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Introduction

Understanding the origin of biological variation is one of the principal goals of biology. The modern synthesis provided an explanation for the evolution of biological diversity by reconciling Mendel's genetic mode of inheritance with Darwin's theory of natural selection (Mayr and Provine 1998). Crucial to the many successes of this synthesis was a focus on the gene as the sole means of inheritance but also as the primary determinant of phenotypes themselves. As a consequence, evolution is commonly studied in terms of genetic variation, yet what we hope to understand is the evolution of traits (Lewontin 1974; West-Eberhard 2003). The limits of an approach that links genes and traits in a one-to-one relationship through a linear and deterministic genotype-to-phenotype map are becoming clear in light of the wealth of investigations that can explain only a small portion of observed trait variation (Manolio et al. 2009; Zuk et al. 2012; Boyle, Li, and Pritchard 2017). At the same time, the study of phenotypic plasticity has re-emerged and offers novel avenues by which to study the causes of trait variation and evolution. The field has shifted its focus away from early debates on, for example, the relative merits of alternative means of characterizing phenotypic plasticity, and whether there exist genes for plasticity (for review see Via et al. 1995). Instead, current theory on phenotypic plasticity incorporates

review see Via et al. 1995). Instead, current theory on phenotypic plasticity incorporates specific patterns of environmental heterogeneity (Snell-Rood et al. 2010; Van Dyken and Wade 2010), genetic characteristics of populations (Crispo 2008; Ledon-Rettig et al. 2014), trait genetic architectures (Snell-Rood et al. 2010; Anderson et al. 2013; Des Marais, Hernandez, and Juenger 2013), and historical and contemporary patterns of selection (Masel 2006; Ghalambor et al. 2007; Wund et al. 2008; Ghalambor et al. 2015).

We suggest that we are on the cusp of integrating these diverse factors into a framework that predicts how environmentally induced variation interacts with genetic variation to influence traits and evolutionary trajectories. Here, we discuss the potential of this new framework in shifting phenotypic plasticity from a distraction or nuisance to a paradigm in evolutionary biology. First, we examine the still prevailing view in which traits emerge from a genetic blueprint. We suggest that evidence from association and population

genetics as well as evolutionary rates highlights the limits of such a view. We devote the majority of this chapter to the theoretical role of phenotypic plasticity in promoting adaptive evolution and shaping evolutionary mechanisms and trajectories. Then, we highlight a limited number of empirical cases that illustrate these theoretical mechanisms. Finally, we conclude by outlining avenues to address outstanding questions and providing an example of how better integrating phenotypic plasticity into our understanding of evolution provides new insights that strengthen evolutionary hypotheses and help resolve long-standing controversies.

Limits of the Genotype-to-Phenotype Map

One of the central organizing principles of the modern synthesis is the direct relationship between genotype and phenotype, in which variation in traits emerges as a direct consequence of information that is stored and inherited at the level of genes. This relationship between genes and traits, or a genetic blueprint, is often framed in terms of a simplifying heuristic referred to as the genotype-to-phenotype map. While contemporary biology has a more nuanced understanding of trait development, the concept of a genotype-to-phenotype map permeates biological thinking and is the core assumption underlying many fields of research (Orgogozo, Morizot, and Martin 2015). As a consequence, causal priority in evolutionary research is often given to natural selection among genetic variants that influence the distribution of traits in a population with less importance given to other factors that determine trait variation, such as phenotypic plasticity and developmental processes.

The utility of the genotype-to-phenotype map heuristic is evident in the number of population and quantitative genetic dissections of evolutionary-relevant traits such as coat coloration in mice (Linnen et al. 2013), lateral plate armor in stickleback (Cresko et al. 2004), wing patterning in *Heliconius* (Joron et al. 2011), flowering time in model plants such as *Mimulus guttatus* and *Arabidopsis thaliana* (Ausin, Alonso-Blanco, and Martinez-Zapater 2004), or the gap gene network in *Drosophila* (Jaeger 2011). Increasingly, however, the limits of this gene-centric view are becoming clear. While it is often a useful heuristic, the genotype-to-phenotype map is not a real attribute of biological organisms. Much of the phenotypic variation in nature is difficult to explain with an analytical framework that views genetic variation as the primary determinant of trait variation. We provide three perspectives drawn from largely independent fields to support this assertion.

Perhaps the most striking evidence comes from the quantitative trait nucleotide (QTN) or genome-wide association study (GWAS) program of research (Rockman 2012). The goal of this massive research effort is to identify the causal factors that underlie phenotypic variation by uncovering statistical associations among genetic variants and traits. While researchers have discovered thousands of such associations, we remain unable to explain the majority of variation even in extensively studied and highly heritable traits (Visscher

et al. 2012; Boyle et al. 2017). For example, 60 to 80 percent of schizophrenia risk is heritable, which suggests that the genetic component of variation in the schizophrenia phenotype is very large, yet the causal variants discovered by GWAS can explain just 3 percent of this heritable variation (Schizophrenia Working Group of the Psychiatric Genomics 2014). Similarly, the genetic variants significantly associated with height, a complex trait that has been the subject of recent natural selection across many human populations (Stulp and Barrett 2016), explain only 20 percent of the heritable variation in this trait (Wood et al. 2014). Some explanations of this so-called missing heritability problem assume the genotype-to-phenotype map is a true aspect of organisms that can be uncovered, and that missing heritability stems from methodological shortcomings. Either we are simply querying the wrong type of genetic variation by focusing on common variant, ignoring structural variation, rare effect variants, microRNAs, and others (Manolio et al. 2009; Eichler et al. 2010), or the disconnect stems from a systematic over-estimation of trait heritability (Kumar et al. 2016).

However, an alternative set of explanations posit that the genotype-to-phenotype map is instead highly complex and dynamic; many genes contribute to traits and their effects vary across environments, genomic context, and ontogenetic and evolutionary time, and it is this complexity that creates the gap between explained and observed heritability. Under one such hypothesis, most traits have a nearly infinitesimal genetic architecture, that is, traits are determined by thousands, or tens of thousands of genetic loci, each with small individual impacts, making their detection statistically intractable (Rockman 2012; Wellenreuther and Hansson 2016; Shi, Kichaev, and Pasaniuc 2016). Under a second, not mutually exclusive hypothesis, non-additive, but heritable effects such as epistasis and interactions between genes and environments (Zuk et al. 2012; Bloom et al. 2013), as well as epigenetic inheritance (Bourrat, Lu, and Jablonka 2017) significantly contribute to trait variation, leading to a gap between narrow and broad sense heritability. These explanations call into question the utility of the genotype-to-phenotype map as a metaphor, because the map is either too complex or too dynamic to be interpretable and predictive.

Shifting focus from the production of phenotypes to their evolution, we observe a similar pattern: with the advent of next-generation sequencing, we have begun to identify the genetic variation that contributes to adaptive evolution in natural populations (Vitti, Grossman, and Sabeti 2013; Hoban et al. 2016), but with the exception of oligogenic traits, we have only limited understanding how this genetic variation is translated into adaptive variation in phenotypes. For example, in humans, natural selection has driven genetic differentiation among populations (Hancock, Witonsky et al. 2010; Wollstein and Stephan 2015), and this differentiation occurs within genomic regions associated with complex traits that may themselves be adaptively significant (Turchin et al. 2012). Yet, for most traits examined, differentiation at the complex trait-associated genomic regions does not appear to have been driven by selection on the trait itself (Zhang et al. 2013; Berg and Coop 2014). Therefore, even as new techniques in genomics have vastly increased the

These observations, drawn from diverse fields (association genomics, quantitative genetics, and population genomics, respectively) cumulatively point to a relationship between genes and traits that is highly nonlinear, complex, and dynamic. Not only do thousands or more genetic loci commonly contribute to a trait but their impacts frequently depend on interactions with other loci and the environment. Therefore, while genetic variation may provide the primary basis of heritability, our current ability to empirically determine the genotype-to-phenotype map places limits on the utility of genetic variation as a predictor of trait variation or evolutionary trajectories. This is not to say that future efforts to uncover the relationship between genetic variation and evolutionarily significant trait variation are futile, nor do we wish to diminish the importance of the results of past investigation under the genotype-to-phenotype map framework. However, we suggest, as have others (e.g., Schlichting and Pigliucci 1998; Pigliucci 2001; West-Eberhard 2003; Sultan 2015), that previous efforts have largely ignored the central role of the environment in determining phenotypes, and that better incorporating the environment into our understanding of the production of traits has the potential to provide new insights.

Consider how incorporating the environment into our understanding of the production of traits has the potential to ameliorate or address each of the three limits outlined above.

(i) If the environment interacts with genetic variation in a non-additive fashion, then we would expect the causative variants discovered by GWAS to explain only a portion of heritable variation in phenotypes, because these gene-by-environment interactions lead to a disconnect between broad and narrow-sense heritability (Flint and Mackay 2009; Zuk et al. 2012). Understanding how such gene-by-environment interactions operate may provide an answer to the missing heritability problem (Eichler et al. 2010; Marigorta and Gibson 2014), yet the primacy of the gene in previous research efforts has meant that neither the frequency, nor the genetic architecture of gene-by-environment interactions in complex traits is clear (Des Marais, Hernandez, and Juenger 2013), despite a long recognition of their evolutionary potential (Waddington 1953; Bradshaw 1965).

(ii) Accumulating evidence suggests that novel or rare environments not only expose genetic variation that does not usually contribute to traits but that the amount of heritable genetic variation for traits increases in these environments (McGuigan et al. 2011; Rokholm et al. 2011; Kuttner et al. 2014; Parsons et al. 2016), for reviews see (Ledon-Rettig et al. 2014; Paaby and Rockman 2014). Future work that considers the role of the environment in generating and releasing this cryptic genetic variation may explain rapid evolution of traits that appear to have limited evolutionary potential under current environmental conditions (Paaby and Rockman 2014) or the origin of novelties and major evolutionary transitions (Moczek et al. 2011).

(iii) Gene-centric approaches to studying adaptation, such as genome scans for signatures of selection, have identified regulatory variation as a major target of natural selection. At the same time, research investigating the role of regulatory variation among species and

populations within ecologically relevant environmental contexts has led to novel insights into trait production and evolution (e.g., Abouheif and Wray 2002; Wellmer and Riechmann 2010; Schneider, Meyer, and Gunter 2014). Findings from these and related fields (e.g., eco-evo devo) are only beginning to be incorporated into broader evolutionary thought (Abouheif et al. 2014; Gilbert and Epel 2015), but they have the potential to make the connections among genetic variation and traits that would prove impossible to decipher without explicit consideration of the environment.

Evolutionary Implications of Phenotypic Plasticity

The brief discussion above highlights some of the limitations of a gene-centric view of the production and evolution of traits and introduces how increased consideration of the environment may offer novel insights. In this section, we summarize the body of theory that hypothesizes how environmental sensitivity, or phenotypic plasticity, influences evolutionary trajectories.

Genetic variation in phenotypic plasticity, usually viewed as the statistical notion of a gene-by-environment interaction (GxE), is commonly observed at the trait level (Schlichting and Pigliucci 1998). The consequences of GxE are twofold. First, by providing the variation for plasticity on which selection can act, GxE permits the evolution of plasticity itself (Pigliucci 2001). Perhaps more importantly, the existence of GxE means that genetic variation is expressed differently across environmental contexts (Bradshaw 1965). Traditional perspectives hold that phenotypic plasticity functions in evolution only to shield genotypes from selection, thus reducing the effectiveness of directional selection and slowing adaptation (S. Wright 1931; Jong 1995; Orr 1999). Yet, there has been a revival of theoretical and empirical research, based on early models (Baldwin 1896; Morgan 1896; Schmalhausen 1949; Waddington 1953), indicating that phenotypic plasticity can play a major role in shaping evolutionary trajectories because it mediates the relationship between selection and genetic variation. This effect is only part of the broader body of evolutionary theory concerning plasticity, but we choose to focus here.

The mechanisms by which phenotypic plasticity can potentially influence evolution fall into three broad categories depending on whether phenotypic plasticity: (i) reduces the cost of selection in novel environments and allows populations to persist; (ii) determines the phenotypes on which selection can act, guiding the pattern of evolution through genetic accommodation *sensu* (West-Eberhard 2003); or (iii) alters the genetic variation available to selection. These mechanisms have been hypothesized to play a role in many evolutionary phenomena with varying levels of empirical support, including adaptation to new environments (Ghalambor et al. 2007), the evolutionary origin of complex traits and novel innovations (Moczek 2008; Moczek et al. 2011; Schlichting and Wund 2014), and the divergence of specialized ecotypes, speciation and adaptive radiations (Pfennig et al. 2010;

Thibert-Plante and Hendry 2011; Fitzpatrick 2012; Schneider and Meyer 2017). The mechanisms are briefly reviewed below.

Much of the theory concerning the role of phenotypic plasticity in evolution is focused on whether adaptive plasticity allows populations to persist in novel environments or after an environmental shift. As a consequence, phenotypic plasticity is often viewed as a phenomenon promoting adaptive evolution because it reduces the cost of selection (*sensu* Haldane 1957) after an environmental shift and allows populations to persist and subsequently adapt through either new mutations or selection on the standing genetic variation (Baldwin 1896; Robinson and Dukas 1999; Price, Qvarnström, and Irwin 2003; Chevin and Lande 2011). For example, in the model of Chevin and Lande (2010), a population with a partially adaptive plastic response can maintain higher population size relative to a population without plasticity, reducing the probability of extinction and therefore increasing the rate of evolutionary adaptation. Extending these predictions to scenarios of a heterogeneous environment with gene flow produces similar results. Gene flow from populations in the ancestral habitat reduces mean fitness and imposes a selection cost in the population experiencing the new conditions, but this cost is offset by adaptive plasticity (Chevin and Lande 2011; Thibert-Plante and Hendry 2011). From this perspective, phenotypic plasticity flattens the adaptive landscape *sensu* (Simpson 1944) and provides a valley crossing mechanism, allowing populations to explore adaptive peaks other than the local optimum (Price, Qvarnström, and Irwin 2003; Frank 2011). This role of plasticity in promoting adaptive evolution may be especially important for populations with high rates of migration and gene flow from ancestral habitats (Sultan and Spencer 2002; Crispo 2008) and for traits with moderately beneficial plasticity (Price, Qvarnström, and Irwin 2003).

After populations are exposed to new environmental conditions, phenotypic plasticity can influence evolutionary trajectories through genetic accommodation (West-Eberhard 2003). This mechanism views "phenotypes as leaders and genes as followers in evolution" (West-Eberhard 2003); novel phenotypes arise in a population, either because of new mutations or exposure to different environmental conditions, and are subsequently refined by selection through quantitative genetic changes at many loci. If the phenotypic variants arise because environmental changes, genetic accommodation can have a major influence on the rate of adaptive evolution because the emergence of the phenotype occurs within a single generation at high frequency in the population and across diverse genetic backgrounds (West-Eberhard 2003). Thus, populations do not need to wait for the emergence of a single adaptive mutation to arise, which would be vulnerable to stochastic loss (Orr 2005).

Instead, because the environment plays a role in both the production of and selection on a trait, adaptation can occur through quantitative genetic changes from the standing genetic variation that adaptively refines the regulatory architecture of the trait's expression (Pfennig et al. 2010; Moczek et al. 2011; Wund 2012; Ehrenreich and Pfennig 2015). This refinement (accommodation) is possible because plastic phenotypes frequently have

complex genetic architectures that not only provide many genetic targets for selection but are also likely to exhibit substantial genetic variation owing to their conditional expression (Windig, De Kovel, and Jong 2004; Aubin-Horth and Renn 2009; Hodgins-Davis and Townsend 2009; Snell-Rood et al. 2010; Van Dyken and Wade 2010) and because plasticity may lead to a bias among genetic effects along the axis of adaptive phenotypic variation (Draghi and Whitlock 2012; Lind et al. 2015). Furthermore, phenotypic plasticity promotes increased genetic variation in populations experiencing novel conditions because adaptive plasticity can increase gene flow from other populations (Crispo 2008; Colautti and Barrett 2011).

Genetic accommodation of environmentally sensitive phenotypes can have several outcomes depending on how the regulation of the phenotype is altered. A loss of plasticity or decreased threshold of induction for the phenotype such that it is constitutively expressed in the new environment may occur if the plastic response is costly to produce or alternate environments are rare, that is, genetic assimilation (Waddington 1953; Pigliucci, Murren, and Schlichting 2006). Alternatively, selection may favor enhanced phenotypic plasticity in the direction of the trait optimum. In this case, accommodation reduces the costs and limits of phenotypic plasticity (Suzuki and Nijhout 2006; Murren et al. 2015). Finally, not all phenotypic plasticity is adaptive. In fact, maladaptive phenotypic plasticity that arises as a consequence of passive responses to the environment by biological molecules may be the primary form of plasticity (Van Kleunen and Fischer 2005; Schulte, Healy, and Fangue 2011). Thus, genetic accommodation may function to reduce the effects of maladaptive phenotypic plasticity, such that local adaptation of populations through accommodation leads to the stabilization of phenotypes across heterogeneous environments (Ho and Zhang 2018). This pattern is frequently observed in nature and is termed countergradient variation because the directions of plastic and genetic effects on traits are in opposite directions with respect to the environment (Conover and Schultz 1995). Genetic accommodation of this form is distinguished from others under the term genetic compensation (Grether 2005). Genetic compensation may be particularly relevant to the role of plasticity in promoting divergence, because it establishes reproductive barriers to gene flow between locally adapted populations (Fitzpatrick 2012).

In the final mechanism, phenotypic plasticity is predicted to impact evolutionary adaptation because it promotes the accumulation and release of genetic variation (Gibson and Dworkin 2004; Hermisson and Wagner 2004; Le Rouzic and Carlborg 2008; McGuigan and Sgro 2009; Paaby and Rockman 2014). Phenotypic plasticity contributes to an increase in genetic variation in two ways. First, homeostatic mechanisms exerted through phenotypic plasticity might buffer the effects of new mutations, reducing genetic constraints. The best studied instance of this phenomenon is the heat shock protein Hsp90's function as a "genetic capacitor" (Rutherford and Lindquist 1998). Thermal induction of Hsp90 canalizes the outcome of protein folding during gene expression and stabilizes protein populations in the cell, thereby simultaneously reducing the effects of environmental and

genetic perturbation and promoting the accumulation of genetic variation by reducing the efficacy of purifying and/or stabilizing selection (Queitsch, Sangster, and Lindquist 2002). Such genetic capacitors may be common (Sangster et al. 2008; Chen et al. 2013). In a second, more pervasive effect, phenotypic plasticity may lead to the conditional expression of genetic variation across space and time such that some genetic variation has an effect in only a subset of individuals or populations (Snell-Rood et al. 2011; Des Marais, Hernandez, and Juenger 2013). This conditional expression can result in relaxed selection in the non-inducing environment and an increase in polymorphism at these loci because purifying selection can only remove deleterious alleles in the subset of individuals experiencing the inducing conditions (Kawecki 1994; Lahti et al. 2009; Snell-Rood et al. 2010; Van Dyken and Wade 2010). Conditional expression of genetic variation may also lead to the evolution of developmental systems that bias the effects of both the standing genetic variation and novel mutants along the axis of plasticity, and ultimately, adaptive phenotypic variation (Draghi and Whitlock 2012).

The genetic variation that accumulates by these mechanisms is studied under the phenomenon of cryptic genetic variation (CGV) (Gibson and Dworkin 2004; Hermisson and Wagner 2004; Paaby and Rockman 2014; Paaby and Gibson 2016). While such variation is simply part of the standing genetic variation, it is considered cryptic when the inducing conditions are uncommon in the evolutionary history of the species. The release of CGV is likely to have a major impact on the rate of evolution because it may be accompanied by an increase in heritable variation of phenotypes under rarely encountered or novel conditions where selection may be strongest (i.e., genetic variance; Waddington 1953; Hoffmann and Merila 1999; Rokholm et al. 2011).

In many ways the concepts of evolution via cryptic genetic variation and genetic accommodation are closely linked. While discussion of CGV in the literature is typically framed in terms that give causal priority to the role of genes in trait production and evolution, the core assumption underlying CGV is that the environment determines how and which genetic variants contribute to a trait. In this way, CGV can be thought of as a population-genetic and quantitative genetic account of how evolution might be thought to proceed in a scenario where phenotypes are leaders, not followers during evolutionary transitions. There are, of course, aspects of genetic accommodation, and plasticity-led evolution more generally, that are not adequately encompassed by cryptic genetic variation. However, in this chapter we focus on how cryptic genetic variation can be leveraged as a bridge for exploring the impacts of genetic accommodation using the empirical tools of population genomics and quantitative genetics. For example, an important aspect of genetic accommodation of an environmentally induced trait is the simultaneous induction of the trait across many members of a population. This prediction is paralleled by the phenomenon that alleles that form the basis of cryptic genetic variation can be present at intermediate frequencies in a population because they are conditionally neutral before the onset of selection (Fry, Heinsohn, and Trudy 1996). Furthermore, the increased genetic variance provided by the

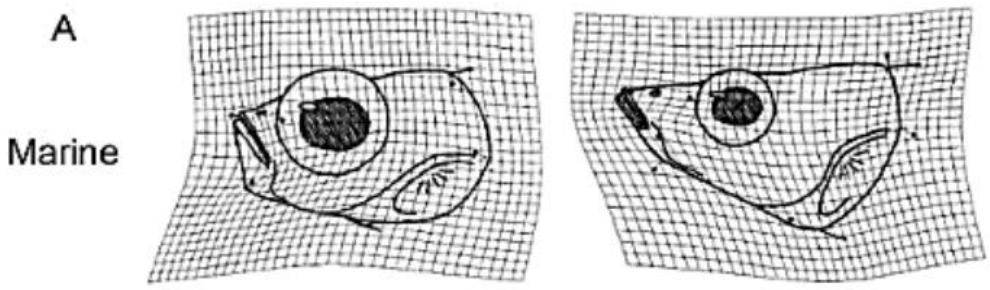
convincing, as plasticity is "ancestral" (was present prior to colonization of novel host plants) and was expressed as clearly adaptive phenotypes.

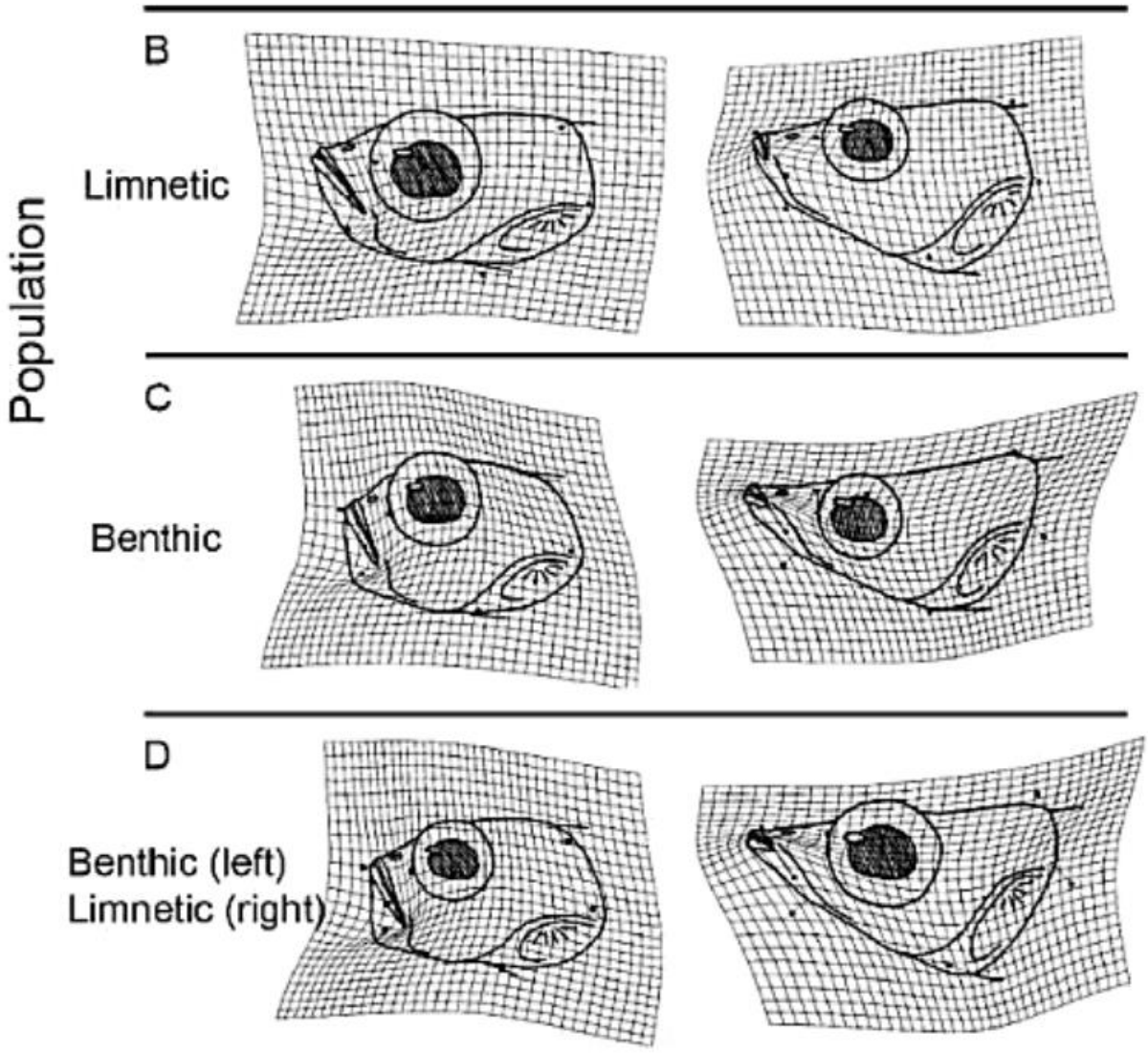
A number of studies offer evidence that plasticity can alter the array of phenotypes available to selection, and hence have potentially altered the direction of evolution through genetic accommodation (point [ii] above). We elaborate two examples we find compelling that are largely compatible with the hypothesis. The first involves phenotypic plasticity of morphology in threespine stickleback—research that also highlights aspects of evolution via genetic accommodation (Wund et al. 2008, 2012). Laboratory-reared oceanic ("ancestral") stickleback exhibit modest but appropriate patterns of plasticity (figure 5.1) when raised in environments simulating those at the extremes of a benthic-limnetic foraging axis (Wund et al. 2008, 2012). This ancestral plasticity parallels the evolutionary transitions made by benthic and limnetic freshwater ecotypes, transitions that have evolved repeatedly and independently in derived freshwater populations. This pattern suggests that an ancestral "flexible stem" (*sensu* West-Eberhard 2003) might have influenced the direction of evolutionary change in the adaptive radiation in freshwater, promoting the environmental match. Further, the patterns of induced phenotypic plasticity in freshwater populations (figure 5.1) differ from those in oceanic populations under the alternative laboratory rearing conditions, demonstrating genetic accommodation (Wund et al. 2008; Wund 2012). Collectively, these studies suggest both that the direction of evolutionary change could have been mediated by plasticity and that genetic accommodation has occurred.

A particularly intriguing example of the way in which plasticity could have influenced subsequent evolution involves the major evolutionary transition in vertebrates from aquatic to terrestrial locomotion. *Polypterus senegalus*, an extant analogue of the fish likely to have given rise to tetrapods, exhibit different limb development when raised in an aquatic environment versus a moist terrestrial environment. In a terrestrial environment, these fish use their fins differently than in the aquatic environment, and they develop limb anatomical characteristics that are similar to those observed in stem tetrapod lineages during the Devonian Period (Standen, Du, and Larsson 2014). Thus, plasticity could well have influenced the ultimate shape and function of tetrapod limbs by altering the range of phenotypes, and underlying patterns of gene expression exposed to selection in the novel, terrestrial environment.

Using Population Genomics to Address the Plasticity Hypothesis

There is substantial evidence that adaptive phenotypic plasticity readily evolves (Schlichting and Pigliucci 1998; Pigliucci 2001; DeWitt and Scheiner 2004) and is often a component of adaptive divergence (Torres-Dowdall et al. 2012; Dayan et al. 2015; Kenkel and Matz 2016). Additionally, a great wealth of studies demonstrate how environmental variation




Population
B
Limnetic
C
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D
Benthic (left)
Limnetic (right)

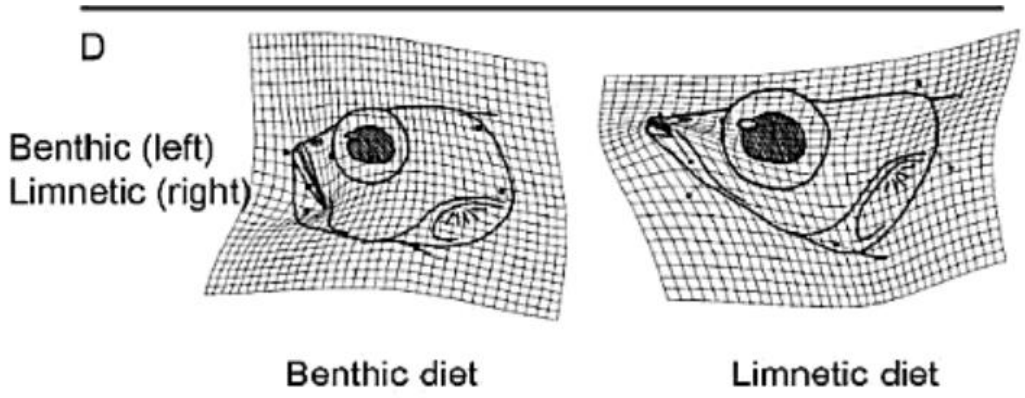

Figure 5.1
Head shape plasticity with respect to food type (benthic items vs. limnetic plankton) in oceanic (ancestral surrogate), limnetic (freshwater plankton feeding ecotype), and benthic (freshwater benthic feeding ecotype) threespine stickleback raised in the laboratory. Deformation grids are based on consensus landmark positions for each experimental group and are exaggerated 4x to make differences more apparent. Benthic diet left column, limnetic diet right column. From Wund et al. (2008).

integrates with developmental process to produce variation in evolutionarily relevant phenotypes (reviewed in Abouheif et al. 2014). However, despite the rich theoretical framework surrounding phenotypic plasticity, empirical evidence pointing to the role of plasticity in directing evolution varies among the models outlined above (see Schlichting and Wund 2014; Levis and Pfennig 2016 for recent reviews). There is suggestive evidence that (i) phenotypic plasticity can promote population persistence in novel or marginal environments (Réale et al. 2003; Yeh and Price 2004; Amarillo-Suárez and Fox 2006; Geng et al. 2006; Orizaola and Laurila 2016), but see (Davidson, Jennions, and Nicotra 2011), that (ii) ancestral plasticity mirrors adaptive divergence (Losos et al. 2000; Gomez-Mestre and Buchholz 2006; Rajakumar et al. 2012) and (iii) that genetic variance increases when current environmental conditions are rare in a population's history (Hoffmann and Merila 1999; Ledón-Rettig, Pfennig, and Crespi 2010; McGuigan et al. 2011; Takahashi 2015; Rowinski and Rogell 2017). Yet many other questions remained unanswered. Below, we discuss how contemporary and emerging techniques in quantitative genetics and population genomics can be leveraged to address some of these outstanding questions.

First, *to what extent does the environment determine genetic architecture of adaptive traits?* In quantitative genetic terms, the extent to which traits are determined by constitutively versus conditionally expressed genetic variation has profound implications for the origin and maintenance of biological variation (Colautti, Lee, and Mitchell-Olds 2012; Paaby and Rockman 2014). Where trait variation is mediated by the same genes across environments (i.e., antagonistic pleiotropy or allelic sensitivity), phenotypic plasticity can slow the rate of adaptation because of pleiotropic constraints (Scarcelli et al. 2007) or drive divergence by establishing reproductive barriers to gene flow (Kawecki and Ebert 2004). On the other hand, conditionally-expressed variation leads to relaxed selection and polymorphism accumulation at conditionally-expressed loci because genetic variation will only be subject to selection in a subset of individuals (Snell-Rood et al. 2010; Van Dyken and Wade 2010). Understanding the extent to which the environment determines which genes matter for evolution provides much needed inference into the relative importance of genetic variation and the environment as causal forces in evolution. At one extreme, the genetic architecture of a trait is static among the environments experienced by a population across time or space. In this case, it is clear how genetic variation can be said to cause the trait variation that is subject to selection and conceptual frameworks such as the genotype-to-phenotype map will be sufficient for evolutionary inference. At the other extreme, the genetic variants that contribute to a trait may completely be determined by the environment. In this case, a clear causal relationship between genes and traits cannot be drawn without invoking the environment, because it is the environment that determines both the distribution of phenotypes and the genetic variants that selection acts on.

Theoretical research into this topic is thorough, but the extent to which it is predictive has yet to be addressed across diverse study systems and at a genome-wide perspective. Recombinant mapping crosses of highly divergent ecotypes or crop varieties suggest that

different genes influence traits across environments (Anderson et al. 2013; Des Marais et al. 2013; Parsons et al. 2016), but aspects of these study populations might predispose them toward conditional expression (Kassen 2002; Hall, Lowry, and Willis 2010; Colautti et al. 2012), leading to ascertainment bias. Recent results in quantitative genetics (Pavličev and Cheverud 2015; Wood and Brodie 2015) point to a greater role for conditional neutrality than antagonistic pleiotropy, and some experimental evolution results suggest that selection acts on conditionally expressed variation (Sikkink et al. 2015), but relatively little is known about the influence of the environment on genetic architecture in highly outbred, large natural populations that are key to our understanding of ecologically relevant mechanisms of evolution. Application of emerging techniques in population genomics and quantitative genetics across environments in such organisms provide promising avenues to address the role of the environment in shaping genetic architecture of ecologically relevant traits. These include pedigree-free estimation of trait heritability (Stanton-Geddes et al. 2013) and polygene-trait association techniques such as regional heritability analysis (Berenos et al. 2015), chromosome partitioning (Santure et al. 2015), and machine learning–based classification and regression algorithms (Brieuc et al. 2018).

Second, *how common is genetic accommodation in nature* (Schlichting and Wund 2014; Levis and Pfennig 2016)? Does plasticity determine evolutionary trajectories? There are ample examples of parallelism between adaptive divergence and patterns of adaptive phenotypic plasticity within a lineage (Losos et al. 2000; Gomez-Mestre and Buchholz 2006; Wund et al. 2012). This suggests that divergence is promoted by high levels of phenotypic plasticity (Pfennig et al. 2010), but these studies do not establish a causal relationship between divergence and plasticity (Kovaka 2017). In order to avoid this pitfall, studies investigating genetic accommodation often rely on comparisons of phenotypic plasticity between derived and extant ancestral populations (Yeh and Price 2004; Wund et al. 2008; Badyaev 2009; McCairns and Bernatchez 2010; Aubret 2015), or cases in which the ancestral state of plasticity can be inferred phylogenetically (Rajakumar et al. 2012). These studies suggest indirectly that adaptation through genetic accommodation may be common, because phenotypic divergence in derived groups mirrors ancestral plasticity. However, the distinguishing feature of genetic accommodation is the initiation of phenotypic diversity followed by refinement through selection (West-Eberhard 2003). Phenotypes are the leaders, not the followers in evolution by genetic accommodation. Therefore, comparisons between populations or species after the onset of selection cannot definitively demonstrate genetic accommodation, because they do not reveal how the phenotype in question arose (Wund 2012; Kovaka 2017). For example, evolution of plasticity in derived lineages may be the result of relaxed selection at genetic loci underlying plastic traits, and not vice versa (Hunt et al. 2011; Leichty et al. 2012).

More direct evidence of genetic accommodation in nature can be inferred from studies that comprehensively assess the evidence of genetic accommodation in natural populations by simultaneously characterizing the genetic and molecular mechanisms underlying plastic

traits while demonstrating adaptive refinement of the traits by selection. For example, work in the horned beetles of the genus *Onthophagus* has revealed in detail how the modularity of developmental plasticity has permitted the reutilization of conserved developmental processes to produce an evolutionarily novel trait and how the emergence of this trait has contributed to diversification within the lineage (Moczek 2009; Snell-Rood et al. 2011).

While such detailed analyses of adaptive traits provide strong direct evidence that plasticity-led evolution can shape evolutionary trajectories for a given trait in a particular lineage, they cannot address the broader significance of genetic accommodation in nature because the intensive nature of this study limits the number of experimental systems in which the question can be addressed. Similarly, experimental evolution studies also provide a strong test of genetic accommodation when demonstrable genetic accommodation of introduced experimental populations mimics the evolved differences in plasticity between ancestral and derived natural populations and the molecular mechanisms underlying the plastic phenotype in both the experimental and natural populations are shared. Such analyses were recently completed by Ghalambor et al. (2015) and Ho and Zhang (2018), but similarly appropriate natural study systems may be too rare to provide easily generalizable examples (Ehrenreich and Pfennig 2015).

We propose that alternative approaches that leverage the power of contemporary population genomic tools provide complementary evidence that can discriminate traditional gene-centered models of evolution and evolution by genetic accommodation. These approaches are readily extendable to non-model systems where little is known. The cryptic genetic variation framework, in particular, provides a link between empirically available evidence, in the form of gene-trait associations and genomic signatures of selection, and the higher order theory of genetic accommodation (Levis and Pfennig 2016). In the case of ancestral-derived comparisons, for example, future studies should strive to address whether cryptic genetic variation is not only released under environmental conditions experienced by the derived populations, but that this cryptic genetic variation is under selection and contributes to adaptive variation in plastic traits in derived populations. If phenotypes are leaders rather than followers during adaptation, genetic variants that conditionally contribute to the focal trait will be enriched for signatures of selection over variants that contribute to the trait in the ancestral environment. In this way, the signature of selection left on genomic variation is a distinctive empirical trace, *sensu* Kovaka (2017), which can be used to discriminate between adaptive traits that emerge via genetic mechanisms or via environmental induction. Until recently, researchers pursuing this research question are faced with the limitation of working with traits for which the mechanisms and underlying genetic architecture are either well-known or experimentally tractable through QTL mapping or association analysis. Such integrated approaches have already suggested that genetic accommodation may shape variation at single loci underlying known adaptive traits (Parsons et al. 2016; Pespeni, Ladner, and Moczek 2017). However, the

(N) in new environments by reducing the cost of selection when plastic responses are adaptive under the new conditions (Chevin and Lande 2010). Phenotypic plasticity also increases the mutational target size, thereby increasing μ, because traits with phenotypic plasticity are likely to have more complex genetic architectures than more constitutively expressed traits (Sultan and Stearns 2005; Moczek 2008). The most profoundly affected of these parameters, however, is likely to be R_a. As discussed above, phenotypic plasticity may be due to conditional expression of genetic variation (Des Marais et al. 2013), leading to accumulation of polymorphism due to conditional neutrality and relaxed selection in the ancestral environment (Snell-Rood et al. 2010; Van Dyken and Wade 2010). Depending on the frequency with which the population has been historically exposed to the new environmental conditions, the fitness effects of genetic variation accumulated under conditional trait expression may not be symmetrically distributed around zero. If environmental conditions are sufficiently rare or entirely novel in the species' evolutionary history, this body of variation may be enriched for deleterious variants, including fixed lethal variants (Kawecki 1994), thereby reducing R_a. On the other hand, previous bouts of purifying selection on this variation during prior exposures can lead to an enrichment of adaptive alleles (Masel 2006). Taken together, it is possible that phenotypic plasticity increases R_a because it reduces deleterious effects of polymorphism in ancestral environments and increases adaptive effects in the new environment. Similarly, evolution of plastic

ments and increases adaptive effects in the new environment. Similarly, evolution of plastic developmental systems can lead to a bias toward adaptive effects of genetic variation (Draghi and Whitlock 2012).

We believe this discussion encapsulates why it is crucial to deepen the integration between the environment and the genotype in our understanding of the production and evolution of traits. From a traditional perspective, the empirical evidence of the role of standing genetic variation in evolutionary adaptation is contentious because it calls into question the utility of many of our best-understood models. Yet, when one appreciates that the environment may be central in determining the genetic architecture of traits and the patterns of genetic variation among and within populations, selection on the standing genetic variation becomes an intuitive component of adaptation.

Conclusion

It is increasingly clear that a gene-centric view of trait development is limited in its power to describe the diversity of phenotypic variation and therefore evolution. Better incorporating phenotypic plasticity into evolutionary thinking offers a potential solution, not only because the environment plays a critical role in determining traits, but because the theoretical development surrounding phenotypic plasticity has provided novel directions for inquiry. Empirical research investigating these questions is accumulating, but much about plasticity-led evolution remains unknown. In this chapter, we have attempted to highlight

how the concept of cryptic genetic variation provides a connection between readily available empirical evidence and theoretical predictions about the role of plasticity in evolution that complements and extends a more developmental, trait-focused approach. We suggest that other investigators should similarly focus on diversifying the set of empirically testable hypotheses that address extent to which plasticity-led evolution is a widespread phenomenon in nature.