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Twenty years on from *Developmental Plasticity and Evolution*: middle-range theories and how to test them

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ABSTRACT

In *Developmental Plasticity and Evolution*, Mary-Jane West-Eberhard argued that the developmental mechanisms that enable organisms to respond to their environment are fundamental causes of adaptation and diversification. Twenty years after publication of this book, this once so highly controversial claim appears to have been assimilated by a wealth of studies on 'plasticity-led' evolution. However, we suggest that the role of development in explanations for adaptive evolution remains underappreciated in this body of work. By combining concepts of evolvability from evolutionary developmental biology and quantitative genetics, we outline a framework that is more appropriate to identify developmental causes of adaptive evolution. This framework demonstrates how experimental and comparative developmental biology and physiology can be leveraged to put the role of plasticity in evolution to the test.

KEY WORDS: Plasticity, Evolution, Evolvability, Development, Adaptive landscape, Morphospace

Introduction

Organisms are inherently responsive to their environment; they modify their morphology and physiology to fit their surroundings, switch between food sources when nutrients become depleted, or move to new locations where conditions are more favourable. Establishing whether or not such short-term environmental responsiveness, or plasticity, is related to long-term evolutionary patterns is a persistent problem in biology.

In an influential attempt to provide a solution, Mary-Jane West-Eberhard in her book *Developmental Plasticity and Evolution*, followed a long tradition of thinkers in arguing that evolutionary biologists should 'put the flexible phenotype first, as the product of development and the object of selection' and she encouraged biologists to 'examine the consequences for the genetic theory of evolution' (West-Eberhard, 2003, p. 3). Following publication of this book 20 years ago, West-Eberhard's advocacy for the constructive role of development in adaptive evolution was both criticised and defended (e.g. de Jong and Crozier, 2003 versus Badyaev, 2005), and developmental plasticity has since become central to debates concerning the structure of evolutionary theory (e.g. Laland et al., 2014 versus Wray et al., 2014; reviewed in Laland et al., 2015). A persistent criticism has been that there is a lack of 'middle-range theories': theories that make specific predictions about the observable traces of developmental plasticity in evolution,

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accompanied with guidance of how to collect evidence that can discriminate between competing hypotheses (Kovaka, 2019).

The aim of this paper is to demonstrate how this domain could be filled. First, we briefly reflect on the extent to which research on developmental plasticity and evolution has come to embrace West-Eberhard's emphasis on 'development, not selection, [as] the first-order cause of design' (West-Eberhard, 2003, p. 141). We suggest that research on 'plasticity-led' evolution in fact has developed in a way that largely bypasses developmental explanations for adaptation, which makes such research more compatible with interpreting adaptive evolution solely in terms of selection on genetic variation. Second, we connect developmental plasticity to the contemporary literature on evolvability to illustrate how to assign adaptive bias in evolution to both developmental and selective processes. We demonstrate that this can help to formulate hypotheses for when, where and why plasticity will 'take the lead' in adaptive evolution, and identify areas that would benefit from further theoretical and empirical development. Third, we provide suggestions for how experimental and comparative biology can put those theories to the test, thereby integrating developmental and selective explanations of adaptation and diversification.

Developmental plasticity and the causes of adaptive change

Evolution is often described as a two-step process, beginning with the origin of a phenotypic variant, followed by the spread and establishment of this variant (e.g. West-Eberhard, 2003, chapter 6). In biological systems, each of these steps can impose an adaptive bias or directionality on evolution. The second step is the more familiar: competition between individuals and 'the struggle for existence' bias evolutionary change towards phenotypes that confer survival or reproductive benefits, making organisms appear designed to their way of life. However, as West-Eberhard argued, adaptive bias also arises from the capacity of individual organisms to accommodate genetic and environmental perturbation through the 'adaptive mutual adjustment among variable parts during development without genetic change' (West-Eberhard, 2003, p. 51). Such 'phenotypic accommodation' results in the appearance of new phenotypic variants whose match to the local environment will be assessed by their effects on survival and reproduction (note that, throughout *Developmental Plasticity and Evolution* and in the present paper, 'development' refers to 'all phenotypic change during the lifetime of an individual'; West-Eberhard, 2003, p. 32).

The constructive role of development in adaptive evolution may appear at odds with the historically wide-spread belief that 'allele frequency change caused by natural selection is the only credible process underlying the evolution of adaptive organismal traits' (Charlesworth et al., 2017; West-Eberhard, 2003, p. 10). It is obviously true that the mechanisms of phenotypic accommodation have evolved, and thus have persisted and been modified through countless rounds of natural selection (although this does not imply

there has been selection for plasticity; Rago et al., 2019). However, it is equally true that the phenotypes that can become selected must arise through development. The two steps of evolution, the generative and selective processes, are intertwined. Moreover, in part because phenotypic accommodation relies on many ancient mechanisms (Gerhart and Kirschner, 2007), past selection for particular phenotypes will not fully determine how individuals will respond to genetic or environmental perturbation in the future. As a result, West-Eberhard considered reference to the 'proximate' mechanisms of development more helpful than referring to past selective regimes to explain why a particular phenotype arises from genetic and environmental perturbation (for example, see the discussion of the morphology of the 'two-legged goat', West-Eberhard, 2003, p. 51–54). Biologists can decide whether they want to explain phenotypes in terms of generative or selective processes, or both, but if one chooses to consider the origin of a novel phenotype to be 'where evolution starts' (West-Eberhard, 2003, p. 28), then 'development, not selection, is the first order cause of design' (West-Eberhard, 2003, p. 28), regardless of the organism's evolutionary history (for more on the conceptual foundations of this position, see Walsh, 2015; Uller and Helanterä, 2019).

A common theme of *Developmental Plasticity and Evolution* is that not just development but specifically environmentally induced phenotypes have exercised persistent and adaptive directionality on evolution. Indeed, compared with mutation, 'environmental induction is superior in terms of evolutionary potential' (West-Eberhard, 2003, p. 145). In brief, the logic is that when environments change, this can affect many individuals (perhaps an entire population), and the plastic responses of those individuals will thereby shape the distribution of phenotypes that is available to selection. Developmental processes often enable organisms to accommodate perturbations, but may do so more or less successfully. Either way, the distribution of phenotypes in a population will depend in part on how development works (Salazar-Ciudad, 2021). This in turn means those phenotypes are non-random with respect to past evolution and the inducing environment, but not fully determined by either. Under this scenario, allelic change under selection is predominantly associated with modification of phenotypic variants originally induced by the environment, a process West-Eberhard referred to as 'genetic accommodation' (West-Eberhard, 2003, p. 29).

Over the past two decades, attempts to develop more specific, 'middle-range', theories on this constructive role of plasticity in evolution have focused on how novel or extreme environments can expose pre-existing genetic variation to selection (see Levis and Pfennig, 2016; Dayan et al., 2019). According to this model, plasticity contributes to the explanation for adaptive change by 'exposing "cryptic" genetic variation to selection, thereby fuelling adaptive evolution' (Pfennig, 2021, p. 75). While this framework captures some possible population-level consequences of plasticity, it is unable to explain why and how those particular phenotypes appeared. Without reference to the developmental processes that generate phenotypic variation, plasticity appears to be an 'add-on[s] to the basic processes that produce evolutionary change: natural selection, drift, mutation, recombination and gene flow' (Wray et al., 2014, p. 164) rather than 'the first-order cause of design' (West-Eberhard, 2003, p.141) that West-Eberhard envisaged. While the benefit of the former perspective is that it is consistent with a standard explanation for adaptive change (i.e. selection on standing genetic variation), which eschews proximate causes, it does not recognise developmental plasticity as a source of adaptive phenotypic variation. As a result, it is unable to shed any light on

whether explanations that integrate developmental and selective accounts of adaptive change provide better accounts of evolution than those that refer to selection alone (Uller et al., 2020; Schlichting, 2021; see also Baedke et al., 2020).

If we were to interpret West-Eberhard's book as a call for the integration of developmental and selective explanations, this arguably requires a different kind of middle-range theory – those that recognise developmental plasticity as a source of adaptive bias in evolution in its own right, rather than something to be explained away by past selection or a way to expose genetic variation to selection. One clue on how this integration can be achieved comes from the literature on evolvability that has developed since the publication of *Developmental Plasticity and Evolution*.

Developmental plasticity and evolvability
While adaptation is concerned with the match between organism and environment, the concept of evolvability directs attention towards the factors that influence the capacity to evolve (Hendrikse et al., 2007; Brown, 2014; Feiner et al., 2021; Riederer et al., 2022; Hansen et al., 2023). Evolvability research aims to understand why organisms or populations appear to differ in their ability to adapt and diversify, and why they adapt in some ways and not others. For example, evolutionary biologists should be able to explain why there are so many different cichlids but so few lampreys; why spots and stripes are common in nature while checkerboard patterns are rare; and why the turtle evolved a protective shell, rather than spikes or fangs, to fend off predators. Other questions include why all the major body plans evolved early in the history of animals, how evolution can bring about coordinated changes in morphology, behaviour and physiology, and whether or not the capacity for adaptive evolution itself evolves.

Finding the answers to those questions will require attention to both the origin of phenotypic variation (i.e. generative processes) and the moulding of phenotypes by selection (i.e. selective processes). With respect to phenotypic variation, evolvability depends on the ability of development to generate novel phenotypes that function well in their environment (Hendrikse et al., 2007). With respect to the response to selection, evolvability depends on the relationship between the distribution of heritable phenotypic variation within a population and the direction of selection (Hansen and Pélabon, 2021). The two perspectives are necessarily linked: how development works influences the distribution of phenotypes that can be selected, and persistent selection can modify the properties of development that are responsible for phenotypic variation and heredity. Note that the problems that evolvability research addresses tend to concern complex, integrated phenotypes that require multivariate representations rather than univariate 'traits' such as body size, flowering time or metabolic rate.

Fig. 1 illustrates how the two perspectives on evolvability can be joined to capture the two steps of adaptive evolution (see also Salazar-Ciudad and Cano-Fernández, 2023). The first step – the origin of phenotypic variation – requires attention to generative processes, perhaps by constructing a mathematical or computational representation of the process that generates phenotypes from genetic and environmental inputs (Brun-Usan et al., 2022; Fig. 1A,B). For example, vertebrate colour patterns are an outcome of the arrangement of chromatophores, which in turn is determined by properties of cells, and interactions between cells and between cells and other features of their surroundings (Kratochwil and Mallarino, 2023). Models of different complexity can capture the macroscopic outcome of these interactions and make it possible to study how
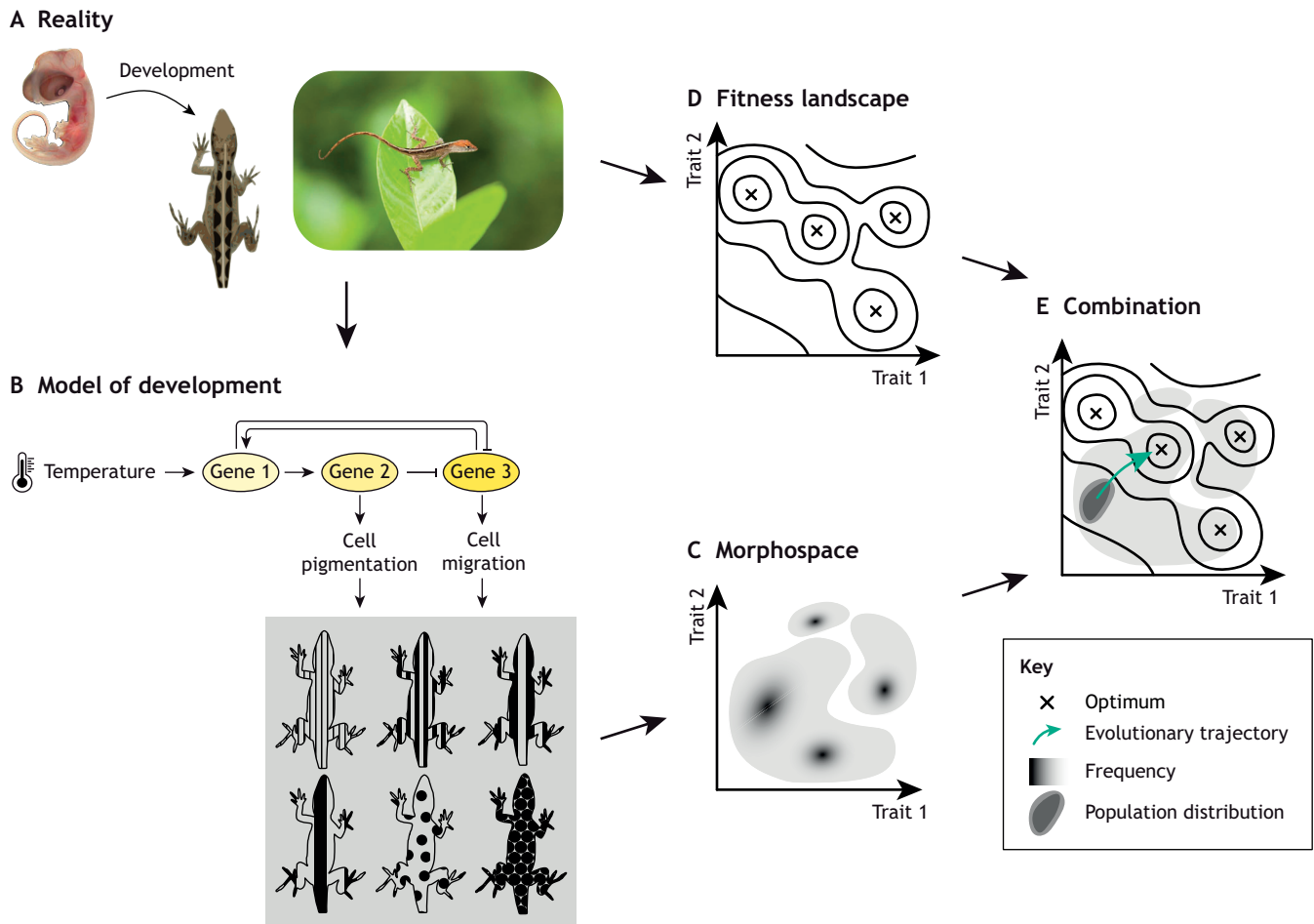
Fig. 1. Representing evolution by combining generative and selective models, using animal colour patterns for illustration. (A) The biological processes that generate colour patterns and consistent variation in fitness between colour variants are causally complex, and those processes must therefore be represented in simplified form in evolutionary theory. (B) Phenotype development (i.e. the generative process) can be modelled mathematically, here as a regulatory network that includes both genetic and environmental inputs. Variation in those inputs affects the nodes or strength of interactions and may therefore influence the phenotype (e.g. colour pattern). (C) For any given developmental mechanism, some phenotypes are likely to be common, others rare, and many patterns will be impossible. As a result, the space of possible phenotypes is bounded and may be gappy. (D) Fitness differences between phenotypes (i.e. the selective process) can be represented as an 'adaptive landscape', here shown for two traits, with phenotypes of high fitness ('fitness peaks') indicated by a cross. (E) Putting together generative and selective processes demonstrates that a population will evolve through the joint effect of development and selection. Note how some high-fitness states may be impossible to reach.

perturbations of the model inputs influence the output pattern (e.g. Miyazawa et al., 2021; Feiner et al., 2022; Jahanbakhsh and Milinkovitch, 2022). If these inputs can be identified with genetic and environmental variables, the consequences of genetic or environmental perturbation on the phenotype distribution can be quantified. For any given mechanism of pattern formation, some phenotypes are likely to be common, others rare, and many patterns will be impossible (e.g. Kavanagh et al., 2007). As a result, for any given developmental mechanism, the space of possible phenotypes is bounded and may be gappy (Fig. 1C).

The second step – the selective elimination and retention of particular phenotypes – requires attention to how the phenotype distribution changes as a result of individual differences in survival and reproduction. One representation envisages that populations occupy an 'adaptive landscape' that describes the fitness of phenotypes (Fig. 1D). Consistent association between phenotype and fitness will result in a shift in the distribution of phenotypes from one generation to the next as long as offspring resemble their parents. For example, a survival advantage for individuals with few, thick stripes will cause this phenotype to increase in frequency at the

expense of alternative colour patterns that are present in the population. However, the fitness advantage of thick stripes can change if the environment changes, and hence the adaptive landscape will change too (fitness can also vary with the phenotype composition of the population but we ignore such frequency dependences here).

Putting together the two concepts of evolvability demonstrates that the movement of the population through phenotype space is jointly determined by generative and selective processes (Fig. 1E). The population evolves through the 'adjacent possible' (Kauffmann, 1996) phenotypes, which are reached through developmental responses to genetic and environmental variation and maintained by their superior fitness (relative to other, realized, phenotypes).

Evolutionary biologists have mostly been concerned with explanations of adaptive change in terms of fitness differences, and tend to assume that development does not impose any bias on the phenotype distribution (this is because it is easier to understand frequency-dependent selection with only two fixed phenotypes, for example, or to understand how body size evolves under directional

selection if the additive genetic variance stays constant). With this assumption, selection becomes the only 'difference maker' that can explain the adaptive movement of the population through phenotype space. However, in reality, some trait combinations tend to be more common than others, for example, because traits regulate each other in development or because different traits rely on the same genes (in quantitative genetics, these 'genetic correlations' are quantified statistically by the G and M matrices; Hansen and Pélabon, 2021).

There is substantial empirical evidence that correlations between traits imposed by development can persist over evolutionary time (for two recent examples on flower and wing morphology, see Opedal et al., 2023 and Rohner and Berger, 2023). As a result, development may also exercise a persistent effect on evolution by skewing the distribution of the adjacent possible phenotypes and making populations evolve along 'lines of least genetic resistance' (Schluter, 1996). This bias imposed by development means that the population may be unable to reach the peaks of the adaptive landscape, it can slow down or speed up the movement towards fitness peaks and, if there are multiple peaks, explain why the population evolves one particular adaptive solution rather than another (Uller et al., 2018; González-Forero, 2022; Salazar-Ciudad and Cano-Fernández, 2023).

What about plasticity? From Fig. 1, it is evident that developmental bias depends not only on the relationship between genetic and phenotypic variation [the 'genotype–phenotype (GP) map'] but also on the relationship between environmental and phenotypic variation (i.e. plasticity or the 'environment–phenotype (EP) map'; Salazar-Ciudad, 2007; Brun-Usan et al., 2022]. How individuals respond to their environment contributes to the shape and location of the phenotype distribution in the space of possible phenotypes. Selection can only act locally on the phenotypes that exist here and now. Thus, one of the key evolutionary implications of the reliance of development on environmental factors is to enable the origin of phenotypic novelty: phenotypes that are difficult or impossible to evolve in one environment could become widely accessible to selection if the environment changes. Whether or not this happens depends on the mechanisms of development, which should not be confused with whether or not there has been selection for plasticity in the past.

An evolvability-inspired perspective on plasticity-led evolution

To investigate the evolutionary implications of development plasticity, we will consider a population that experiences a sudden but persistent change in environment. This scenario is generally considered conducive for plasticity-led evolution (e.g. Price et al., 2003; West-Eberhard, 2003; Ghalambor et al., 2007; Levis and Pfennig, 2016). Assuming that all individuals in a population share the same developmental mechanism, the response to environmental perturbation is likely to be roughly similar but with individual differences due to genetic variation. It will further be assumed that the environmental perturbation can have substantial phenotypic consequences, while the adjacent possible phenotypes that arise from recombination and mutation do not deviate substantially from those that already exist in the population (but, for both kinds of inputs, the phenotype distribution will depend on the developmental system).

Fig. 2 provides a simple graphical representation of the movement of a population on an adaptive landscape given these assumptions. We use this to make five points that are relevant for taking into account both developmental and selective processes in adaptive evolution.

Focus on generative processes reveals why plasticity is important to evolution

In contemporary literature on plasticity and evolution, the 'release of cryptic genetic variation' (an 'increase in heritability of greater phenotypic variation'; Levis and Pfennig, 2016) is commonly considered necessary for plasticity to 'take the lead' in adaptive evolution (reviewed in Levis and Pfennig, 2016; Fig. 2B). However, the release of cryptic variation may not be sufficient to cause a significant change in the evolutionary trajectory, and individual responses to environmental change can direct evolution towards particular adaptive peaks even if the heritable variation decreases rather than increases (Fig. 2A versus B). Whether or not developmental plasticity makes a difference to adaptive evolution depends primarily on how the responsiveness of development to environmental conditions shifts the phenotype distribution relative to the fitness landscape, not on how much heritable variation there is.

The explicit combination of generative and selective processes (Fig. 1) can capture West-Eberhard's claim that development is a cause of adaptation. It does so by locating the adaptive bias generated by plasticity partly in development (evolvability as the origin of variation), rather than exclusively in the opportunities for selection on standing genetic variation (evolvability as a response to selection). As a result, the explanation for the (adaptive and non-adaptive) bias introduced by developmental plasticity requires attention to how living systems work.

Mechanistic models can enrich theory on plasticity and evolution

Properties of development contribute to adaptation and diversification because evolution must always proceed through adjacent possible phenotypes (Fig. 1). The evolutionary consequences of development are often modelled statistically, as trait covariation (Hansen and Pélabon, 2021). However, mechanistic models are useful in this context because phenotype development is characterised by the existence of attractors: points or areas of phenotype space that the developmental system tends to converge upon after perturbation (Jaeger and Monk, 2014). A complete description of these attractors is not possible to deduce from trait variance and covariance (Fig. 2C). Moreover, because interactions in development are non-linear, developmental bias estimated from trait covariation can be highly local and environment dependent (e.g. Wood and Brodie, 2015). This, in turn, can make estimates of 'lines of least genetic resistance' (e.g. as G or M matrices) of limited use for inferring evolutionary trajectories (Milocco and Salazar-Ciudad, 2020, 2022; Fig. 2C).

By providing an internal logic for the space of possible phenotypes in development, the existence of developmental attractors can help to explain non-gradual evolutionary change and the repeated evolution of similar phenotypes. Attention to the mechanisms of development is particularly important to understand whether and how developmental plasticity contributes to the evolution of novel, complex phenotypes, whose variational properties cannot be quantified statistically from standing phenotypic variation.

Ancestor–descendant comparisons need to be interpreted carefully

Claims for 'plasticity-led' evolution have centred on demonstrating that an evolved adaptation resembles an environmentally induced phenotype in (putative) ancestors, or simply by showing that the trait was plastic in ancestors (West-Eberhard, 2003; Levis and Pfennig, 2016). Unfortunately, such comparisons may not be sufficient to show that plasticity initiates adaptive evolution
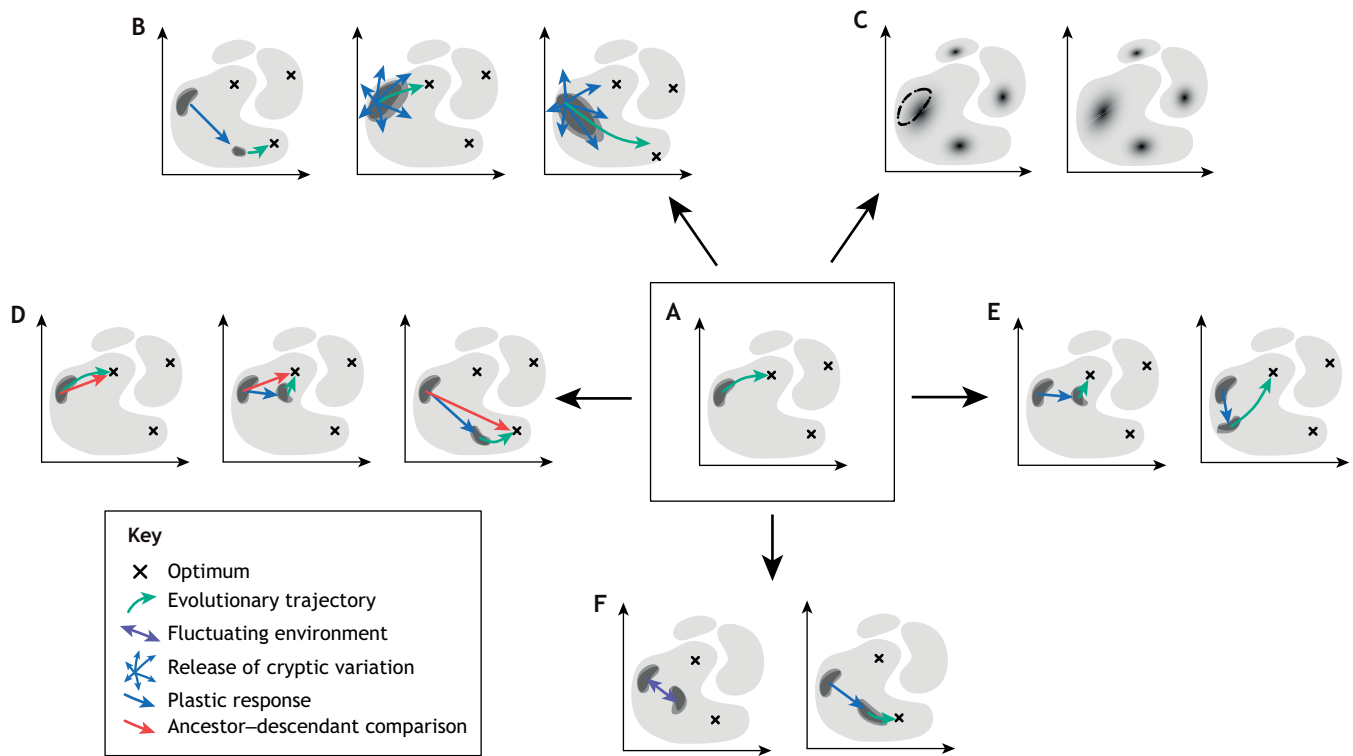
Fig. 2. Combining generative and selective processes illustrates opportunities to develop middle-range theories on plasticity and evolution. (A) In the absence of plasticity, evolution proceeds towards high fitness peaks through the adjacent possible phenotypes that become available by recombination and genetic mutation. Note that the phenotype distribution is expected to be biased as a result of development, resulting in a non-linear trajectory towards the fitness peak even on a smooth adaptive landscape. Fitness contours (see Fig. 1D) and density of phenotypes generated by development (see Fig. 1C) are omitted for clarity of representation unless needed to make the relevant point. (B) Focus on generative processes reveals why plasticity is important to evolution. The evolutionary consequences of development plasticity depend primarily on how environmental perturbation shifts the phenotype distribution, not the amount of (cryptic) heritable variation. Even a reduction in the overall phenotypic variation can result in the evolution of an alternative phenotype. An increase in phenotypic variation in a novel environment can speed up adaptation, but is not sufficient to make plasticity a difference maker with respect to the outcome of the adaptive process (i.e. the population can end up at the same fitness peak as in A). (C) Mechanistic models can enrich theory on plasticity and evolution. Estimates of developmental bias based on the observed phenotypic variation are local and may therefore not reflect the 'true' bias imposed by developmental processes (e.g. the likelihood that diamond patterns will arise in a population of striped individuals). (D) Ancestor–descendant comparisons provide inconclusive evidence for plasticity-led evolution. A plastic response in an ancestor may shift the trajectory of evolution only marginally, and fail to cause a difference in the evolutionary outcome. Hence, other explanatory contrasts are needed to demonstrate that plasticity is a significant difference maker in evolution. (E) Plasticity can both facilitate and constrain adaptive evolution. Plasticity facilitates adaptation by shifting the phenotype distribution closer to an adaptive peak, and constrains adaptation by shifting the phenotype distribution away from fitness peaks. The same plastic response can either facilitate or constrain adaptive change depending on what response it is compared with. (F) The evolution of plasticity can make organisms better at adapting. Theoretical models suggest that fluctuating environments that impose selection for plasticity will cause changes in developmental interactions. These changes in development will tend to impose a similar skew on the adjacent possible phenotypes that can be reached by genetic change.

(Kovaka, 2019). To see why, consider that plasticity will tend to leave a signature on phenotypic evolution simply because adaptive change must proceed through the adjacent possible phenotypes (Fig. 2D). For example, reciprocal transplant experiments demonstrate that locally adapted plants commonly resemble the environmentally induced phenotype of ancestral populations (Radersma et al., 2020). This is the expected pattern if adaptive divergence occurs by genetic accommodation of environmentally induced phenotypes, and hence could be taken as evidence for plasticity-led evolution.

However, those locally adapted phenotypes may also have been reached by (cumulative) genetic mutation alone. This is particularly likely if the developmental bias caused by plasticity is small and similar in direction and magnitude to the developmental bias associated with mutation and recombination (Fig. 2D). In other words, environmentally induced phenotypes can contribute to developmental bias without plasticity being a difference maker (and hence an explanation) for why the plants adapted or why they

adapted as they did. More generally, the existence of developmental bias does not mean that developmental bias explains why evolution proceeded one way or another: there is almost always some bias, and studies need to demonstrate that this bias made a difference to the phenomena to be explained.

Convincing evidence that 'genes are followers, not leaders, in adaptive evolution' (West-Eberhard, 2003, p. 20) would show that plasticity caused the population to evolve along a different trajectory, or to evolve a different adaptation, from how it would have evolved in the absence of plasticity. Alternatively, one could demonstrate that similar organisms (or populations) that differ in their phenotypic response to the same environmental perturbation evolve along different trajectories and end up with different adaptations (Fig. 2D).

Plasticity can both facilitate and constrain adaptive evolution
An adaptive developmental bias implies that plasticity shifts the phenotype distribution closer to an adaptive peak (Ghalambor et al.,

2007; Fig. 2E; remember that environmental change may shift the adaptive landscape too). Conversely, developmental plasticity imposes a non-adaptive bias on evolution if plasticity shifts the phenotype distribution away from fitness peaks. Which of these patterns is the most common is debated, and it is likely that an extreme or novel environment will involve phenotypic changes of both kinds. Moreover, an inevitable ('passive') response in one trait, such as a small body size in nutrient-poor environments, that may seem maladaptive will tend to be accompanied by compensatory 'mutual adaptive adjustment' of other traits (e.g. metabolic rate) that make individuals fitter than they otherwise would have been.

The implication is that it can be difficult to infer whether developmental plasticity facilitates or constrains adaptive evolution without good knowledge of biologically realistic alternative responses and their fitness. Explanations ought to be contrastive, and the same developmental response can either facilitate or constrain adaptive change depending on the alternative it is compared with (Uller et al., 2020). Comparisons with hypothetical non-plastic phenotypes may not be very informative, especially if it is difficult to infer whether or not those phenotypes would be fit (see also the discussion regarding interpretation of gene expression data; e.g. Ghalambor et al., 2015; van Gestel and Weissing, 2018; Ho and Zhang, 2019). Moreover, the fitness landscape is typically known only locally, which limits the conclusions that can be drawn about the adaptive significance of phenotypes that are rare.

The evolution of plasticity can make organisms better at adapting

Discussions of plasticity and evolvability tend to emphasise that plasticity can reduce the strength of selection, resulting in the accumulation of (cryptic) genetic variation that subsequently can be exposed to selection (e.g. Levis and Pfennig, 2016; Snell-Rood et al., 2016). However, organisms that evolve environmental regulation of development may become intrinsically better at evolving if plasticity allows them to track the adaptive landscape associated with environmental change, thereby reducing the amount of genetic change needed to maintain an adaptive fit (Fig. 2E). The significance of this process is well recognised (e.g. Price et al., 2003; Ghalambor et al., 2007; Frank, 2011), but to what extent developmental systems will have this capacity to respond adaptively to extreme or novel environments remains poorly understood.

Models of the evolution of development have demonstrated another important side-effect of adaptive plasticity: the developmental interactions that enable switching between different phenotypes in response to environmental cues will also bias the distribution of phenotypes that arise from genetic change (i.e. the GP map; Draghi and Whitlock, 2012; Brun-Usan et al., 2021; Fig. 2F). For example, selection for producing broad, thick leaves in shaded environments and narrow, thin leaves in sunlight would tend to modify leaf development such that most genetic mutants exhibit the same correlation between leaf shape and thickness. Thus, the 'lines of least genetic resistance' that skew evolutionary responses to selection may owe their existence to the evolution of adaptive plasticity (Brun-Usan et al., 2021). More generally, such results demonstrate how studies of developmental plasticity and evolution can benefit from explicitly considering the reciprocal causal relationships between generative and selective processes that play out over evolutionary time.

Developmental plasticity, evolution and experimental biology

Throughout most of the 20th century, developmental biology and physiology have been peripheral to evolutionary biology: how

organisms work seemed to have had little, if any, bearing on why they evolve (Laland et al., 2011). *Developmental Plasticity and Evolution* can be read as one long argument for why 'proximate mechanisms represent more than just different levels of analyses or research styles. They are the causes of the variation upon which selection acts' (West-Eberhard, 2003, p. 11). Three examples will illustrate how knowledge and understanding of those proximate causes can fill some of the knowledge gaps identified above, and help put the role of developmental plasticity in evolution to the test.

First, further development of middle-range theories will require suitable representations of generative processes in evolutionary models. This requires knowledge about how biological systems work. One well-known example is the implementation of models of tooth development to explain within-population variation and adaptive diversification of tooth morphology in mammals (Kavanagh et al., 2007; Salazar-Ciudad and Jernvall, 2010; Christensen et al., 2023; Couzens et al., 2021; Machado et al., 2023). Recently, this model has also been used in conjunction with an adaptive landscape (Fig. 1) to simulate evolution. By accurately representing the complex relationship between genotype and phenotype that arises from development, it was shown that variational properties summarised by G-matrices can evolve rapidly, and that this has important consequences for evolutionary trajectories (Milocco and Salazar-Ciudad, 2020, 2022). Importantly, these trajectories can differ substantially from those expected from models that do not include a generative process (e.g. Jones et al., 2012). These models of how development influences evolution in general were possible only because of decades of experimental work on tooth development (Thesleff, 2003). An increasing understanding of the mechanistic basis of developmental plasticity [e.g. in beetle horns (Casasa et al., 2020), leaf morphology (Fritz et al., 2018) and the castes of ants (Qiu et al., 2022)], now makes it possible to establish whether patterns of diversification are consistent with the selective modification of environmentally induced phenotypes, and to identify conditions that favour plasticity-led evolution.

Second, experimental work that establishes how phenotypes vary in response to genetic and environmental perturbation – that is, quantification of the GP and EP maps – provides data that are crucial to understand the role of plasticity in evolution (Chevin et al., 2022; Fig. 2). For example, not all mechanisms of developmental plasticity will be equally conducive to adaptive developmental bias. Exploratory processes in brain development, such as the formation and retraction of neuronal connections in response to sensory activity, can cause adaptive developmental bias even in radically different circumstances (e.g. in the darkness of a cave or in a new social context; Knudsen, 2004). Other mechanisms of plasticity, such as the temperature-dependent seasonal polyphenism of the butterfly *Bicyclus anynana*, produce highly specific phenotypes only in particular environments (Brakefield and Frankino, 2009). Demonstrating how such differences in EP maps influence phenotypic evolution will require systematic experimental and comparative studies.

Third, there is much to learn from comparative experimental studies, even though ancestor–descendant comparisons can be less informative than it may seem at first (see point above). Research on plasticity-led evolution commonly makes use of instances of convergent evolution (e.g. Feiner et al., 2020; Wood et al., 2023), but another informative test of plasticity-led evolution would be to contrast populations or species that have evolved different adaptations to the same environment. If 'genes are followers, not leaders, in adaptive evolution' (West-Eberhard, 2003, p. 157), those

differences in adaptive phenotypes should reflect differences in how the (putative) ancestors responded to environmental perturbation. Unfortunately, the plasticity of ancestors can be impossible to establish, and knowledge of mechanisms will be useful when there is a need to contrast the observed phenotypes to hypothetical plastic responses. It will often be biologically unrealistic to compare the observed plastic response to a hypothetical 'non-plastic' phenotype. Finally, following the example set by Waddington (1942) and Suzuki and Nijhout (2006), experimental evolution in laboratory settings can give researchers control over both the organism and the adaptive landscape, allowing them to design studies that test predictions concerning the relative importance of plasticity at different stages of the evolutionary process.

Concluding remarks

Developmental Plasticity and Evolution is a book of enormous scope. In comparison, the domain of the middle-range theory we describe can seem narrow and too system specific to deliver genuine insight into how evolution works. However, taking development seriously does not merely add detail and complexity; it re-introduces a putative source of adaptive bias without which biologists may simply be unable to satisfactorily explain evolutionary diversification. That such evolutionary explanations require input from fields that have traditionally received less attention, such as experimental biology and physiology, is a sign of progress. Twenty years on from *Developmental Plasticity and Evolution*, there is still much to do.

Special Issue
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