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Review

Evaluating 'Plasticity-First' Evolution in Nature: Key Criteria and Empirical Approaches

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Many biologists are asking whether environmentally initiated phenotypic change (i.e., 'phenotypic plasticity') precedes, and even facilitates, evolutionary adaptation. However, this 'plasticity-first' hypothesis remains controversial, primarily because comprehensive tests from natural populations are generally lacking. We briefly describe the plasticity-first hypothesis and present much-needed key criteria to allow tests in diverse, natural systems. Furthermore, we offer a framework for how these criteria can be evaluated and discuss examples where the plasticity-first hypothesis has been investigated in natural populations. Our goal is to provide a means by which the role of plasticity in adaptive evolution can be assessed.

Need for a Predictive Framework

Among the enduring problems of evolutionary biology is explaining how complex, adaptive traits originate [1,2]. Although it is widely assumed that new traits arise solely from genetic factors [3], many researchers are asking whether environmentally initiated phenotypic change – in other words **phenotypic plasticity** (see Glossary) – precedes and facilitates adaptation [4–12].

This alternative route, dubbed the **plasticity-first hypothesis** [4,13], rests on the observation that phenotypic plasticity often produces developmental variants that can enhance fitness under stressful conditions [4,5,14]. If underlying genetic variation exists in the tendency or manner in which individuals produce such variants (as is often the case [15]), then selection can refine the trait from an initial, potentially suboptimal version through quantitative genetic changes over time; in other words **genetic accommodation** occurs [4]. Furthermore, depending on whether or not plasticity is favored [16], this selection can respectively promote either increased environmental sensitivity – which maintains the trait as a **polyphenism** (*sensu* [17]) or decreased environmental sensitivity – which can result in the constitutive expression of the trait; in other words **genetic assimilation** (*sensu* [18]). By 'jump-starting' phenotypic change in an adaptive direction [19], environmentally induced phenotypic change precedes, and promotes, the evolutionary origins of a complex, adaptive trait (Figure 1; Box 1).

When initiated by plasticity, refinement of a developmental variant into an adaptive trait (whether **novel** or not) does not require new genes. Instead, environmentally induced phenotypic change sets in motion an evolutionary sequence in which selection promotes adaptation by acting on existing genetic variation (e.g., [15,20–22]). In essence, such selection refines a trait through evolutionary adjustments in both the form and regulation of trait expression. The outcome of this process is an adaptive phenotype that, relative to its initial state, has been modified both in its

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Phenotypic plasticity has long been proposed to precede and possibly facilitate adaptive evolution.

This 'plasticity-first hypothesis' is controversial because skeptics argue that it lacks compelling evidence from natural populations.

A chief difficulty with demonstrating plasticity-first evolution in natural populations is that, once a trait has evolved, its evolution cannot be studied *in situ*. To get around this difficulty, researchers can study extant lineages that act as ancestral-proxies to the lineage possessing the focal trait.

Using such an approach, key criteria of the plasticity-first hypothesis can be evaluated using a relatively simple experimental design.

Applying these criteria to various systems, the plasticity-first hypothesis has some empirical support. However, more studies are needed to conclusively determine the role of plasticity in adaptive evolution.

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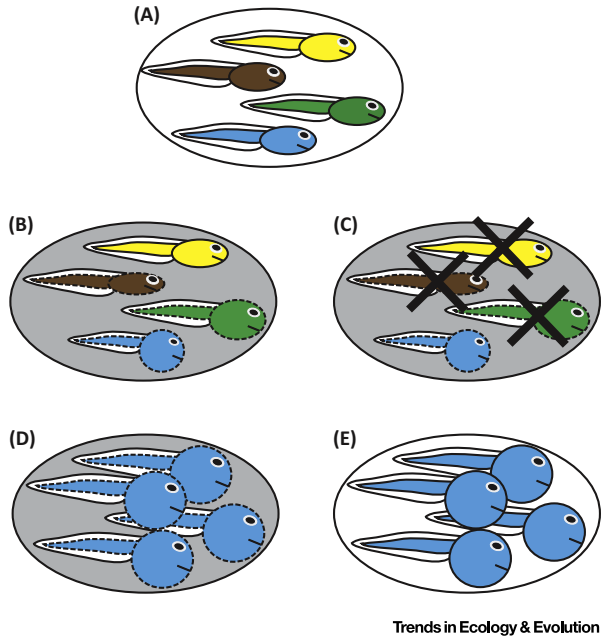

Figure 1. An Idealized Representation of How Plasticity-First Evolution Leads to a Novel, Adaptive, Complex Trait. (A) A genetically variable population (here, different-colored tadpoles represent different genotypes) (B) experiences a novel environment (shading), which immediately induces novel developmental variants (dashed lines). However, different genotypes vary in the manner in which they respond (or indeed, if they respond at all). (C) Selection acts on this formerly cryptic genetic variation (revealed by the change in environment) by disfavoring those genotypes that produce phenotypes that are poorly adapted in the new environment (here, the round-bodied phenotype is favored, whereas all others are disfavored, as indicated by the 'X'). (D) This selection also leads to the adaptive refinement of the favored phenotype (depicted here by the enlargement of the blue tadpole). If the ancestral phenotype (i.e., narrow-bodied tadpole) is still maintained in the ancestral environment (see A), then the result is a novel polyphenism. (E) However, selection might instead favor the loss of plasticity (i.e., genetic assimilation), resulting in a novel phenotype that is now produced constitutively, even when the population experiences the ancestral environment (indicated here by the loss of shading and dashed lines). Note that observations from natural systems likely will not be as clear-cut as the process described here. Furthermore, although we have shown how a plasticity-first process promotes novelty, this process could also explain the evolution of traits that are not novel (e.g., body size).

morphological and physiological attributes as well as in its environmental sensitivity. Of course, other evolutionary forces (e.g., genetic drift, mutation) could alter the degree of plasticity. However, the plasticity-first hypothesis assumes that any such change occurred via genetic accommodation, which (by definition) is driven by selection.

Although lab studies have demonstrated that the plasticity-first hypothesis can promote adaptation (e.g., [18,23–25]), and there are suggestive field studies (e.g., [26–30], reviewed in [31]), whether plasticity, followed by genetic accommodation, has actually contributed to the evolution of any complex trait in any natural population is controversial [32–35]. Part of the difficulty is that the key criteria of the plasticity-first hypothesis have not been made clear. However, if as stated in two recent prominent reviews: 'what remains to be done is to generate creative approaches to collecting empirical data from natural populations to test predictions. . .' [11], and if 'the best way to elevate the prominence of genuinely interesting phenomena such as phenotypic plasticity... is to strengthen the evidence for their importance' [35], then these criteria and predictions must be made clear and rigorously tested.

We describe here key criteria for testing the plasticity-first hypothesis. We also present a general framework in which these criteria could be evaluated in natural populations, and we discuss case

Glossary

Cryptic genetic variation: genetic variation that normally has little or no effect on phenotypic variation except under atypical conditions.
Genetic accommodation: a mechanism of evolution wherein a phenotype, generated by either a mutation or environmental change, is refined into an adaptive phenotype through selection driving quantitative genetic changes. Accommodation can also promote either increased or decreased environmental sensitivity of the focal phenotype; when environmentally induced phenotypes lose environmental sensitivity, they undergo 'genetic assimilation'.
Genetic assimilation: an extreme form of 'genetic accommodation' that occurs when selection causes environmentally induced (i.e., plastic) phenotypes to lose their environmental sensitivity over evolutionary time.
Novel trait: broadly, any major developmental innovation; sometimes defined as a body part that is neither homologous to any body part in the ancestral lineage nor serially homologous to any other body part of the same organism; a difficult concept to define.
Phenotypic accommodation: the maintenance of a novel, induced trait or phenotype that is an automatic consequence of multidimensional adaptive physiological, morphological, and/or behavioral plasticity in the face of a developmental change.
Phenotypic plasticity: the ability of an organism to alter its behavior, morphology, and/or physiology in response to changes in environmental conditions; sometimes used synonymously with developmental plasticity.
Plasticity-first hypothesis: a mechanism of adaptive evolution in which environmental perturbation leads, via phenotypic plasticity, to developmental reorganization (via, e.g., altered gene expression) and uncovers 'cryptic genetic variation' for, and ultimately production of, a novel developmental variant (i.e., trait) that immediately undergoes 'phenotypic accommodation' and is subsequently refined through 'genetic accommodation' some definitions include cases in which mutation initiates trait origin (not discussed here; see Box 1).

Box 1. Evolutionary Potential of Environmentally Initiated Versus Mutationally Initiated Phenotypic Change

Stresses – such as mutation or environmental change – can generate novel developmental variants [4,5]. The subsequent genetic accommodation of such variants can occur whether they are induced by mutation or a change in the environment [4], and these two modes of induction are often interchangeable [4,12,13]. However, environmentally triggered novelties likely have far greater evolutionary potential than mutationally induced ones, for at least three reasons [4]. First, changes in the environment often affect many individuals simultaneously, in contrast to a genetic mutation, which initially affects only one individual and its immediate descendants (also, the vast majority of mutations are deleterious [58,59]). This widespread impact of environmental change enables a newly induced trait to be tested among diverse genotypes, thereby providing fertile ground for selection to act and increasing the chances that genetic accommodation will occur [4]. Second, although the chance that a particular mutation will occur is not influenced by whether or not the organism is in an environment in which that mutation will be advantageous – in other words, adaptively directed mutation does not occur [60] – the situation is different for environmentally triggered traits. Such traits are always associated with a particular environment – the one that triggered it. Therefore, environmentally induced traits are more likely than mutationally induced novelties to experience consistent selection and directional modification [4]. This allows new environments to immediately produce and select among new phenotypes and rapidly refine their expression [5]. Third, plasticity promotes the storage and release of cryptic genetic variation – in other words variation that is expressed only under atypical conditions (e.g., [41,45,46]). The release of such variation ultimately makes genetic accommodation possible [9,44]. For these reasons, environmental initiation might have greater evolutionary potential than previously appreciated.

Polyphenism: environmentally induced alternative phenotypes.
Reaction norm: a graphical representation of the set of phenotypes that a single genotype produces in response to some specific environmental variable(s); individuals show plasticity if their reaction norm is non-horizontal.

studies that have utilized this framework to find support for these criteria. Our goal is to provide a roadmap for testing the plasticity-first hypothesis and thereby clarify the role of plasticity in adaptive evolution.

Key Criteria for the Plasticity-First Hypothesis of Adaptive Evolution

Before outlining criteria for demonstrating plasticity-first evolution in natural populations, we note that the most compelling evidence for this process would be to actually observe it taking place. Indeed, plasticity-first evolution could potentially be observed in real-time in: (i) cases in which naturally occurring populations experience rapid environmental change (e.g., climate change or introduced species [26]), or (ii) resurrection studies; for example, using seeds from datable sediment as ancestral contrasts to modern, derived individuals from the same population. In both contexts, the ancestral condition would be known, and environmental change can occur swiftly enough to observe an evolutionary response. Note that a third context, studying the plasticity-first hypothesis in lab populations of rapidly evolving organisms [36,37], would be worthwhile but would not clarify whether plasticity has contributed to adaptation in any natural population [32–35].

For most systems, however, it is likely that only the final products of any putative plasticity-first process will be present in modern-day populations. In such situations the chief difficulty with demonstrating plasticity-first evolution is that, once a trait has evolved, its evolution cannot be studied *in situ*. To get around this difficulty one could either study lineages (i.e., species and/or populations) that are ancestral-proxies to the lineage possessing the focal trait [5] or, alternatively, evaluate whether environmentally induced differences within taxa reflect adaptive (fixed) differences among these same (or related) taxa [31]. Of these approaches, ancestral-derived comparisons produce stronger evidence of genetic accommodation [31]. Although such comparisons are only feasible in systems with well-understood natural histories and readily accessible ancestral-proxy and derived lineages (Box 2), we focus on this approach in suggesting four key criteria of the plasticity-first hypothesis.

Importantly, validation of any one criterion, by itself, is insufficient to establish that plasticity-first evolution has occurred. For instance, many systems appear to satisfy criterion 1 below – that a trait is present in ancestral-proxy lineages as an environmentally induced variant [13,31]. However, the mere existence of such ancestral plasticity is insufficient to demonstrate that the trait evolved via a plasticity-first process. As noted above, several evolutionary mechanisms

Box 2. Considerations When Choosing a Study System

As noted, only the final products of plasticity-first evolution might still exist for most systems, making *in situ* investigation difficult. One way to circumvent this difficulty is to use other lineages (i.e., species or populations) as proxies for the ancestral condition and compare them to derived lineages that possess the focal trait. To do so, one must know the phylogenetic relationships between lineages that differ in the form of, or propensity to produce, an induced phenotype. This is important because it is essential to know which lineages can represent the ancestral and derived states. However, this means that some systems that lack phylogenetic information might not be suitable for evaluating the plasticity-first hypothesis using the approaches we have outlined here.

In addition, our criteria depend on knowing the environmental stimulus that might have initially led to production of the trait as well as the selective pressures leading to its refinement or change in frequency of expression in derived lineages. Without this information, evaluating the plasticity-first hypothesis is not possible.

While knowledge of the phylogenetic relationships and relevant environmental factors is required, other non-essential characteristics could improve the utility of a system in studying the plasticity-first hypothesis [61], including:

(i) Multiple, parallel derived lineages with varying divergence times from the ancestral-proxy lineage.
(ii) Knowledge of the ecological circumstances experienced by, and the selective agents impinging on, both ancestral-proxy and derived lineages.
(iii) A quantifiable trait that is readily induced under laboratory conditions.
(iv) Adequate genomic resources for investigation of molecular underpinnings.
(v) Other features of 'typical' model organisms (e.g., fast generation time, easy to rear in large numbers in the lab, numerous offspring, etc.).

can lead to the subsequent change in the degree of plasticity, but the plasticity-first hypothesis requires that selection favored any such changes. Thus, confirmation of several (ideally, all four) criteria increases support for this mode of evolution.

Criteria 1 and 2 focus on detecting pre-existing plasticity and developmental reorganization (i.e., **phenotypic accommodation**), whereas criteria 3 and 4 focus on the subsequent refinement of expression and form of the focal phenotype (i.e., genetic accommodation). Note that we have omitted criteria specifically dealing with how developmental reorganization can produce a novel phenotype in ancestral-proxy lineages because such reorganization has extensive support (reviewed in [4]) and is widely accepted. However, we expect any changes in the measured trait between ancestral-proxy and derived lineages to be accompanied by concurrent mechanistic changes (e.g., hormones, alternative splicing, transcription factors, *cis*-regulatory elements, etc.) [4,9,38–40] underlying production and/or regulation of that trait.

Below we list each criterion followed by the rationale behind it. Although each of these criteria has been discussed previously (e.g., [4,5,9,22,29]), they have not been collectively assembled, and clear methods for testing them have not been provided before now.

Criterion 1. The Focal Trait Will Be Environmentally Induced in Ancestral-Proxy Lineages

The most fundamental criterion of the plasticity-first hypothesis is that the trait of interest should exhibit ancestral plasticity. By 'ancestral plasticity' we mean that a developmental variant (or character state), similar to the derived (possibly fixed) trait, should be expressed among individuals from ancestral-proxy lineages when these individuals experience the derived environment; in other words the environmental conditions in which the focal trait is normally expressed in derived lineages [4]. In addition, if there is phylogenetic support that a taxon with an inducible trait resembles the ancestral condition, and/or if the developmental mechanisms for producing the trait are conserved in related species where the trait does not regularly occur, then this would suggest that the trait started out as an environmentally induced developmental variant [4]. It is important to note that the developmental variant need not be expressed to the same degree as is seen in derived lineages.

Criterion 2. Cryptic Genetic Variation Will Be Uncovered When Ancestral-Proxy Lineages Experience the Derived Environment

If a trait only exists as an environmentally induced variant, and therefore is infrequently (or never) exposed to selection, then genetic variation should accumulate in the response of the trait to a novel environment (or in components that make up the trait) that can be revealed when environmental conditions change (Figure 1C). In a novel environment, this **cryptic genetic variation** would be uncovered – manifest as an increase in heritability or greater phenotypic variation resulting from perturbation of an evolutionary capacitor [30,41–43] – and act as a selectable substrate (Figure 1D) [4,9,44–46]. Subsequently, however, lineages in the novel (derived) environment should experience a selective sweep and lose this variation as the trait undergoes genetic accommodation [4,9,47]. Thus, once derived lineages have undergone genetic accommodation, they should exhibit reduced heritability and/or genetic variation in the trait of interest.

Criterion 3, The Focal Trait Will Exhibit Evidence of Having Undergone an Evolutionary Change in its Regulation, Form, or Both in Derived Lineages

This criterion can be manifest as a change in the slope or elevation (or both) of the **reaction norm** in derived versus ancestral-proxy lineages. Because a change in slope represents a change in the regulation of a trait and a change in elevation indicates a change in its form [30,48], finding either in derived lineages would suggest genetic accommodation [4]. Furthermore, finding that reaction norms are fixed across different environments would suggest that the trait has been genetically assimilated. Finally, changes in reaction norms should be mirrored by changes in the mechanisms underlying the trait (e.g., hormones, alternative splicing, transcription factors, *cis*-regulatory elements, etc.) [4,9,38–40].

Criterion 4. The Focal Trait Will Exhibit Evidence of Having Undergone Adaptive Refinement in Derived Lineages

Under the plasticity-first hypothesis, the frequency that a trait is expressed will determine the degree to which it is refined by selection [4]. Therefore, compared with individuals from ancestral-proxy lineages, individuals from derived lineages should produce superior versions of the trait when both types of lineages experience environments that induce the trait. For example, derived individuals should produce a version of the trait with improved functionality, fewer side effects, or a lower threshold for expression [4]. This criterion is based on two assumptions: (i) individuals in derived lineages should express the trait more frequently than individuals in ancestral-proxy lineages (which produce the trait infrequently), and (ii) a trait in a population in which it is expressed (and exposed to selection) more frequently should evolve greater and more rapid refinement [4]. As a corollary, the fitness consequences of a trait – in other words its contribution to individual fitness – should be higher in derived lineages than in ancestral-proxy lineages when both are in the derived environment [4,49].

Evaluating the Plasticity-First Hypothesis in Nature: General Framework

Rather than describe how each criterion could be tested individually, we present a scheme for testing all criteria simultaneously (Figure 2). Our scheme consists of two phases: (i) identification of ancestral-proxy and derived lineages, and (ii) common-garden experimentation in which both types of lineages are reared under environmental conditions similar to those that they would experience in the wild.

Before starting, it is essential that background information about the study system is known (Box 2). Assuming this information is available, the first phase requires inferring ancestral character states to help to clarify which lineages could serve as ancestral-proxies (to ensure that the ancestral-proxy is adequate, the directionality of any phylogenetic reconstruction should be well supported by using multiple outgroups). These ancestral-proxy lineages should

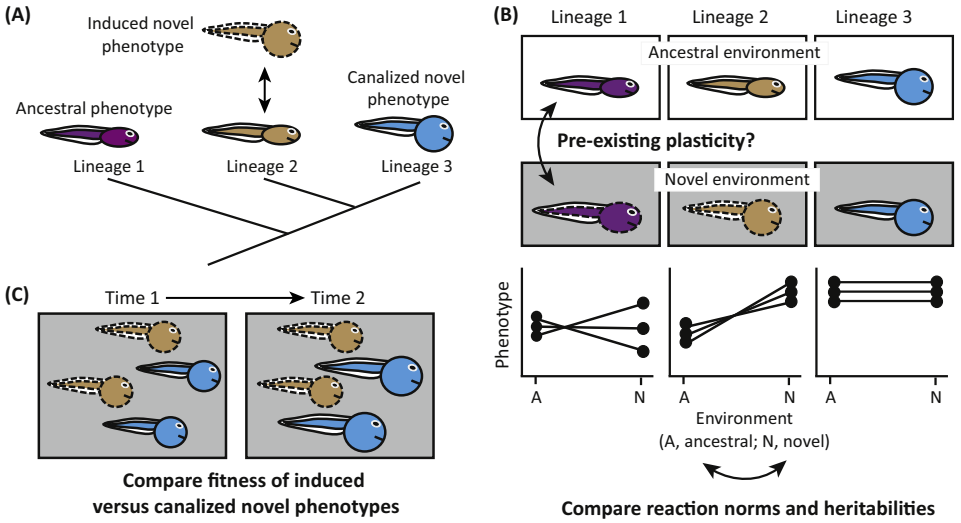

Figure 2. Approaches for Testing the Key Criteria of the Plasticity-First Hypothesis. (A) As an initial step, identify lineages to serve as ancestral-proxies to the lineage(s) that produce(s) the focal trait. In this example, lineage 1 does not produce a novel trait during normal development; lineage 2 produces the novel trait as part of normal development depending on environmental conditions (i.e., it exhibits adaptive plasticity in trait expression); lineage 3 produces the novel trait regardless of environmental conditions (i.e., it is canalized, meaning that it exhibits no plasticity in the trait). (B) Next, use a common-garden approach to determine if: (i) ancestral-proxy lineages that normally lack the trait of interest (lineage 1) produce developmental variants of the trait through phenotypic plasticity when experiencing the novel environmental stimulus (criterion 1); (ii) the novel environment uncovers cryptic genetic variation in these ancestral-proxy lineages (criterion 2); (iii) phenotypic variation revealed in the ancestral-proxy lineages is greater than in derived lineages (and might be random with respect to its adaptive value in the novel environment); and (iv) reaction norms have evolved in a manner suggesting that selection has refined trait expression in the novel environment (indicated by directional reaction norms in lineage 2 versus flat reaction norms in lineage 3; criterion 3). (C) Finally, use a common-garden approach to determine if the trait has indeed undergone adaptive refinement by comparing the fitness of individuals from lineages that produce the novel phenotype facultatively versus constitutively (criterion 4). If genetic accommodation has occurred, the latter should outperform the former in the novel environment [indicated by increased growth (larger size) of the blue (canalized) phenotype at time 2], but not in the ancestral environment.

be closely related to the derived lineages, but they should not possess the derived (potentially fixed) trait when in their natural (ancestral) environment that lacks the inducing stimulus.

In the second phase of testing the plasticity-first hypothesis, all key criteria can be tested simultaneously. This requires rearing multiple sibships from ancestral-proxy and derived lineages (Figure 2A) in conditions representing ancestral and derived environments (e.g., without and with the inducing stimulus, respectively). This could be done with a common-garden or reciprocal transplant design. An advantage of this approach is that it allows one to estimate how much evolutionary distance (from ancestral to derived trait) was covered when an ancestral lineage experienced a novel environment (indeed, such environmentally induced variants typically cover only part of this evolutionary distance; e.g., [22,27,29,50]). Multiple sibships are necessary, both because the expression of phenotypic plasticity often varies among genotypes [15] and because criterion 2 specifically involves comparing genetic variability across ancestral and derived environments.

From such an experiment, observations and measurements that could validate the criteria include: a wide variety of phenotypes produced by ancestral-proxy sibships in derived environments, some of which are along the same axis of variation as the focal phenotype possessed by derived individuals (criteria 1 and 2; Figure 2B); an increase in heritability of the trait(s) (or components of the trait) among ancestral-proxy sibships when reared in derived environments

Box 3. Alternative Methods for Evaluating Criterion 4 of the Plasticity-First Hypothesis

Our criteria might be validated using methods other than those presented above. This is especially likely to be true for criterion 4 because what constitutes a superior trait is system-specific. We highlight here some alternative approaches for testing criterion 4.

(i) Compare in different lineages the amount of stimulus required to induce the focal trait (e.g., [62]). For example, in the case of light-induced response in plants, one could measure the amount of light needed to induce the trait and, in the case of predator-induced responses in either plants or animals, one could measure the intensity of predation or abundance of predators needed to induce the trait. Moreover, if the underlying endocrine signals are known, one could manipulate concentrations of hormones to determine the amount needed to induce the trait. Generally, the amount of stimulus required to elicit induction of a trait should be inversely proportional to the amount of evolutionary time a population has been exposed to that stimulus. Thus, the threshold of induction should be lower (or zero) in derived sibships than in ancestral-proxy ones.

(ii) Perform experiments in which individuals from ancestral-proxy and derived lineages are directly set against one another (Figure 2C) [28]. For example, if derived individuals have a novel resource-acquisition trait, then they could be directly competed with ancestral-proxy individuals in obtaining that resource. One could then determine if (as predicted) derived individuals obtain more resource and exhibit improved survival or growth than ancestral-proxy individuals.

(iii) Measure selection on the trait in ancestral-proxy, derived polyphenic, and derived constitutive expression lineages in the wild [54]. Ideally, one would identify individuals with different trait values and measure their fitness. The expectation is that derived lineages with constitutive expression should exhibit the strongest selection favoring the trait. In practice, however, it might only be possible to measure a component of fitness, such as survival, mating success, or fecundity, or (even less directly) a trait that correlates with these fitness components, such as body size. Regardless, from the slope and shape of the regression line relating phenotype to fitness (or some proxy of fitness), one could determine the strength and mode of selection acting on the trait of interest in different lineages.

(criterion 2; Figure 2B); a change from reaction norms lacking consistent directionality among ancestral-proxy sibships to having a relatively consistent slope among derived sibships (criteria 2 and 3; Figure 2B); a change in the slope or elevation of the reaction norm in derived sibships relative to ancestral-proxy sibships (criterion 3; Figure 2B); greater variance in trait values for ancestral-proxy sibships compared to derived sibships when reared in derived environments (criteria 2 and 3; Figure 2B); greater fitness (e.g., survival, growth, size, development, fecundity, etc.) of derived sibships than ancestral-proxy sibships when reared in derived environments (criterion 4; Figure 2C); and a stronger correlation between fitness and trait value in derived sibships than ancestral-proxy sibships when reared in derived environments (criterion 4; see Box 3 for other suggestions for testing criterion 4).

Evaluating the Plasticity-First Hypothesis in Nature: Illustrative Case Studies

To illustrate the above framework for testing the key criteria of the plasticity-first hypothesis, we describe two case studies. Although these studies are not necessarily the most compelling examples of plasticity-first evolution, they illustrate how to test the plasticity-first hypothesis in a natural population.

Spadefoot Toads

Spea tadpoles exhibit a novel polyphenism not seen in other species [22] consisting of an omnivore ecomorph, which eats detritus primarily, and a morphologically and behaviorally distinctive carnivore ecomorph, which specializes on shrimp and which expresses a suite of unique, complex traits [51]. Omnivores are the default morph; carnivores are induced when a young omnivore eats shrimp or other tadpoles [28,52]. However, most populations harbor heritable variation in the propensity to produce carnivores [51]. Carnivores arise developmentally from an omnivore-like form via accelerated growth of features [53], and frequency-dependent, disruptive selection – stemming from resource competition – maintains both ecomorphs within most populations [54]. Several studies, taken together, suggest that this novel carnivore ecomorph arose through plasticity-first evolution [22,41,55].

One study found support for criteria 1, 3, and 4 [22]. Using ancestral character state reconstruction, *Scaphiopus couchii* was chosen as a proxy for non-plastic *Spea* ancestors

(*Sc. couchii* produce only omnivores). Gut length (carnivores produce shorter guts than omnivores) and gut cell proliferation (a measure of gut performance) were compared in *Sc. couchii*, *Spea multiplicata*, and *Sp. bombifrons*. *Sc. couchii* produced a wider range of gut lengths when fed shrimp [a novel diet for this species, but representing the derived diet (environment) for *Spea*] than when fed detritus, and the variation was not directional: both shorter and longer gut lengths were produced on shrimp. By contrast, both *Spea* species consistently produced shorter guts when fed shrimp than when fed detritus. Furthermore, whereas *Sc. couchii* tadpoles did not exhibit increased gut cell proliferation when fed shrimp, both *Spea* species did, suggesting that shrimp digestion has undergone genetic accommodation in *Spea*.

Two subsequent studies further support criteria 2, 3, and 4 [41,55]. Cryptic genetic variation in *Sc. couchii* was detected when fed different diets and exposed to corticosterone (a stress hormone). These *Sc. couchii* tadpoles developed and grew more slowly, had increased corticosterone levels, and exhibited greater heritability in size, development, and gut length when fed shrimp than when fed detritus. In addition, *Sc. couchii* tadpoles exposed to corticosterone had greater heritability in development and gut length. Therefore, these studies demonstrated a release of cryptic genetic variation in the ancestral condition when tadpoles were exposed to the derived stimulus, and they also identified a possible hormonal mediator of the carnivore ecomorph.

Moreover, this novel ecomorph appears to have undergone genetic assimilation in some derived populations of *Spea*. In ancestral populations containing only a single species, both *Sp. multiplicata* and *Sp. bombifrons* produce similar, intermediate frequencies of both ecomorphs. By contrast, in derived populations where these species co-occur, each becomes nearly monomorphic, with *Sp. multiplicata* producing mostly omnivores, and *Sp. bombifrons* producing mostly carnivores, regardless of resource availability [28]. This near fixation of one ecomorph is adaptive because it minimizes competition between the two species [28]. More generally, this selection-driven shift from plastic to fixed ecomorph production supports criterion 3.

Note, however, that *Sc. couchii* might not represent the ancestral condition (they might have evolved the omnivore feeding strategy secondarily as an adaptive response to competition with, or predation by, sympatric *Spea* tadpoles), and support for criteria 1–3 could therefore be questioned. In addition, support for criterion 4 is limited. Nevertheless, this system illustrates how plasticity might have contributed to the evolution of a novel, complex phenotype in natural populations.

Cavefish

Eye loss in cave-dwelling populations of Mexican tetra (*Astyanax mexicanus*) also provides an excellent setting for testing the plasticity-first hypothesis. The cave environment is an evolutionarily novel environment, and it is known that cave populations are derived from surface populations [56].

The greatest abiotic difference between surface and cave environments (other than light availability) is lower conductivity of cave water [43]. When surface *A. mexicanus* were reared under low conductivity, they displayed greater variation in eye and orbit size, and they upregulated HSP90 [43]. Moreover, when HSP90 was manipulated to mimic environmental stress (i.e., its chaperone ability was reduced), surface fish displayed greater variation in eye and orbit size beyond the range observed in controls. Cavefish did not increase trait variation under HSP90 manipulation. In addition, when HSP90-manipulated fish with the smallest eyes were crossed, their untreated F2 progeny had eyes and orbit sizes at the lower end of the range in parental fish, and the sizes were comparable with the smallest eyes of treated fish. These observations

Table 1. Examples of Species, Conditions, and Traits for which Two or More Key Criteria of the Plasticity-First Hypothesis Are Supported in Naturally Occurring Systems

Species	Novel Condition(s)	Trait	Criteria Supported	Refs
Plants				
Arabidopsis thaliana	Shade, HSP90 inhibition	Morphology; growth	1,2	[42,63]
Acacia spp.	Ant guards	Ant mutualism	1,3,4	[64,65]
Nematodes				
Rhabditina spp.	Alternative diets	Mouth morphology	1,3	[66]
Crustaceans				
Daphnia melanica	Fish predators	Melanization	1,3	[26]
Insects				
Drosophila mojavensis	Alternative hosts	Host preference	1,3	[67–69]
Polites sabuleti (skipper butterfly)	Low temperatures	Wing patterning; coloration	1,3	[70]
Nymphalis antiopa (mourning cloak butterfly)	Low temperatures	Wing patterning; coloration	1,3	[71]
Fishes				
Fundulus spp. (killifish)	Various salinities	Salinity tolerance	1,3	[72–76]
Cyprinodon diabolis (Devils Hole pupfish)	High temperatures; reduced resources	Pelvic fin loss	1,3	[77,78]
Gasterosteus aculeatus (threespine stickleback)	Reduced cannibalism	Antipredator and courtship behavior	1,3	[79,80]
Gasterosteus aculeatus (threespine stickleback)	Alternative resources	Resource use ecotypes	1,3	[29]
Gasterosteus aculeatus (threespine stickleback)	Fresh water	Growth (size); salinity tolerance	1,2,3,4	[30,81–84]
Astyanax mexicanus (Mexican tetra)	Caves	Eye loss	1,2,3,4(?)	[43,56]
Amphibians				
Spadefoot toad spp.	Ephemeral ponds	Development time	1,3	[85]
Notophthalmus viridescens (eastern newt)	Altered pond hydroperiod	Developmental strategy	1,3,4	[86–88]
Lithobates sylvaticus (wood frog)	Insecticide	Insecticide tolerance	1,3,4	[89]
Spea spp. (spadefoot toad)	Alternative diets; competitors	Resource use ecomorph	1,2,3,4	[22,28,41,55]
Reptiles				
Anolis spp.	Alternative perch diameters	Hindlimb length	1,4	[27,50,90,91]
Notechis scutatus (tiger snake)	Alternative diets	Head size	1,3,4	[20,92,93]
Birds				
Agelaius phoeniceus (red-wing blackbird), *Parus major* (great tit), and other urban birds	Urban landscapes	Song	1,3	[94,95]
Carpodacus mexicanus (house finch)	Various	Reproductive attributes; offspring morphology	1,3,4	[96]

suggest that: stressful conditions (i.e., low conductivity) induce similar changes in HSP90 function as in laboratory manipulation of HSP90; both stressful conditions and HSP90 manipulation result in uncovering of cryptic genetic variation for eye size; and individuals that develop small eyes when HSP90 is inhibited contain alleles that contribute to the inheritance of reduced eyes, even in the absence of treatment (i.e., become genetically assimilated).

Subsequent refinement of this induced eyeless phenotype is associated with improved functionality in cave conditions. When competed directly, cavefish forage better than surface-dwelling fish in the dark [56].

Thus, the transition from surface to cave likely involved the production of a range of novel phenotypes (satisfying criterion 1), which was facilitated by the uncovering of cryptic genetic variation (satisfying criterion 2). This was followed by selection favoring fixation of the eyeless phenotype (satisfying criterion 3) and possible refinement of this phenotype, such that cavefish outcompete surface fish for food in the dark (potentially satisfying criterion 4). Note, however, that it is unclear if derived (cave-dwelling) populations exhibit enhanced resource acquisition because of refinement of the focal trait *per se* (reduced eye size; as required by criterion 4) as opposed to some other aspect of the phenotype (e.g., olfaction), which could have arisen via new mutations. Thus, further studies will be necessary to determine if criterion 4 is satisfied in this system. Nevertheless, this system again illustrates how plasticity might have contributed to the evolution of a complex trait.

Evaluating the Plasticity-First Hypothesis in Nature: General Assessment of the Evidence

Beyond these case studies, researchers have (intentionally or not) demonstrated portions of the plasticity-first hypothesis in numerous natural systems. Indeed, two recent reviews have evaluated the empirical support for plasticity-first evolution and found many systems in which an adaptive trait is present in ancestral (or ancestral-proxy) lineages as an environmentally induced variant [13,31], thereby satisfying criterion 1 above. However, as noted previously, the mere existence of such ancestral plasticity is not sufficient to demonstrate that a trait evolved via a plasticity-first process; demonstrating that plasticity-first evolution has likely occurred also requires evidence that any evolutionary changes in expression of plasticity reflect selection (criteria 3 and 4).

In Table 1 we provide examples of naturally occurring systems in which our literature survey revealed that two or more criteria were validated. Our general assessment is that few systems have fulfilled all four criteria. In particular, although many systems have satisfied criteria 1 and 3, few satisfy criteria 2 (accumulation and release of cryptic genetic variation in ancestral-proxy lineages) and 4 (increased refinement in derived lineages). While criterion 2 is the least crucial of the four criteria (and among the most difficult to evaluate), criterion 4 is essential to rule out alternative evolutionary explanations (see above).

We hasten to add, however, that although few systems support all four criteria, taken together the body of evidence is reminiscent of Darwin's approach to supporting evolution by natural selection [57]: multiple lines of partial evidence point toward the same process operating in many, diverse taxa.

Concluding Remarks

We have described key criteria for evaluating the plasticity-first hypothesis in natural populations, provided a roadmap for testing these criteria (Figure 2 and Box 3), and presented examples that serve as a guide for testing the criteria (Table 1). More tests are needed before the plasticity-first hypothesis can be regarded as a general explanation for how complex, adaptive traits originate.

Outstanding Questions

How pervasive is plasticity-first evolution in nature?

Can we observe plasticity-first evolution in real-time in natural populations?

What are the molecular signatures of the plasticity-first hypothesis?

Are particular taxonomic groups and traits more likely to experience plasticity-first evolution than others, and, if so, why?

What are the selective and proximate bases (e.g., molecular mechanisms) of genetic accommodation and assimilation?

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