D., Cartwright, N., Bateson, P., Dupre J., and K. Laland, 2017 New trends in evolutionary biology: biological, philosophical and social science perspectives. Interface Focus **7:** 20170051.

Oettler, J., T. Platschek, C. Schmidt, R. Rajakumar, M. J. Favé *et al*., 2018 Interruption points in the wing gene regulatory network underlying wing polyphenism evolved independently in male and female morphs in *Cardiocondyla* ants. J. Exp. Zool. B Mol. Dev. Ecol. 332: 7–16. https://doi.org/10.1002/jez.b.22834

Phillips, P. C., 2016 Five heads are better than one. Curr. Biol. 26: R283–R285. https://doi.org/10.1016/j.cub.2016.02.048

Pigliucci, M., 2001 *Phenotypic Plasticity: Beyond Nature and Nurture: Syntheses in Ecology and Evolution*. Johns Hopkins University Press, Baltimore.

Projecto-Garcia, J., J. F. Biddle, and E. J. Ragsdale, 2017 Decoding the architecture and origins of mechanisms for developmental polyphenism. Curr. Opin. Genet. Dev. 47: 1–8. https://doi.org/10.1016/j.gde.2017.07.015

Ptashne, M., 2004 *A Genetic Switch*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Ragsdale, E. J., M. R. Muller, C. Rodelsperger, and R. J. Sommer, 2013 A developmental switch coupled to the evolution of plasticity acts through a sulfatase. Cell 155: 922–933. https://doi.org/10.1016/j.cell.2013.09.054

Rechavi, O., and I. Lev, 2017 Principles of transgenerational small RNA inheritance in *Caenorhabditis elegans*. Curr. Biol. 27: R720–R730. https://doi.org/10.1016/j.cub.2017.05.043

Rechavi, O., G. Minevich, and O. Hobert, 2011 Transgenerational inheritance of an acquired small RNA-based antiviral response in *C. elegans*. Cell 147: 1248–1256. https://doi.org/10.1016/j.cell.2011.10.042

Rechavi, O., L. Houri-Ze'evi, S. Anava, S. Siong, W. Goh *et al*., 2014 Starvation-induced transgenerational inheritance of small RNAs in *C. elegans*. Cell 158: 277–287. https://doi.org/10.1016/j.cell.2014.06.020

Reuter, M., M. F. Camus, M. S. Hill, F. Ruzicka, and K. Fowler, 2017 Evolving plastic responses to external and genetic environments. Trends Genet. 33: 169–170. https://doi.org/10.1016/j.tig.2017.01.004

Rödelsperger, C., N. Prabh, and R. J. Sommer, 2019 New gene origin and deep taxon phylogenomics: opportunities and challenges. Trends Genet. 35: 914–922. https://doi.org/10.1016/j.tig.2019.08.007

Sanger, T. J., and R. Rajakumar, 2018 How a growing organismal perspective is adding new depth to integrative studies of morphological evolution. Biol. Rev. Camb. Philos. Soc. 94: 184–198. https://doi.org/10.1111/brv.12442

Schlichting, C. D., and M. Pigliucci, 1998 *Phenotypic Evolution*. Sinauer Associates, Sunderland.

Schmalhausen, I. I., 1949 *Factors of Evolution: The Theory of Stabilizing Selection*. University of Chicago Press, Chicago.

Schneider, R. F., and A. Meyer, 2017 How plasticity, genetic assimilation and cryptic genetic variation may contribute to adaptive radiations. Mol. Ecol. 26: 330–350. https://doi.org/10.1111/mec.13880

Serobyan, V., and R. J. Sommer, 2017 Developmental systems of plasticity and trans-generational epigenetic inheritance in nematodes. Curr. Opin. Genet. Dev. 45: 51–57. https://doi.org/10.1016/j.gde.2017.03.001

Serobyan, V., H. Xiao, S. Namdeo, C. Roedelsperger, B. Sieriebriennikov *et al*., 2016 Chromatin remodelling and antisense-mediated up-regulation of the developmental switch gene *eud-1* control predatory feeding plasticity. Nat. Commun. 7: 12337. https://doi.org/10.1038/ncomms12337

Seth, M., M. Shirayama, W. Gu, T. Ishidate, D. Conte *et al*., 2013 The *C. elegans* CSR-1 Argonaute pathway counteracts epigenetic silencing to promote germline gene expression. Dev. Cell 27: 656–663. https://doi.org/10.1016/j.devcel.2013.11.014

Shirmayama, M., M. Seth, H.-C. Lee, W. Gu, T. Ishidate *et al*., 2012 piRNAs initiate an epigenetic memory of nonself RNA in the *C. elegans* germline. Cell 150: 65–77. https://doi.org/10.1016/j.cell.2012.06.015

Sieriebriennikov, B., and R. J. Sommer, 2018 Developmental plasticity and robustness of a nematode mouth-form polyphenism. Front. Genet. 9: 382. https://doi.org/10.3389/fgene.2018.00382

Sieriebriennikov, B., N. Prabh, M. Dardiry, H. Witte, C. Röseler *et al*., 2018 A developmental switch generating phenotypic plasticity is part of a conserved multi-gene locus. Cell Rep. 23: 2835–2843.e4. https://doi.org/10.1016/j.celrep.2018.05.008

Sieriebriennikov, B., S. Sun, J. W. Lightfoot, H. Witte, E. Moreno *et al*., 2020 Conserved hormone receptors controlling a novel plastic trait target fast-evolving genes expressed in a single cell. PLoS Genet. https://www.biorxiv.org/content/10.1101/809350v1 (Preprint)

Smythe, A. B., O. Holovachov, and K. M. Kocot, 2019 Improved phylogenomic sampling of free-living nematodes enhances resolution of higher-level nematode phylogeny. BMC Evol. Biol. 19: 121. https://doi.org/10.1186/s12862-019-1444-x

Sommer R. J., (Editor), 2015 Pristionchus pacificus – a nematode model for comparative and evolutionary biology. Leiden; Boston: Brill. https://doi.org/10.1163/9789004260306_003

Susoy, V., and R. J. Sommer, 2016 Stochastic and conditional regulation of nematode mouth-form dimorphisms. Front. Ecol. Evol. 4: 23. https://doi.org/10.3389/fevo.2016.00023

Susoy, V., E. J. Ragsdale, N. Kanzaki, and R. J. Sommer, 2015 Rapid diversification associated with a macroevolution-

ary pulse of developmental plasticity. Elife 4: e05463. https://doi.org/10.7554/eLife.05463

Suzuki, Y., and H. F. Nijhout, 2006 Evolution of a polyphenism by genetic accommodation. Science 311: 650–652. https://doi.org/10.1126/science.1118888

Tandonnet, S., and A. Pires daSilva, 2016 Phenotypic plasticity and developmental innovations in nematodes. Curr. Opin. Genet. Dev. 39: 8–13. https://doi.org/10.1016/j.gde.2016.05.018

Uller, T., A. P. Moczek, R. A. Watson, P. M. Brakefield, and K. N. Laland, 2018 Developmental bias and evolution: a regulatory network perspective. Genetics 209: 949–966. https://doi.org/10.1534/genetics.118.300995

van den Hoogen, J., S. Geisen, D. Routh, H. Ferris, W. Traunspurger *et al.*, 2019 Soil nematode abundance and functional group composition at a global scale. Nature 572: 194–198. https://doi.org/10.1038/s41586-019-1418-6

van Gestel, J., and F. J. Weissing, 2018 Is plasticity caused by single genes. Nature 555: E19–E20. https://doi.org/10.1038/nature25495

Waddington, C. H., 1975 The Evolution of an Evolutionist. Cornell University Press, Ithaca, NY.

Wedeles, C. J., M. Z. Wu, and J. M. Claycomb, 2013 Protection of germline gene expression by the *C. elegans* Argonaute CSR-1. Dev. Cell 27: 664–671. https://doi.org/10.1016/j.devcel.2013.11.016

Werner, M., B. Sieriebriennikov, T. Loschko, S. Namdeo, M. Lenuzzi *et al.*, 2017 Environmental influence on *Pristionchus pacificus* mouth-form through different culture methods. Sci. Rep. 7: 7207. https://doi.org/10.1038/s41598-017-07455-7

West-Eberhard, M. J., 1989 Phenotypic plasticity and the origins of diversity. Ann Rev Ecol Syst. 20: 249–278. https://doi.org/10.1146/annurev.es.20.110189.001341

West-Eberhard, M. J., 2003 *Developmental Plasticity and Evolution*. Oxford University Press, New York.

Whitman, D. W., and T. N. Ananthakrishnan (Editors), 2009 *Phenotypic Plasticity of Insects: Mechanisms and Consequences*. Science Publishers, Jersey.

Williams, G. C., 1966 *Adaptation and Natural Selection*. Princeton University Press, Princeton.

Wund, M. A., 2012 Assessing the impacts of phenotypic plasticity on evolution. Integr. Comp. Biol. 52: 5–15. https://doi.org/10.1093/icb/ics050

Xu, L., Y. Fong, and S. Strome, 2001 The *Caenorhabditis elegans* maternal-effect sterile proteins MES-2, MES-3, and MES-6, are associated in a complex in embryos. Proc. Natl. Acad. Sci. USA 98: 5061–5066. https://doi.org/10.1073/pnas.081016198

Communicating editor: A. S. Wilkins