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SYMPOSIUM

Cryptic Genetic Variation in Natural Populations: A Predictive Framework

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Synopsis Understanding how populations respond to rapid environmental change is critical both for preserving biodiversity and for human health. An increasing number of studies have shown that genetic variation that has no discernable effect under common ecological conditions can become amplified under stressful or novel conditions, suggesting that environmental change *per se* can provide the raw materials for adaptation. Indeed, the release of such hidden, or "cryptic," genetic variants has been increasingly viewed as playing a general and important role in allowing populations to respond to rapid environmental change. However, additional studies have suggested that there is a balance between cryptic genetic variants that are potentially adaptive in future environments and genetic variants that are deleterious. In this article, we begin by discussing how population and environmental parameters—such as effective population size and the historical frequency and strength of selection under inducing conditions—influence relative amounts of cryptic genetic variation among populations and the overall phenotypic effects of such variation. The amount and distribution of cryptic genetic variation will, in turn, determine the likelihood that cryptic variants, once expressed, will be adaptive or maladaptive during environmental transitions. We then present specific approaches for measuring these parameters in natural populations. Finally, we discuss one natural system that will be conducive to testing whether populations that vary in these parameters harbor different amounts, or types, of cryptic genetic variation. Generally, teasing apart how population and environmental parameters influence the accumulation of cryptic genetic variation will help us to understand how populations endure and adapt (or fail to adapt) to natural environmental change and anthropogenic disturbance.

Introduction

Transitions to new environments are associated with phenotypic diversification in many taxa. For example, the spectacular adaptive radiations of Darwin's finches, Hawaiian silversword plants, and East African Rift Lake cichlids are all thought to have occurred when a single ancestral lineage encountered novel environmental pressures (reviewed in Schluter 2000). Environmental transitions also characterize anthropogenic disturbance: Species introductions often result from transport by humans, and habitat destruction leads to alterations in species interactions and their access to resources. How do populations adapt to such changing conditions?

Traditional models of evolution assume that, when a new phenotype is favored by a change in the environment, populations must rely on standing genetic variation (Barrett and Schluter 2008), mutation, recombination, and/or hybridization/gene flow to generate novel genetic variants that are necessary for producing the newly favored phenotype (Futuyma 2013). Standing genetic variation has been shown to predict short-term evolutionary responses to moderate environmental change (Barton and Keightley

2002), whereas novel mutations have traditionally been thought to underlie macroevolutionary phenotypic changes. If substantial phenotypic change is needed to respond to drastic and rapid environmental change, the waiting time for adaptive genetic variants to arise in a population can be prohibitively long, and the probability of subsequent loss through drift is often high (under the assumption that only a few individuals will originally carry these new genetic variants) (Phillips 1996). However, it has often been noted that when a population encounters a novel or stressful environment, additive genetic variance (population-level phenotypic variation underlain by genetic variation) actually increases. That is, the effects of individual genetic variants on a trait, which may be nominal under typical environmental conditions, increase substantially in the novel environment (Hoffman and Merilä 1999). Thus, an alternate view of how populations adapt to rapidly changing conditions suggests that the environment itself influences the phenotypic expression of genetic variation, thereby providing the raw material for adaptation to occur (Gibson and Dworkin 2004). Because such genetic variance is not observed under typical environmental conditions, it is referred to as ''cryptic'' genetic variation.

That cryptic genetic variation can fuel evolutionary change was first demonstrated by Waddington's (1953) seminal laboratory experiments with *Drosophila*. Waddington observed that when *Drosophila* pupae were exposed to heat shock, a few individuals consistently developed wings as adults lacking an otherwise characteristic vein (a condition called ''crossveinless'') (Waddington 1953). He used this phenomenon to determine whether a trait that was initially environmentally induced could become fixed in a population. In one set of selection experiments, Waddington heat-shocked pupae each generation and selected either individuals that developed the crossveinless phenotype or random individuals (the latter served as a control group). In the crossveinless group, the percentage of individuals developing the crossveinless phenotype increased in frequency until nearly all individuals developed the phenotype when exposed to heat shock as pupae. Remarkably, by the 23rd generation, it was no longer necessary for individuals to be heat shocked in order to produce the crossveinless phenotype; all individuals developed the crossveinless phenotype when raised under normal conditions, a phenomenon Waddington referred to as ''genetic assimilation.''

Two aspects of Waddington's *Drosophila* experiments provided critical evidence for the evolutionary potential of cryptic genetic variation. First, the number of generations necessary for the crossveinless phenotype to go to fixation in the population was too few for novel mutations of large effect to arise. Second, when Waddington's graduate student, K.G. Bateman, repeated the experiment with isogenic flies, she found that the same phenomenon could not be replicated in such a genetically depauperate population (Bateman 1959). These results suggested that selection of preexisting (i.e., standing) genetic variation, which had no phenotypic effect under typical environmental conditions, led to the genetic assimilation of an originally environmentally induced phenotype; that is, the expression of trait was resistant to subsequent environmental variation. In the years since Waddington and Bateman's experiments, cryptic genetic variation has been revealed in several, diverse laboratory systems (e.g., *Caenorhabditis elegans*, *Manduca sexta*, *Arabidopsis*, yeast, and ribozymes) (True and Lindquist 2000; Queitsch et al. 2002; Suzuki and Nijhout 2006; Félix and Wagner 2008; Hayden et al. 2011). Thus, ample evidence suggests that, in principle, cryptic genetic variation can play a major role in trait evolution during environmental transitions.

Despite such compelling evidence from laboratory populations, we still have a poor idea of the availability or role of cryptic genetic variation in *natural* populations. We also lack information on how often cryptic genetic variation fuels or inhibits adaptation during environmental change. Quantitative genetic studies can be used to infer cryptic genetic variation in natural populations by estimating changes in additive genetic variance across environments (Paaby and Rockman 2014). Although a handful of studies using this approach have helped fill this gap (Auld 2010; Ledón-Rettig et al. 2010; McGuigan et al. 2011), these studies were conducted using single populations, and it is unclear whether such findings can be generalized to other species or even other populations within the same species that experience different environmental and population parameters.

In this article, our goal is to develop a predictive framework for determining relative amounts of cryptic genetic variation among populations and the overall phenotypic effects of such variation. To do so, we first discuss the best-studied phenomenon leading to the accumulation of cryptic genetic variation: Conditional trait expression (Kawecki 1994; Van Dyken and Wade 2010). We then outline parameters that have been used to model either the amount or nature of cryptic genetic variation that accumulates in populations, and describe how these parameters might be determined empirically in

natural populations. Finally, we describe a system that will be useful for assessing whether these parameters predict levels and phenotypic effects of cryptic genetic variation in natural populations. Our goal is to encourage field studies of cryptic genetic variation that span multiple populations and environmental conditions, and thereby uncover general principles governing the nature of cryptic genetic variation and the conditions that influence the likelihood that such variants, once expressed, will be adaptive or maladaptive.

Origins of cryptic genetic variation

Conditional trait expression

Processes that result in the accumulation of cryptic genetic variation have been discussed in depth elsewhere (Paaby and Rockman 2014). Here, we focus on one key process—conditional trait expression—but only in enough detail to illustrate how this process might vary among populations.

Conditional trait expression—the situation in which only a fraction of individuals in a population express a particular trait—often arises when individuals change their phenotype in direct response to different environmental conditions; that is, when individuals exhibit phenotypic plasticity (West-Eberhard 2003). It is thought that when phenotypically plastic traits change in value (i.e., modified body size in response to salinity), they are expressing (phenotypically) different genes or the same genes at different levels. Conditional trait expression may also occur when individuals express different phenotypes owing to sex-limited genes (e.g., maternal effect genes or male-specific genes in facultatively sexual species; Chasnov and Chow 2002; Brisson and Nuzhdin 2008; Cruickshank and Wade 2008). Conditional trait expression should foster the accumulation of genetic variation for the simple reason that variants influencing the values of conditionally produced traits are only exposed to selection when phenotypically expressed. By contrast, in situations where these traits are not expressed, variants influencing the traits should experience relaxed selection. Thus, relative to genes underlying constitutively expressed traits (where selection has the greatest potential to remove genetic variation), we expect a higher degree of mutation accumulation and polymorphism in genes underlying conditionally expressed traits (Kawecki 1994; Snell-Rood et al. 2010; Van Dyken and Wade 2010), as the cumulative effects of selection are relatively weak. Therefore, all else being equal, the degree of mutational accumulation in genes underlying conditionally expressed traits should be inversely correlated with the frequency of their expression among individuals or across generations.

Thus far, we have described conditional gene expression as if genes are either ''on'' or ''off'' throughout the lifetime of an individual. This, of course, is rarely the case, as genes may vary in the degree of their expression over different environments or life stages. Describing the consequences of variation in gene expression levels is an active area of research, both conceptually and empirically, and we discuss the implications of variable gene expression patterns for cryptic genetic variation in our conclusions and future directions.

Empirical support for the predictions from natural systems

Is there any empirical support for the prediction that the degree of mutational accumulation (via relaxed selection) in genes underlying conditionally expressed traits is inversely correlated with the frequency of trait expression? Recently, researchers have found support for this prediction in natural populations in which the environmental conditions that trigger a phenotypic response are novel or rarely encountered. For instance, although most anuran tadpoles will opportunistically consume invertebrates (such as fairy shrimp), those of certain species of spadefoot toads (genus *Scaphiopus*) rarely have the opportunity to consume the shrimp that co-occur in their ponds, possibly owing to an antipredator behavior that other predaceous species elicit (tadpoles of another spadefoot genus, *Spea*, have the potential to develop as large, cannibalistic morphs; Ledón-Rettig and Pfennig 2012). Thus, in populations in which these tadpoles have historically experienced recurrent predation pressure, they have also experienced relaxed selection on traits involved with consuming shrimp. Relaxed selection on such traits should, in turn, lead to the accumulation of cryptic genetic variants. As predicted by the theory, tadpoles from these populations that are fed shrimp (a rare diet) express greater genetic variance in resource-use traits than those fed a standard diet of detritus (Ledón-Rettig et al. 2010). Similarly, McGuigan et al. (2011) found that marine sticklebacks reared in low salinity (a novel environment) express high genetic variance for body size. These authors speculated that such environmentally dependent genetic variance may have provided the raw materials for the repeated evolution of a smaller body size in sticklebacks colonizing postglacial freshwater lakes.

Environments need not be entirely *novel* to reveal hidden genetic variation. As discussed above, relaxed selection occurs as a by-product of any plastic trait (whether the trait is expressed in only a fraction of individuals or possible generations; Van Dyken and Wade 2010). For instance, freshwater snails (*Physa acuta*) express two types of reproductive modes: Selfing when predators are present or outcrossing when predators are absent (Auld 2010). In one population, the more common reproductive scenario is one with mates, and thus genetic variation expressed under these conditions was expected to be more quickly depleted than genetic variation expressed under selfing conditions (Auld 2010). Indeed, Auld (2010) found that genetic variance was higher in reproductive traits when these snails were exposed to cues from predators (that induce selfing conditions) than in those same traits in the absence of such cues.

Finally, the patterns of genetic variance underlying plastic traits in natural populations might actually reveal the nature of the selective pressures on those populations. For instance, Gomez-Mestre et al. (2008) looked at genetic variance in hatching rates when *Bufo americanus* eggs were ''induced'' to hatch by water mold infection. They found that the level of genetic variance in hatching rate was less in the presence of the water mold. In light of evidence that the frequency of infected clutches was high at their field site (Gomez-Mestre et al. 2006), the authors concluded that water mold infection was actually the more common environmental condition (as opposed to an infection-free environment), and that molds had imposed historical selection on hatching in these tadpoles.

To summarize, the expectation that conditionally expressed traits engender the accumulation of cryptic genetic variation has been corroborated in natural populations by studies that link higher genetic variance with novel, rare, or fluctuating environmental conditions (but for examples in which dampened genetic variance is associated with rare environments, see McGuigan and Sgro 2009).

Parameters that influence cryptic genetic variation and its effects

The aforementioned examples are informative, because they demonstrate that cryptic genetic variation can accumulate (and be subsequently expressed) in natural populations under the expectations of conditional trait expression (Kawecki 1994; Van Dyken and Wade 2010). If this is a general trend in natural populations, selection on such variation may, in part, explain instances of rapid adaptation to novel or changing environments (assuming that some of the alleles are indeed adaptive). Nonetheless, these studies assessed cryptic genetic variation in single populations, and populations vary in demographic and environmental parameters that might influence both the amount of genetic variation accumulated and the distribution of the fitness effects of the mutations when they are expressed. In particular, the same process that leads to the accumulation of genetic variants that are adaptive in future environments (relaxed selection) can lead to the increased maintenance and fixation of deleterious mutations, which might impair the function and evolution of the trait in question (Kawecki 1994; Masel 2006). For instance, mutational accumulation in genes with male-limited expression in the 99.9% hermaphroditic *C. elegans* has resulted in poor mating efficiency of males relative to closely related dioecious species (Chasnov and Chow 2002). Likewise, mutations that accumulated under relaxed environmental conditions have been implicated in reduced fitness under stressful conditions in wild radish (Roles and Conner 2008), *Drosophila* (Shabalina et al. 1997), and *Daphnia* (Schaack et al. 2013). Therefore, it is important to determine whether, based on the demographic parameters and evolutionary history of a population, the adaptive potential of cryptic genetic variants accumulated in conditionally expressed traits outweighs their mutational load.

In the next section, we focus on two parameters that likely influence the probability that conditionally expressed genetic variance will play a role in the direction and speed of the evolution of future traits: (1) effective population size and (2) the historical pattern of selection on the variants under inducing conditions.

Effective population size

The effective population size (N_e) of an observed population reflects the number of individuals it would possess if it met the genetic assumptions of a randomly mating, idealized population (Wright 1931, 1940). For various reasons (e.g., historical bottle necks and skewed sex-ratios due to biases in production of offspring, viability, and non-random mating), a population's effective size is often considerably smaller than its census size. The effective population size influences standing genetic variation through genetic drift, the loss of genetic variants by random sampling (Nei et al. 1975). Although genetic variants that differentially influence fitness can be lost due to selection(s), both neutral and non-neutral genetic variants can be lost due to drift. In particular,

small populations experience larger stochastic changes in gene frequencies, and are therefore more vulnerable to drift (specifically, drift outweighs selection when $s < [2N_e]^{-1}$) (Crow and Kimura 1970).

How do effective population size and its relationship with genetic drift influence conditionally expressed genes? Similar to what we would expect for neutral genetic polymorphism, the amount of conditionally expressed genetic variation (which is neutral while it is hidden) is expected to increase with population size (Masel 2006). However, when genes are only conditionally expressed, they are neutral for a fraction of the time, and therefore subject to drift (Van Dyken and Wade 2010). Thus, the property of being "hidden" from selection protects conditionally expressed genes from removal by selection, and also makes them more susceptible to genetic drift. In simulations, the increased influence of drift on conditionally expressed genes is manifested as an increase in variance of mean allele frequency across replicated simulations (Van Dyken and Wade 2010). Although the effect of drift on conditionally expressed genes while they are hidden is not predicted to be substantial for populations with $N_e > 10^3$, in very small populations the loss of polymorphism through drift might reduce the amount of cryptic genetic variation available for future environmental change, and the fixation of potentially deleterious mutations might increase (i.e., the "drift load") (Van Dyken and Wade 2010).

To summarize, populations harboring a conditionally expressed trait should generally possess greater levels of cryptic genetic variation for that trait, but this amount should be disproportionally less in populations with a small N_e as a result of genetic drift.

Historical patterns of selection

Another parameter that will shape cryptic genetic variation in natural populations is the historical pattern of selection on that variation when it is expressed; specifically, the frequency and magnitude of selection. The historical pattern of selection is a function of both the frequency with which a population has historically encountered the inducing environment and the strength of selection on otherwise hidden variants when they are exposed (Masel 2006; Van Dyken and Wade 2010). Below, we discuss the challenges with empirically disentangling these two aspects of selection. Here, we note that, generally, the historical pattern of selection is an important parameter to quantify because it influences both the amount of cryptic genetic variation in a population as well as the distribution of the resulting phenotypic variation when it is expressed.

The frequency with which a trait is expressed in a population (ϕ) can be signified as either the fraction of individuals encountering the inducing environment or the fraction of generations over time experiencing the inducing environment: each situation produces approximately the same degree of relaxed selection on genes underlying that trait (Van Dyken and Wade 2010). This means that, as ϕ decreases, the range of phenotypic effects caused by expressed cryptic genetic variation should increase. By contrast, as ϕ increases, the range of phenotypic effects caused by expressed cryptic genetic variation should decrease. That is, rarely expressed genes should harbor the most polymorphism, which could translate into greater mutational load when those alleles are deleterious in future environments, or greater evolutionary potential when those alleles allow populations to reach new adaptive peaks.

The second aspect of the historical pattern of selection—the *strength* of selection on genes underlying conditional traits while they are expressed—will influence both the amount and distribution of cryptic genetic variation under subsequent inducing conditions (Eshel and Matessi 1998). If a population has experienced the same inducing environment in the past, *and* that inducing environment exerted a selective pressure on the resulting phenotypic variants, deleterious alleles will be reduced in frequency, leaving the pool of cryptic genetic variants enriched with neutral and adaptive variants (Eshel and Matessi 1998). Thus, in situations where cryptic genetic variation is exposed under recurrent environmental conditions, the distribution of fitness effects will have less variance, but the mean value of these effects will be shifted along the axis of selection (Eshel and Matessi 1998; Paaby and Rockman 2014).

How does the historical pattern of selection influence the probability that some cryptic genetic variants will be adaptive under changing environmental conditions? Theoretically, when the history of selection with an inducing environment has been either rare or weak, the genetic variation exposed by that environment will have a wide range of effects, but will be centered symmetrically over the mean trait values in the ancestral environment; essentially, relatively little of the exposed variation will be "preadapted" to the new environment (Eshel and Matessi 1998). Nevertheless, this type of variation might be critical for populations that need to reach globally higher fitness peaks that are very different from the ancestral fitness peak (Van Dyken and

Wade 2010). By contrast, if a population's history of selection with an inducing environment is frequent or strong, the phenotypic variation exposed by that environment will be more limited, but will deviate from the original phenotypic mean in a direction that is adaptive, meaning that more of the expressed variation will be seemingly preadapted to the environmental shift. This type of distribution has greater potential to fuel adaptive evolution to recurrent, fluctuating environments or even drastic environmental changes as long as they favor phenotypes along the axis of previous selection, but has less potential to cope with environmental changes that favor phenotypes in any other direction (Eshel and Matessi 1998).

Thus far, we have assumed that the inducing environment is qualitatively similar every time it is experienced, and that selection imposed by the inducing environment favors a similar phenotype. However, in some instances, generic buffering systems evolve that suppress the expression of genetic variants across most developmental or environmental variation (reviewed by Paaby and Rockman 2014). These buffering systems store cryptic genetic variation until it is exposed by *any* extreme environmental deviation, and can therefore be subjected to different selection pressures. Thus, a third potential component of historical patterns of selection on cryptic genetic variation is the direction of selection. However, what effect random selection will have on the subsequent distribution of phenotypes generated by cryptic genetic variation is largely an open question (see the "Conclusions and future directions" section).

How do we measure these parameters?

Although effective population size and the historical pattern of selection might determine how cryptic genetic variation influences the direction and speed of evolution, no study, to our knowledge, has attempted to quantify these parameters in conjunction with estimates of cryptic genetic variation. Below, we suggest methods for quantifying these parameters in natural populations.

Effective population size

Effective population size is generally indirectly estimated from patterns and frequency of molecular polymorphism and population heterozygosity, assuming neutrality (Fu 1994; Wang 2005; Li and Stephan 2006; Charlesworth and Charlesworth 2010). The heterozygosity (i.e., amount of genetic variation) at neutral loci is determined by the balance between the influx of mutations versus the loss of variants due to stochastic sampling (genetic drift). Mutation rates can be determined empirically, or by comparing patterns of sequence variation with closely related species. Variation at such neutral loci can be measured empirically by quantifying the number of segregating nucleotide sites among sequences, the average number of nucleotide differences between sequences, or the number of alleles among a number of DNA sequences (Wang 2005). For a diploid population at drift–mutation equilibrium, heterozygosity at a neutral locus is modeled as $4N_e\mu$, where μ is the mutation rate. In simple situations, when mutation rate has been independently estimated, the effective population size can be inferred by dividing the estimated heterozygosity by 4μ. When the mutation rate is unknown, we can at least obtain relative measurements of effective populations sizes by comparing different populations (Wang 2005). Estimating N_e from current heterozygosity and other estimated molecular population-genetic parameters from genomic data is becoming increasingly common (Haddrill et al. 2005; Li and Stephan 2006; Thornton and Andolfatto 2006; Charlesworth 2009). Such methods can be used not only to estimate current N_e, but also, to some extent, patterns of drift, bottlenecks, and selection.

Historical patterns of selection

As mentioned previously, the historical pattern of selection on a population under inducing conditions will be an amalgamation both of the frequency of the inducing environment and the strength of selection on phenotypes revealed by the inducing environment. These two aspects can be teased apart in models, but they are hard to differentiate empirically. In some cases, as with fine-scaled climatic data, differences in strength and frequency can be dissociated. For instance, if drought is the inducing environment of interest, reconstructions of tree-rings can be used to determine how often the inducing environment has occurred (frequency of selection) and how severe it has been when it did occur (strength of selection) (Herweijer et al. 2007). However, with more complex selective factors (e.g., intensity of predation), the frequency and strength of the selecting environment may be necessarily collapsed into one variable ($\phi = s$). Although this simplification may make the relationship between the historical pattern of selection and cryptic genetic variation noisier, some basic expectations might still be met; for example, a population experiencing frequent induction *or* strong selection whereas induced might harbor

cryptic genetic variation that is less variable and aligned with the axis of selection.

A second factor to consider when choosing a proxy for the historical pattern of selection is the generation time of the organism. Specifically, the proxy should have a record that is long relative to the organism's generation time, such that there is sufficient opportunity for mutations to accumulate. Environmental records that span timescales that are short relative to the organism's generation time are unlikely to predict amounts or distributions of cryptic genetic variation. More accurate and fine-grained information (e.g., as obtained from instrumental temperature records) might be available for organisms with short generation times, whereas organisms with longer generation times might benefit from more long-term environmental data (e.g., paleoclimatic data).

There are several methods for inferring abiotic environmental trends that both expose phenotypic variation in, and impose selection on, populations. Human-generated records, such as records of insecticide applications, species counts (e.g., the Christmas bird count) and the instrumental temperature record, often are detailed, but only extend, at most, a few hundred years into the past. Dendrochronology (the study of tree rings, usually taken from cores) also yields valuable environmental data such as an area's history of wildfires, precipitation, and temperature, and has the potential to span thousands of years into the past (Herweijer et al. 2007). Likewise, sediment cores taken from lakes can yield thousands of years of information about an area's fire history (from charcoal content), tree cover, and species composition (from pollen content), and the lakes invertebrate community assemblage (from invertebrate remains) (Dunwiddie 1987; Millspaugh and Whitlock 1995; although see Rautio et al. 2000). Finally, paleoclimatologists use a combination of these proxy methods, plus others such as isotopes from rocks and icesheets, to model climatic changes over longer periods of time (e.g., 20,000 years before the present) (Richards et al. 2007).

All of these methods have the potential to give researchers information about abiotic inducing and selecting environments, but what do we do when the selecting pressure is biotic, such as selection caused by species interactions? Understanding the historical ranges of species can be difficult, especially when the fossil record is poor or absent. One option is to use ecological niche modeling in conjunction with paleoclimatic data to determine historical ranges of species (e.g., Walker et al. 2009) and, in turn, infer historical interactions between species. For niche modeling, we rely on observed distributions of species in combination with fine-scaled information on current climatic variables such as precipitation, isothermality, and temperature, to develop a model of their ecological niche (Austin 1985). To determine the species' *historical* distribution, we project the ecological model (based on current climatic variables) onto models of past climate (Carstens and Richards 2007; Richards et al. 2007). This approach has the potential to reveal how organismal ranges have changed over time. Thus, if trait expression in one species is conditional on the presence of another species (i.e., trait-mediated indirect interactions) (Werner and Peacor 2003), such paleoclimatic modeling can shed light on the inducing and selective environment for that trait.

A system for testing predictions in nature

Spadefoot toad species of North America promise to be a useful system for assessing how cryptic genetic variation might vary across populations of different sizes and selective histories. Spadefoots are found throughout the continental United States, and different populations experience distinct ecological conditions. For example, the larvae differ in exposure to predation. Interestingly, this predation pressure is exerted on spadefoot larvae by other spadefoot larvae. Tadpoles of the genus *Spea* have the potential to develop into a distinctive, large-mouthed ''carnivore'' morph. Carnivores feed mostly on fairy shrimp, but they also prey on other tadpoles. In contrast, tadpoles of the genus *Scaphiopus* (which do not produce carnivores) are potential prey of *Spea*.

Carnivorous *Spea* tadpoles tend to inhabit the center of ponds (where the highest concentrations of shrimp prey are located). When they occur in these same ponds, *Scaphiopus* tadpoles choose microhabitats at the edges of ponds (Ledón-Rettig and Pfennig 2012), presumably minimizing the risk of predation from *Spea*. However, in doing so, these *Scaphiopus* tadpoles limit themselves from consuming shrimp (Ledón-Rettig and Pfennig 2012). This conditional behavior has allowed them to change the selective pressures on some of the traits that they express (Price et al. 2003, Ledón-Rettig et al. 2012). In areas where these tadpoles have historically experienced a predator regime, they have experienced relaxed selection on traits involved with consuming shrimp, which, in turn, has lead to the accumulation of cryptic genetic variation in trophic traits

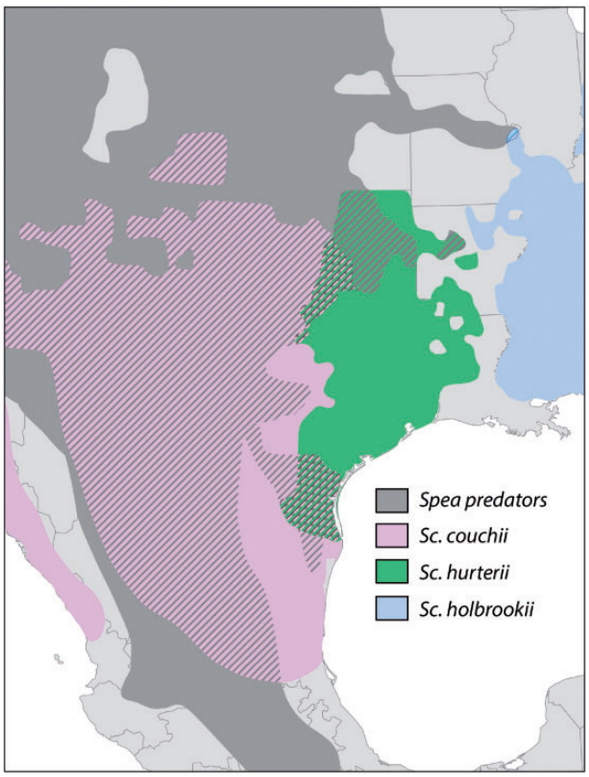

Fig. 1 Species and populations of *Scaphiopus* vary in whether they coexist with predaceous *Spea* species (all species in dark gray). For instance, *Sc. holbrookii* (blue) occurs east of the Mississippi River and never experiences predation pressure from *Spea*. In contrast, *Sc. hurterii* (green) and *Sc. couchii* (purple) exist in populations that are both allopatric (solid colors) and sympatric (mixed colors) with predaceous *Spea*.

associated with the consumption of shrimp (Ledón-Rettig et al. 2010).

However, populations and species of *Scaphiopus* vary in whether they currently coexist with predators upon larvae (Fig. 1), and might also vary in how long they have coexisted with such predators. The previously described niche-based historical-distribution modeling methods can be used to estimate each *Scaphiopus* population or species' history with *Spea* species, and thereby provide an estimate of their historical strength of selection with respect to predators. Additionally, heritabilities of trophic traits related to the consumption of shrimp, which are only expressed in the absence of predators, can be estimated using quantitative genetic methods. In conjunction with effective population sizes (estimated using population genomics), such approaches will allow us to determine the relative contributions of population size and historic patterns of selection on conditional genetic variance.

Conclusions and future directions

To predict population responses to environmental change, we need to understand levels both of constitutive and conditionally expressed genetic variation. Conditionally expressed genetic variation, especially in situations in which such variation is commonly hidden (cryptic genetic variation), has become largely accepted as a reservoir of variation that can allow populations to adapt to novel environmental change. However, the amount of, and balance between, variants that are adaptive or deleterious in the new environment will depend both on population size and on historical patterns of selection in the inducing environment, parameters that have not yet been measured alongside cryptic genetic variation in natural populations.

If we measure these parameters, what kinds of relationships would we expect them to have with cryptic genetic variation? Cryptic genetic variation should be greatest for traits that are expressed infrequently (the inducing environment is rare relative to the generation time of the organism) in populations that are relatively large. Such variation could potentially fuel the evolution of novelties, traits that are far beyond the normal range of phenotypes expressed in the ancestral environment (e.g., microorganisms expressing antibiotic resistance to a new drug therapy) (Van Dyken and Wade 2010). Additionally, genes under weak constraint—and that therefore might exhibit high levels of polymorphism—may be the ones that are, in turn, co-opted to regulate conditional trait expression (Hunt et al. 2011; Leichty et al. 2012). Alternatively, cryptic genetic variation should be relatively low for traits expressed frequently or in small populations. This lack of variation might not inhibit persistence of a population insofar as the population's history with the inducing environment prepares it for subsequent encounters. For example, if a historically small population (i.e., low N_e) experienced a new environment, but the environmental change is within the range of environmental variation the population encountered in its native habitat (frequent or recurrent environmental change), cryptic genetic variation underlying induced traits might play only a small role in further adaptation to the new environment. Further, if that small population experienced environmental conditions outside the range of what it would experience in its ancestral habitat (infrequent or completely novel), cryptic genetic variation underlying induced traits could be overwhelmingly deleterious. Here, the mutation load would be relatively high due to the joint actions of genetic drift and the wider range of neutral genes

(while they are hidden), rendering most of the exposed variation maladaptive for the changing environmental conditions.

Additionally, some aspects of cryptic genetic variation are completely unexplored—theoretically or empirically—and deserve investigation. For instance, what if there is little or no correlation between the inducing environment and the direction of selection? In all examples we have presented from natural populations, the inducing environment (e.g., predator-free regimes cues for tadpoles and brackish water for sticklebacks) selects for the same phenotype each time it is experienced. In contrast, for organisms that have evolved generic buffering systems that suppress genetic variants, cryptic genetic variation would be expressed in response to different environmental stressors that potentially impose different selection pressures. What are the implications of such buffering systems for the dynamics of cryptic genetic variation in natural populations? Because the direction of selection with each inducing event will be random with respect to past selection events, it is possible that the amount and phenotypic effects of cryptic genetic variation in buffering systems will be similar to that of systems in which the frequency of induction is very low; that is, the range of expressed phenotypic variation will be relatively broad, but relatively little of it will be adaptive for the inducing environment. However, the dynamics of buffering systems in nature are basically unknown; describing buffering systems in natural populations would make the elegant demonstrations of buffering systems in laboratory populations more relevant to ecological and evolutionary theory.

In a similar vein, it is incompletely understood whether the expectations of genes that are conditionally expressed in an "on–off" fashion (e.g., as might occur among castes in social insects) (Abouheif and Wray 2002) can be extended to traits that are expressed in a differential fashion (e.g., as might occur with thermal performance curves) or vary within an individual's lifetime (Snell-Rood et al. 2010). Evidence is accumulating that levels of gene expression within individuals do, in fact, correlate with rates of divergence (Subramanian and Kumar 2004; Lemos et al. 2005), suggesting that the expectations for conditional gene expression may be generalizable to different levels of expression. Van Dyken and Wade (2010) suggested that, in such scenarios, a regression coefficient relating the level of gene expression to selection (β) could be used in place of ϕ to model the expected levels of accumulation of mutations (and, by extension, levels of cryptic genetic variation). More complex models may be conceived, not only by modeling differences in levels of gene expression among individuals, but also by integrating different levels over the course of an individual's lifetime (for instance, differences in gene expression that are exhibited over larval and adult stages).

Finally, here we have only discussed how different population and environmental parameters might influence relative levels and types of cryptic genetic variation in the same trait among populations of the same species. Another poorly understood aspect of cryptic genetic variation is whether organisms and traits differ fundamentally in their potential to accumulate and maintain cryptic genetic variants. For instance, is cryptic genetic variation more common in certain types of traits than in others (e.g., morphological, physiological, or behavioral traits)? Is cryptic genetic variation more common in animals than in plants? Theoretical and empirical studies that help us understand these potential differences will undoubtedly improve our ability to predict how specific populations will respond to environmental perturbations.

Designing a research program that will successfully quantify environmental and population parameters in conjunction with estimates of cryptic genetic variation will be a complex task that will likely require diverse approaches, from population genetic analysis to niche modeling. However, the rewards of measuring these parameters are potentially very great if they allow us to predict the persistence of populations and the evolution of traits in a world currently undergoing rapid and drastic environmental change.

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