SYMPOSIUM

Can a Network Approach Resolve How Adaptive vs Nonadaptive Plasticity Impacts Evolutionary Trajectories?

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Synopsis
Theoretical and empirical work has described a range of scenarios in which plasticity may shape adaptation to a novel environment. For example, recent studies have implicated a role for both adaptive and non-adaptive plasticity in facilitating adaptive evolution, yet we lack a broad mechanistic framework to predict under what conditions each scenario is likely to dominate evolutionary processes. We propose that such a framework requires understanding how transcriptional, protein, and developmental networks change in response to different rearing environments across evolutionary time scales. Our central argument is that these hierarchical networks generate and maintain phenotypic variation in populations, both by buffering organisms from developmental noise and mutational inputs and by exhibiting flexible responses to environmental cues. These network properties in turn lead to predictions about how plasticity should influence adaptive evolution. Because buffering mechanisms allow the build-up of cryptic genetic variation (i.e., genetic variation without phenotypic consequences), the initial response of individuals colonizing novel environments should be a release of genetic and phenotypic variation that selection acts upon; some of which is adaptive and some of which is not. Thus, in the early stages of adaptation, strong selection against maladaptive phenotypes should result in rapid evolution acting on standing cryptic variation. However, over longer time scales, evolutionary change should largely be compensatory, to rebuild robust developmental processes and promote integrated phenotypes. We argue that considering how hierarchical networks respond over developmental and evolutionary time encompasses a more mechanistic understanding of the genotype–phenotype map, and will result in a more predictive framework for understanding the role of plasticity in adaptive evolution.

Introduction
A major goal of integrative biology is to understand how the complex interactions between the genome and the environment generate hierarchically integrated phenotypes, and how these interactions in turn influence evolutionary processes. Central to this question are contrasting views on how environmentally induced phenotypic variation (i.e., phenotypic plasticity) influences adaptive evolution in novel environments. A novel environment broadly includes any conditions outside the range that a population has historically experienced; we use this term rather than referring to stressful environments, as novel environments need not induce physiological stress (Hoffmann and Hercus 2001; Badyaev 2005; Bijlsma and Loeschcke 2005). At the individual level, the initial response to novel conditions will be through plasticity; how plasticity in development, physiology, and behavior influences subsequent evolutionary change at the population level remains a long-standing and unresolved question (Baldwin 1896; Schmalhausen 1949; Robison 1999; Price et al. 2003; Badyaev 2005; Ghalambor et al. 2007).

On the one hand, plasticity in novel environments may shield organisms from strong directional selection and constrain evolution, if responses to the novel environment mask underlying heritable variation or yield near-optimal phenotypes (Huey et al. 2003; Price et al. 2003). On the other hand, empirical
and theoretical research indicates several distinct ways in which phenotypic plasticity may facilitate adaptive evolution. One possibility is that novel environments induce phenotypic changes in an adaptive direction (i.e., toward the phenotypic optimum in the novel environment), immediately increasing survival and population persistence in the novel environment and allowing for subsequent adaptation (Baldwin 1896; Robinson 1999; West-Eberhard 2003; Price et al. 2003; Ghalambor et al. 2007; Lande 2009; Chevin et al. 2010). In contrast, novel environments that induce plasticity in a non-adaptive direction (i.e., away from the phenotypic optimum in the novel environment) will increase the strength of directional selection such that adaptive evolutionary responses must overcome environmentally induced shifts (Price et al. 2003; Grether 2005; Ghalambor et al. 2007; Conover et al. 2009; Morris and Rogers 2013; Ghalambor et al. 2015). A complementary possibility is that novel environmental conditions may reveal cryptic genetic variation, creating a range of unpredictable phenotypic diversity (both adaptive and non-adaptive) that was not visible to selection in the ancestral environment but potentiates a population’s ability to respond to selection in the novel environment (Rutherford 2000; Hermisson 2004; Schlichting 2008; McGuigan and Sgro 2009; Rohner et al. 2013; Paaby and Rockman 2014). Further, the underlying genetic architectures that give rise to plastic phenotypes may also confer greater evolutionary potential and facilitate population divergence (Fierst 2011; Espinosa-Soto et al. 2011; Draggi and Whitlock 2012). The conceptual framework for many of these views has historically been based in traditional population and quantitative genetic theory that postulates simple statistical relationships between genotypes and phenotypes. However, current research increasingly demonstrates that evolutionary divergence is not easily characterized by simple relationships between genetic variation and phenotypic innovations because the genotype–phenotype map is an emergent property of interacting networks influenced by genetics, environment, and stochasticity (Morris et al. 2014). Thus, there is a need for new perspectives and conceptual frameworks that can better predict how plasticity influences evolution.

The diverse roles of phenotypic plasticity in evolution continue to be vigorously debated from theoretical (de Jong 2005; Braendle and Flatt 2006; Ghalambor et al. 2007; Conover et al. 2009; Chevin et al. 2010), mechanistic (Kelly et al. 2012; Schlichting and Wund 2014; DeBiasse and Kelly 2015), and ecological (Robinson 1999; Agrawal 2001; Richards et al. 2006; Agrawal et al. 2007) viewpoints. In this perspective piece, we argue that examining molecular, physiological, and developmental networks underlying phenotypic variation will help derive general principles characterizing adaptation to novel environments. We highlight how shifts in such hierarchical networks during the process of adaptation may alter the relationship between plasticity and evolution across time scales. Below, we first discuss conflicting evidence largely from molecular studies for the role of adaptive versus non-adaptive plasticity in driving adaptation to novel environments. We then briefly summarize the extensive work characterizing the properties of biological networks and examine how robust networks respond via plasticity to either buffer or mediate organismal consequences of environmental variation, and how such responses drive phenotypic divergence both under historical and novel environmental conditions. We build on these ideas to consider how plasticity and divergent selection interact in novel environments and how network mechanisms differ across evolutionary time-scales (Fig. 1). We present a framework that considers how molecular and physiological networks influence adaptive evolution in novel environments across distinct time windows: (1) initial plastic responses during development, (2) short-term evolutionary change, and (3) long-term evolutionary change.

**Relationship between evolution and plasticity in molecular networks**

What should be the relationship between plasticity and evolution when populations are exposed to novel environments? How might this relationship vary over different time periods during adaptive evolution? Examining plasticity and evolutionary divergence in transcriptional and protein networks offers unique perspectives into these questions for several reasons. First, these networks represent the genetic, developmental, and homeostatic mechanisms acting to maintain integrated phenotypes and thus focus attention on how underlying mechanisms may constrain or contribute to phenotypic evolution. Second, unlike typical morphological, physiological, and behavioral traits, transcriptional and protein networks produce very large numbers of relatively easily measured molecular phenotypes that can provide more power in identifying general trends in the relationship between the direction of plasticity and evolution. A major drawback of these molecular phenotypes is that they are often further removed from correlates of fitness, posing challenges to infer the
fitness consequences of specific molecular traits without extensive research characterizing functional pathways and examining the phenotypic consequences of targeted manipulations. While this challenge is shared by gene and protein expression studies generally, these types of data are being increasingly and successfully applied to studies of adaptation, especially in situations in which likely fitness benefits of individual molecular phenotypes can be inferred (see example below).

A growing number of studies have taken advantage of transcriptome and proteome surveys to examine whether evolutionary divergence occurs predominantly in the molecular phenotypes that exhibit adaptive (i.e., plasticity in the same direction as evolved divergence) vs. non-adaptive (i.e., plasticity...
in the opposite direction as evolved divergence) plasticity (Fig. 2A), but with contrasting results. Several studies have found a positive relationship between the direction of evolutionary divergence in transcriptomes or proteomes and the direction of plasticity (e.g., Scoville and Pfrender 2010; Fraser et al. 2014; Shaw et al. 2014; Gleason and Burton 2015; Mäkinen et al. 2016). These studies capture a diversity of ecological conditions, only some of which likely act as physiological stressors. Scoville and Pfrender (2010) determined that population divergence in mRNA expression matched predator-induced plasticity in levels of a candidate gene for melanization in water fleas. Shaw et al. (2014) found that the small number of genes implicated in developmental responses to salinity and arsenic challenges also showed parallel expression changes in populations of killifish with greater salinity tolerance. Mäkinen et al. (2016) found an overwhelmingly positive relationship between the direction of plasticity and divergence in grayling proteomes in distinct thermal environments, as did Fraser et al. (2014) in comparisons of transcriptional signatures of alternative reproductive tactics in sailfin mollies. The results of these studies suggest that, in response to novel environments, initial plastic responses at the transcriptional or proteome level are adaptive and do a good job of predicting the direction of subsequent evolutionary change in these molecular phenotypes.

Conversely, other studies have found a negative relationship between the direction of plasticity and evolutionary divergence (Pespeni et al. 2013; Schaum and Collins 2014; Dayan et al. 2015; Ghalambor et al. 2015). Schaum and Collins (2014) conducted a laboratory selection experiment and found the plastic response to elevated pCO2 in green algae was to increase cell division rates, but the evolved response was to decrease cell division rates. Dayan et al. (2015) found a negative relationship between the direction of plasticity in gene expression in cold- vs. warm-acclimated fish and the direction of transcriptional divergence in cold- vs. warm-adapted fish. Ghalambor et al. (2015) found that the direction of evolutionary change in guppy brain gene expression in response to a novel environment lacking predators was in the opposite direction of the ancestral developmental plasticity in brain gene expression when animals were reared in the absence of predators (Fig. 2B). The results of these studies reveal that initial plastic responses are in the opposite direction of evolved responses, suggesting selection in novel environments acts most strongly on non-adaptive plastic responses, and that expression of these molecular phenotypes are likely to be the first to evolve (Ghalambor et al. 2015).

How might we reconcile these contrasting sets of results? Whether non-adaptive or adaptive plasticity dominate evolutionary transitions may depend on the extent to which fitness in the novel environment is severely compromised immediately after colonization. For example, if most colonists to a new environment have zero fitness, then the absolute fitness gain of adaptive plasticity in even a small number of individuals may enable population persistence (Chevin et al. 2010). Consequently, a signature of initial adaptive plasticity may remain present as a positive relationship between the direction of ancestral plasticity and the direction of subsequent adaptive evolutionary change. In contrast, if the novel environment is sufficiently benign such that most colonists have some fitness, then the relative fitness differences among individuals will determine the response to selection (Orr 2009). Under such conditions, a combination of adaptive and non-adaptive plastic responses will determine the distribution of fitness effects, and influence the net strength of selection. Specifically, traits exhibiting non-adaptive plasticity may be the first to exhibit evolutionary responses because such traits tend to be under stronger directional selection than traits exhibiting adaptive plasticity in relatively benign environments (Ghalambor et al. 2015).

A complementary hypothesis is that adaptive and non-adaptive plasticity both contribute to adaptive evolution in novel environments, but their importance varies across different time courses. Non-adaptive plasticity may be particularly important in the very earliest stages of adaptive divergence if directional selection acts predominantly on relative fitness differences, as mentioned above. In this scenario, traits exhibiting adaptive plasticity may be under relatively weaker selection initially and therefore respond more slowly to selection (Ancel 2000; Price et al. 2003), but may nonetheless contribute to compensatory changes in developmental and physiological processes that stabilize phenotypes, optimize phenotypic integration, and minimize antagonistic pleiotropy in the long term (discussed in more detail below).

These two hypotheses are not mutually exclusive: how benign the novel environment is and how much time since colonization might both contribute to the opposing findings that signatures of non-adaptive or adaptive plasticity dominate divergence in distinct evolutionary transitions. Unfortunately, we generally lack relevant data to assess the extent to which these hypotheses predict evolutionary dynamics in natural
The above studies did not report comparable measure of reproductive success in the novel environments, so we cannot assess directly whether signatures of adaptive plasticity are associated with adaptation to harsher environmental conditions. We propose that the alternative reproductive tactics described in Fraser et al. (2014) are one likely counter-example because the positive relationship between plasticity and divergence in brain gene expression is associated with relative differences in mating success rather than low absolute fitness. Further, time since colonization in the novel environment is rarely available except in experimental evolution scenarios. For example, Schaum and Collins (2014) report results of artificial selection after a known period of time (100 and 400 generations), and Ghalambor et al. (2015) is the only study to capture evolutionary changes in gene expression after the first few generations in a novel field environment.

The time since colonization hypothesis outlined above predicts a consistent temporal pattern of evolutionary divergence in gene and protein networks: traits with non-adaptive plastic responses are altered during the earliest stages of adaptation to benign novel environments (as explained above), and subsequent fine-tuning may modify diverse traits, including those with adaptive plastic responses. We tested this prediction by comparing our findings that non-adaptive plasticity potentiates rapid evolution of brain gene expression during the earliest stages of adaptation to low-predation environments (Ghalambor et al. 2015) with gene expression patterns in pairs of established high- and low-predation populations with longer divergence times (E. K. Fischer, K. A. Hughes, C. K. Ghalambor, K. L. Hoke unpublished data; Supplementary Materials).

Fig. 2 Adaptive and non-adaptive plasticity across time scales. Guppies (Poecilia reticulata) are small, live-bearing, freshwater fish native to the Island nation of Trinidad and Tobago and the adjacent South American mainland. Much ecological, evolutionary, and behavioral research has focused on guppies, in large part due to their remarkable ability to rapidly adapt to changing environmental pressures. In independent evolutionary transitions, ancestral guppies from high predation ancestors have colonized low-predation sites upstream in river drainages across northern Trinidad. Experimental introductions have mimicked those natural colonizations to chart early stages of evolution in novel habitats. Common garden experiments decompose natural variation into variation due to genetic differences among populations reared in the same habitat, and phenotypic differences due to rearing with exposure to chemical cues of predators. (A) Whether adaptive colonization events. The above studies did not report comparable measure of reproductive success in the novel environments, so we cannot assess directly whether signatures of adaptive plasticity are associated with adaptation to harsher environmental conditions. We propose that the alternative reproductive tactics described in Fraser et al. (2014) are one likely counter-example because the positive relationship between plasticity and divergence in brain gene expression is associated with relative differences in mating success rather than low absolute fitness. Further, time since colonization in the novel environment is rarely available except in experimental evolution scenarios. For example, Schaum and Collins (2014) report results of artificial selection after a known period of time (100 and 400 generations), and Ghalambor et al. (2015) is the only study to capture evolutionary changes in gene expression after the first few generations in a novel field environment.

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Fig. 2 Continued versus non-adaptive plasticity dominates adaptation is reflected in a positive (adaptive, black) or negative (non-adaptive, gray) relationship between direction of plasticity in the novel environment and direction of divergence during adaptation to that environment. (B) In artificial introduction experiments, genes that diverge after four generations in parallel in two independent introductions are those that exhibit non-adaptive plasticity in the ancestral population (gray symbols), suggesting non-adaptive plasticity drives adaptation in the short term (adapted from Ghalambor et al. 2015). (C) In contrast, in long established populations, genes that diverged in expression during evolution in the derived environment tend to be those that exhibit adaptive plasticity in the ancestral population (black symbols), suggesting adaptive plasticity contributes to long-term evolution (E. K. Fischer et al. unpublished data; see Supplementary Materials).
Consistent with the time since colonization hypothesis, we found that the negative direction of the relationship between plasticity and divergence at short time scales (Fig. 2B) is reversed when longer time periods are considered; evolutionary divergence in gene expression is positively correlated with plasticity in abundance of those transcripts in response to predator cues (Fig. 2C). While these results compare different populations evolving in similar environmental conditions, they are intriguing enough to have motivated us to consider the more general questions of (1) how complex hierarchical networks respond across developmental time to current environmental conditions, and (2) how those developmental trajectories change across evolutionary timescales. Most current empirical work on the role of plasticity in evolution—especially in natural populations—is restricted to long-term evolutionary timescales, examining populations that have adapted to distinct environmental conditions over many generations. While network and systems approaches are increasingly considered in the context of evolvability (Draghi and Wagner 2009; Garfield et al. 2013), few attempts have been made to link plasticity to evolutionary change within such a framework.

Network homeostasis during development: robustness and plasticity

The reliable production of a phenotype depends not only on the complex interactions between developmental, physiological, and biochemical mechanisms at different levels of biological organization, but also on how these mechanisms respond to environmental inputs and stochastic noise. Biological networks are characterized as being “robust” because of their ability to buffer environmental and mutational sources of variation during development (e.g., de Visser et al. 2003; Raman and Wagner 2011). The robustness of a network is based on processes that can broadly be classified as mechanisms that maintain homeostasis, in which feedback mechanisms maintain overall network output despite variability in individual network nodes. Homeostatic processes regulate the output of biological networks generally (e.g., gene, protein, cellular, organ, or neural networks), as compensatory changes exhibited by network components enable these networks to maintain network output despite environmental or genetic perturbations and despite stochasticity or noise in the system. For example, simulations predict that a wide range of neuronal parameters can produce nearly identical outputs given the basic design of the pyloric circuit in crustaceans (Prinz et al. 2004), and empirical work confirms consistent rhythmic outputs of pyloric circuits despite dramatic variation in individual neural components (e.g., ion channel abundances; Schulz 2006; Schulz et al. 2007) and synaptic strengths (Goaillard et al. 2009). Similarly, diverse patterns of metabolic gene expression predict consistent Fundulus heart metabolic phenotypes (Oleksiak et al. 2005). That diverse network states can give rise to consistent system output (i.e., consistent phenotypes) underlies the complexity of the genotype–phenotype map (Fig. 1B) and also complicates our ability to infer fitness consequences of individual molecular or physiological measures.

In theory, stabilizing selection should shape network structure to buffer organismal phenotypes from stochasticity and environmental variability via homeostatic processes. The network properties that confer developmental and environmental robustness, such as feedback or inhibitory feed-forward loops, also promote mutational robustness, in which biochemical networks maintain functional output despite mutations in individual genes (Masel and Siegal 2009; Siegal and Leu 2014). These network features complement other mechanisms that promote robust developmental processes such as chaperone proteins and microRNAs (Siegal and Leu 2014), all of which stabilize network output despite diverse network configurations, and thereby ultimately stabilize phenotypes. Thus, cryptic genetic variation accumulates because the phenotypic consequences of mutations are buffered for organisms developing under typical environmental conditions, both as a consequence of stabilizing selection for developmental robustness and as an intrinsic feature of complex regulatory networks (Rutherford 2000; McBride et al. 2008; Draghi et al. 2010; Wagner 2012; Paaby and Rockman 2014). This accumulation of cryptic genetic variation in turn increases evolutionary potential, as elegantly demonstrated in the context of RNA virus evolution (reviewed in Lauring et al. 2013).

If biological systems are characterized by redundant networks and homeostatic processes that buffer organismal-level phenotypes from mutations, stochasticity, and environmental noise, then how can these networks promote adaptive plasticity of organismal phenotypes in response to different environmental conditions? While robustness and plasticity need not be thought of as opposing each other (Siegal and Leu 2014), little empirical work has addressed this fundamental question due to the challenges of studying complex networks. If buffering or homeostatic mechanisms must be restricted in order
for adaptive plasticity to match organismal phenotypes to environmental conditions, plasticity may occur at the cost of developmental stability (Wolfe and Tonsor 2013). Alternatively, different environments may induce alternative “set points” for the homeostatic or feedback mechanisms and thereby promote robust phenotypic alternatives (Vinay and Reece 2013; Woods and Wilson 2015). The phenotypic variation that results from robust alternatives may manifest as either categorical differences (e.g., polyphenism, castes, etc.) or quantitative trait variation (e.g., any continuously distributed trait). The need to balance network robustness with exquisite sensitivity to the environment may have led to the evolution of developmental processes that are perched just at the edge of instability between alternative network states, each of which are robust (Hidalgo et al. 2014).

In sum, the complexity of the genotype–phenotype map encompasses interacting hierarchical networks that buffer against stochasticity while amplifying and coordinating responses to relevant environmental triggers throughout development to maintain integrated phenotypes. Natural selection has shaped both homeostatic processes and developmental mechanisms to reliably produce a range of environment-dependent phenotypes, but only within the range of environments historically experienced.

**Network evolution during early stages of adaptation to novel environments**

How can biological networks influence patterns of adaptive evolutionary change, given their capacity for developmental robustness, the build-up of cryptic genetic variation, and the ability to exhibit adaptive plasticity to environmental cues commonly encountered (Fig. 3A)? Theoretical and empirical work suggest that when organisms colonize novel environments outside the range to which the population is adapted, plasticity in the novel environment may release cryptic genetic variation (Rutherford 2000; Gibson and Dworkin 2004; Hermisson 2004; Schlichting 2008; McGuigan and Sgrò 2009; McGuigan et al. 2010). We can consider the release of such cryptic variation as a breakdown in the robustness of biological networks, but at the same time an opportunity for selection to re-shape the components of the network (Siegal and Leu 2014).

Novel environments induce diverse phenotypes in the earliest colonists, including both adaptive and non-adaptive outcomes that depend on individual genotypes, developmental experiences, and stochastic developmental noise (Fig. 3B). For example, sea urchins raised in pH conditions lower than their typical range preserve morphology and calcification by upregulating genes implicated in the relevant developmental processes at the cost of slowed developmental rate; however below a pH threshold, phenotypic buffering is incomplete and gene upregulation does not occur (Martin et al. 2011). Similarly, Rohner et al. (2013) demonstrated that the disruption of heat shock protein Hsp90 function in cave environments reduced its buffering capacity and revealed standing genetic variation in eye size that presumably led to the evolution of eyelessness in cave fish. While only a subset of novel environments will be stressful in ways that overwhelm generic buffering mechanisms (discussed broadly as evolutionary capacitors; Bergman and Siegal 2003; Masel and Siegal 2009; Masel and Trotter 2010), networks operating outside their typical range may nonetheless be generally more variable (Hermisson 2004; Draghi and Whitlock 2012), (Fig. 3B). Either a decreased capacity for homeostatic mechanisms to buffer phenotypic outcomes or novel gene–environment interactions may increase phenotypic variation and decrease integration of organismal phenotypes immediately after colonization of novel environments (Hermisson 2004; Draghi and Whitlock 2012; Rohner et al. 2013).

What are the consequences of the release of cryptic genetic variation for the earliest stages of adaptive evolution? The increased phenotypic variation in novel environments provides the variation among individuals necessary for selection to act on, amplifying the populations’ response to selection if that variation is heritable (Rutherford 2000; Paaby and Rockman 2014). However, the relationship between the release of this phenotypic variation and the path to adaptive evolutionary change could depend on the relationship between phenotypes and fitness. As discussed above, depending on the colonists’ ability to survive and thrive in the novel habitat, traits exhibiting adaptive plasticity may enable survival of only a subset of genotypes (e.g., in environments with extremely low absolute fitness) and/or non-adaptive plasticity may increase the strength of selection (e.g., in environments where relative fitness is most important). As a result, the expected increase in population-level phenotypic variation immediately following colonization due to variability among genotypes in plasticity in the novel environment is likely to decrease within the first few generations when directional selection in the novel environment is strongest (Fig. 3C). Mechanisms, such as epigenetic changes, that can act rapidly to bias network set points and stabilize phenotypes may be
particularly important during early stages of adaptation (Badyaev 2014; Schlichting and Wund 2014). Thus, we would predict the early stages of adaptation to a novel environment to be characterized by a breakdown in robustness of complex networks that leads to an increase in phenotypic variation and decrease in phenotypic integration for selection to act upon. High frequency of novelties and asymmetries in organismal phenotypes in early colonists suggest compromised network robustness, prompting comprehensive mechanistic studies such as those comparing connectivity patterns among gene regulatory networks or feedback responses in neuroendocrine systems in organisms experiencing novel and typical environments. As adaptation proceeds in the long term, selection will act to build new networks from the variation generated in the short term in order to re-establish patterns of integration, homeostasis, and robustness.

Network evolution during later stages of adaptation to novel environments

At longer evolutionary time scales, the proposed roles of plasticity in the earliest stages of adaptive evolution may no longer apply. In the long term, selection will favor compensatory genetic changes that act to reconfigure networks in a manner that fine tunes homeostatic mechanisms and reestablishes stable network set points that are flexible within the range of environments the derived population now experiences (Fig. 3D). Simulated gene regulatory network evolution demonstrates that a history of plasticity in gene network nodes confers greater additive genetic variance that influences the direction of evolution in the earliest stages of divergence in novel environmental conditions, but that this signature decays within ca. 20 generations (Draghi and Whitlock 2012). Whether signatures of ancestral plasticity typically remain in natural populations after generations of adaptive evolution in a novel environment is an open question, although several
Adaptive vs nonadaptive plasticity

Our work with guppies suggests that signatures of non-adaptive plasticity dominating the earliest stages of divergence are eclipsed at later stages, when other processes may promote the co-option of transcriptional plasticity (Fig. 2C). Why would a signature of processes that characterize gene and protein networks make it challenging to devise general predictions as to whether signatures of the original relationship between adaptive or non-adaptive plasticity and evolution in novel environments should persist after longer periods of evolution, and what other homeostatic and network factors might dominate patterns of long-term evolution.

Our work with guppies suggests that signatures of non-adaptive plasticity dominating the earliest stages of divergence are eclipsed at later stages, when other processes may promote the co-option of transcriptional plasticity (Fig. 2C). Why would a signature of adaptive plasticity characterize divergence at longer time scales of evolution if not present initially? We propose that the increased mutational variance that accompanies developmental plasticity in gene expression remains relatively stable and dominates later stages of adaptation to low-predation habitats, as predicted by Draghi and Whitlock (2012). At longer divergence times, many compensatory changes in developmental, biochemical, and physiological networks occur due to processes such as random drift and selection on both novel mutations and favorable combinations of ancestral genetic variation. These processes allow selection to act on more subtle differences in fitness on longer evolutionary time scales. In addition, continued selection in the novel environment can recalibrate homeostatic mechanisms and reshape network relationships, the extent of stochastic variation, and the environmental sensitivity of individual network components (Draghi and Whitlock 2012; Jones et al. 2014; Woods 2014). Empirical evidence for the long-term evolution of compensatory network mechanisms includes (1) comparing the network states (e.g., covariances among levels of interacting proteins, or the variability in network outputs) among populations in different environments or across time during adaptation, and (2) detailing evolution of feedback mechanisms themselves. For example, human populations adapted to living at high altitudes share some common organismal and physiological adaptations that are consequences of different mutations in a few key regulatory pathways (Beall 2006; Alkorta-Aranburu et al. 2012; Huerta-Sanchez et al. 2013). Mutations in Tibetan populations may change the set points of homeostatic processes that underlie ancestral acclimation to high altitudes but are maladaptive for long-term residents (Storz 2010; Storz et al. 2010). Selection may thus alter the relationships among network components (Siegal and Bergman 2002; Draghi and Whitlock 2012; Jones et al. 2014; Shaw et al. 2014), as alternative network configurations that yield similarly favorable phenotypes may nonetheless be subject to distinct trade-offs. Network plasticity, homeostasis, and stochasticity will therefore differ across developmental and evolutionary time scales, with important consequences for phenotypic integration and evolution.

Conclusions and future prospects

In sum, the role of plasticity in evolution is influenced by a diversity of processes that operate across developmental and evolutionary time scales. We have presented a mechanistic framework in which hierarchical networks change during development in response to rearing environments, and shift over short and long divergence times to shape phenotypic diversity and adaptation to novel environments. We argue that developmental plasticity and evolution of hierarchical networks may differ between the early and long term stages of adaptation to a novel environment, as networks reestablish robustness while simultaneously accommodating phenotypic divergence. The few empirical studies of network plasticity rarely capture the earliest stages of adaptation to novel environments, or compare initial and longer time scales of evolution. Integrating the network mechanisms that confer robustness and flexibility in phenotypic outputs into the empirical and theoretical debate will allow us to better understand the relationship between plasticity and evolutionary divergence. While this hierarchical network view of the genotype–phenotype map may be a more realistic representation of how organisms develop and how complex traits evolve, it also raises fundamental challenges to our current theoretical models and statistical analyses. The phenotypic and fitness consequences of individual changes in network components remain difficult to interpret, and we caution researchers to consider carefully their assumptions about the relationships between individual mutations, transcriptomic or proteomic network responses, and the organismal phenotypes that
Supplementary data
Supplementary data available at ICB online.

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