DISSERTATION

FLEXIBLE PHENOTYPES: THE DIVERSE ROLES OF PHENOTYPIC PLASTICITY DURING ADAPTIVE EVOLUTION IN EXPERIMENTAL POPULATIONS OF TRINIDADIAN GUPPIES

Submitted by

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In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

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Fall 2015

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ABSTRACT

FLEXIBLE PHENOTYPES: THE DIVERSE ROLES OF PHENOTYPIC PLASTICITY DURING ADAPTIVE EVOLUTION IN EXPERIMENTAL POPULATIONS OF TRINIDADIAN GUPPIES

The colonization of novel environments can lead to rapid adaptive evolution. However, the mechanisms that facilitate or constrain adaptive evolution during the initial stages of divergence are poorly understood. Adaptive evolution can happen rapidly when natural selection acts on genetic variation that is harbored by a population. But natural selection acts on phenotypes, not genotypes, and phenotypic variation can also emerge from phenotypic plasticity, which is the ability of a genotype to produce a range of phenotypes in response to the environment.

For more than a century, evolutionary biologists have recognized an ability for plasticity to influence evolution. Yet, traditional models of adaptive evolution ignore a mechanistic role for phenotypic plasticity, because it has been assumed to represent non-heritable phenotypic variation that slows evolution by masking underlying genetic variation from the effects of selection. However, in new environments, plasticity can have important direct and indirect roles in moderating the pressures of natural selection on an individual, and, in turn, affect the rate and pattern of evolution. For instance, plasticity can result in the expression of novel phenotypes or reorganize trait correlations that are targeted by selection. Any beneficial phenotypes or trait combinations increase fitness while maladaptive phenotypes should experience stronger selection acting against them. Alternatively, plasticity in one trait can modify behavior and alter

how species interact or utilize available resources, indirectly changing the strength of selection on other traits. Even if plastic changes merely facilitate the survival of some individuals in a novel environment, plasticity would play an important role in range expansions, dispersal, and setting the stage for evolutionary divergence.

A powerful framework for testing how selection acts on phenotypically plastic traits is to track genetic and plastic changes following the colonization of a new environment. Such an approach allows for direct comparisons of how trait means and their plasticity diverge between the ancestral source population and descendant derived populations, and for testing specific predictions on how phenotypically plastic traits evolve. I used recently established populations of Trinidadian guppies (*Poecilia reticulata*) to characterize the nature of plasticity and evolution in a suite of traits that are known to diverge among locally adapted native populations in response to predators and resource abundance.

Trinidadian guppies have become a model system for studying adaptive evolution. The Northern Range Mountains on the island of Trinidad are home to tropical streams that exhibit environmental gradients in their assemblages of piscivorous predators and available resources. Guppy populations are found throughout these streams and show patterns of local adaptation to downstream locales that are abundant in predators and food resources and upstream habitats that lack predators and are limited in food resources. Natural guppy populations throughout the streams in the Northern Range Mountains show patterns of convergent evolution within these contrasting environments, and translocation experiments have demonstrated that local adaptation happens on contemporary time scales. Previous experimental work has focused on the timing, direction, and magnitude of evolutionary divergence, but little is known about the biological processes involved immediately following a colonization event and leading up to evolutionary

divergence. I monitored four populations that were experimentally translocated into replicate low predations streams from a single high predation source population in the springs of 2008 and 2009. By combining field observations with laboratory experiments, I was able to elucidate the source of phenotypic variation in several important morphological, physiological, and life history traits and evaluate how plasticity influenced their evolution.

Chapter 1 explored how the trait correlations in body morphology changed in the field immediately following the introduced populations' release from predation in the experimental translocations. Specifically, first generation recruits were compared to the ancestral population and found to exhibit trait correlations that were novel. Moreover, reshuffling the phenotypic correlations led to adaptive combinations of traits that have been observed in nearby natural populations that are adapted to the low predation environment.

Chapter 2 expanded on the field observations of body morphology and size to look at i) the pattern of divergence in the field during the first year after the populations were introduced, ii) the genetic basis of changes in body morphology, and iii) whether body morphology and size exhibited plasticity, by rearing guppies in the presence and absence of simulated predation risk. The initial patterns of divergence observed in chapter 1 were maintained or increased over the first year after introduction. Additionally, the introduced populations had started diverging from the ancestral phenotype and toward locally adapted phenotypes in body shape but not size, which was highly plastic and sensitive to the presence of predator cue in the rearing environment in all populations. Body shape, in contrast, evolved plasticity from a non-plastic ancestor in ways that are presumably adaptive based on contrasts with locally adapted low predation populations.

In chapter 3, I explored how growth and metabolic rates changed following the release from predation, and tested whether the nature of phenotypic plasticity was indicative of

evolutionary divergence. The ancestral and introduced populations exhibited non-adaptive plasticity, growing slower in the presence of predator cue. However, the introduced populations evolved a slower growth rate, as would be expected in the low predation environments they colonized. For metabolic rate, plasticity evolved and the trait mean diverged toward an adaptive phenotype, but only when reared without predator cue. Most populations exhibited an anchor effect, where they converged on the ancestral phenotype when predator cue was present in the rearing environment.

Chapter 4 assessed plasticity and evolutionary change in life history traits over the four years following the colonization of low predation habitats by guppies translocated from a high predation environment. I looked at the stability of plasticity and the nature of divergence in age and size at maturity and growth rate. Plasticity appeared to be mostly beneficial and largely unchanged over four years. Nonetheless, trait means in age and size at maturity evolved in patterns consistent with local adaptation. Moreover, evolutionary divergence in age and size at maturity did not appear to be hindered by the presence of adaptive plasticity.

Overall, this research demonstrated that phenotypic plasticity can rearrange trait correlations, produce both adaptive and non-adaptive phenotypes, and evolve *de novo* during the course of adaptive evolution. I did not find evidence for the long-held assumption that plasticity impedes evolutionary divergence. However, it did appear that novel trait combinations may induce phenotypic novelty in combination with adaptive phenotypes and that non-adaptive plasticity may invoke adaptive divergence, because selection is stronger on maladaptive phenotypes. Thus, there seems to be multiple roles for plasticity during the early stages of evolutionary divergence, depending on how the genotype interacts with the environment.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Cameron Ghalambor, for his mentorship and contribution to my intellectual development. His ability to always see the big picture and help me focus on the broader relevance of my work has been instrumental to my success. I would also like to thank Lisa Angeloni for her support and intellectual contribution to this work. My committee members, Mike Antolin and Chris Myrick provided insightful suggestions during the planning and execution of this project. David Reznick has made important intellectual contributions and was essential to the success of the field experiments that this entire project hinged on. This project could not have succeeded without the field and laboratory technicians that were willing to go above and beyond expectations to ensure the collection of high quality data. I am also thankful for my colleagues in the in the Ghalambor and Angeloni labs. The support of my family and friends has helped me stay motivated and reach my goals. I could not imagine having done this without them. Emily Ruell, my partner in crime, has been the foundation of my success. Her contributions to this project are embedded in this work at all levels from experimental design to publication. The opportunities and experiences we have shared while working together and traveling the world have been the highlight of my life.

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Chapter 1. Phenotypic plasticity changes correlations of traits following experimental introductions of Trinidadian guppies (*Poecilia reticulata*)[1]

Introduction

Colonization of novel environments can alter selective pressures and act as a catalyst for rapid evolution in nature (Thompson 1998; Hendry and Kinnison 1999; Reznick and Ghalambor 2001; Hairston et al. 2005; Carroll et al. 2007). Indeed, most empirical examples of rapid adaptive evolution follow colonization events and are associated with shifts in the selective landscape (Reznick and Ghalambor 2001). Theory and empirical studies suggest that the ability of a population to exhibit an adaptive evolutionary response to these novel selection pressures should reflect the presence of sufficient additive genetic variance and covariance for individual and correlated traits (Lande 1979; Lande and Arnold 1983; Roff 1997).

At the genetic level, genetic covariance among correlated traits arises from the pleiotropic effects of genes and linkage, and is characterized as the genetic variance-covariance matrix (**G**) in multivariate selection models (Lande 1979). At the phenotypic level, **G** is manifested as the degree to which phenotypic traits are correlated with each other and can be similarly described by the phenotypic variance-covariance matrix (**P**) (Olson and Miller 1958; Cheverud 1982; Cheverud 1988; Revell et al. 2007; Revell et al. 2010). The, structure of **G** and **P** jointly determine how a set of correlated traits should respond to selection (Lande 1979; Lande and Arnold 1983; Roff 1997). When there is a high degree of genetic and phenotypic integration, traits are not free to evolve independently, thus biasing the path of evolutionary divergence in multiple traits away from the directional selection gradient (Lande 1979; Lande and Arnold

[1] This chapter has been reprinted with permission from Oxford University Press: Handelsman CA, Ruell EW, Torres-Dowdall J, Ghalambor CK. 2014. Phenotypic plasticity changes correlations of traits following experimental introductions of Trinidadian guppies (*Poecilia reticulata*). Integrative and Comparative Biology 54:794–804.

1983; Björklund 1996; Schluter 1996; Walker 2007; Kirkpatrick 2009; Chenoweth et al. 2010; Revell et al. 2010). Conversely, when directional selection gradients parallel the major axis of genetic variance, evolution should be rapid and the path of phenotypic divergence should be toward the new fitness-optimum (Schluter 1996; Merilä and Björklund 2004). Yet the ability of **G** and **P** to provide predictive insight into patterns of evolutionary divergence and constraint depends on the stability of genetic correlations across space and time.

The stability of **G** is a fundamental assumption in quantitative genetic models of evolution (Lande 1979; Lande and Arnold 1983; Falconer and Mackay 1996; Schluter 1996; Lynch and Walsh 1998). Indeed, **G** has been found to show remarkable stability among populations (Spitze et al. 1991) and species (Shaw et al. 1995; Roff and Mousseau 1999), and inform patterns of divergence across species (Bégin and Roff 2003). However, others have recognized that **G** is likely to evolve (Steppan et al. 2002; Arnold et al. 2008) and empirical work supports the evolution of **G** and **P**, particularly in response to selection, inbreeding, and genetic drift (Phillips et al. 2001; Cano et al. 2004; McGuigan et al. 2005; Doroszuk et al. 2008; Revell et al. 2010). Thus, evolutionary forces have the potential to modify the bias of **G** and alter trajectories of phenotypic divergence.

Another important, but less explored, mechanism by which patterns of genetic and phenotypic integration can change, is phenotypic plasticity. Phenotypic plasticity is the ability of a single genotype to express different phenotypes in response to the environment that individuals experience during ontogeny (Schlichting 1986; Scheiner 1993; Via et al. 1995; Pigliucci 2001). Typically, such responses are non-reversible. Plasticity changes mean values of traits and thereby shifts the phenotypic distribution exposed to selection; however, the plasticity of multiple traits and their correlations has rarely been considered (Parsons and Robinson 2006). In theory,

phenotypic plasticity can alter **G** and **P**, and in turn either alleviate existing constraints on the response to selection or induce new ones (Gillespie and Turelli 1989; Stearns et al. 1991; Parsons and Robinson 2006; Pitchers et al. 2013). Despite the potential of plasticity to reshuffle correlations of traits, alter the strength of selection on multivariate phenotypes, and bias evolutionary trajectories, few studies have explicitly compared patterns of integration between ancestral and derived populations.

Phenotypic plasticity and evolution in Trinidadian guppies

Natural populations of Trinidadian guppies are found throughout the tropical streams of Trinidad's Northern Range Mountains and provide a model system for studying rapid evolutionary change in nature (Reznick et al. 1997). Guppies that occupy larger rivers and streams experience intense predation from a suite of piscivorous fishes (Reznick 1982; Reznick and Endler 1982; Reznick et al. 1996). In contrast, guppies in smaller headwater and tributary streams experience lower extrinsic mortality due to predation (Reznick 1982; Reznick and Endler 1982; Reznick et al. 1996). Differences in predation and other environmental covariates, such as food-resources or stream-velocity, are correlated with rapid adaptive divergence in life histories (Reznick and Endler 1982), behavior (Seghers 1974; Endler 1995; Godin and Briggs 1996; Templeton and Shriner 2004), and body-morphology (Layman et al. 2003; Langerhans and Dewitt 2004; Alexander et al. 2006; Hendry et al. 2006; Burns et al. 2009). Prior studies have experimentally translocated guppies from streams where they experience high predation into streams with low predation and found rapid evolution in the aforementioned traits (Reznick and Bryga 1987; Reznick et al. 1990; Reznick and Bryga 1996; Reznick et al. 1997). However, these

studies have not captured the initial phenotypic changes that arise immediately following colonization.

Body-shape in fishes shows consistent patterns of integration and divergence due to the functional constraints imposed by aquatic environments (Langerhans and Dewitt 2004; Walker 2010). Moreover, guppies show parallel patterns of divergence in body-shape that correspond to predation regimes in the wild (Alexander et al. 2006; Hendry et al. 2006), making morphology of the body a candidate phenotype to investigate the stability of trait-correlations during divergence. Specifically, guppies from high-predation locales have more fusiform bodies and a dorsal orientation of the mouth (Alexander et al. 2006) that show genetic and plastic responses to stream-velocity, predation-risk, and foraging behavior (Robinson and Wilson 1995). In contrast, patterns of water-flow and the acquisition of resources in low-predation habitats leads to a phenotype characterized by deeper bodies with a more terminal and anterior orientation of the mouth (Alexander et al. 2006).

We simulated the historical colonization of streams with low predation by translocating guppies native to a high-predation stream into four streams characteristically similar to habitats with low predation but lacking in guppies. We investigated how phenotypic plasticity refines body-morphology in the novel environment and tested whether it alters **P**. By monitoring an environmental shift that parallels the evolutionary history of natural populations of guppies colonizing low-predation streams, this approach provides novel insight into the ability of phenotypic plasticity to change the combinations of traits that are exposed to a new selection-regime and sets the stage for describing the conditions leading to repeated patterns of adaptive evolution.

Methods

Sampling of guppy populations

We sampled a natural population of guppies subjected to high predation and four experimental populations that were descendants from that population in the Guanapo River drainage in the Northern Range Mountains of Trinidad, West Indies (Handelsman et al. 2013). Briefly, the natural high-predation population, hereafter referred to as the ancestral population, is subject to high levels of predation from a variety of predatory species, including the common predator on guppies, the pike cichlid *Crenicichla frenata* (Gilliam et al. 1993; Torres-Dowdall et al. 2012). The experimental populations were established in upstream tributaries of the Guanapo River in reaches that previously lacked guppies and contained only one species of fish, a small killifish (*Rivulus hartii*). *Rivulus hartii* are gape-limited omnivores that occasionally prey on juvenile or small guppies (Mattingly and Butler 1994). Thus, the experimental reaches mimic low-predation habitat for guppies.

Paired introductions were conducted across two consecutive years (Handelsman et al. 2013; Arendt et al. 2014). In March 2008, descendants from the ancestral population were introduced into the Lower Lalaja and Upper Lalaja tributaries of the Guanapo River (hereafter Intro–1 and Intro–2, respectively). Each stream was stocked with 38 gravid females and 38 mature males. To minimize the potential for founder effects and standardize genetic diversity in each stream, males and females were randomly crossed and introduced into alternate streams with the consequence that the introduced females carried sperm stores from the 38 males that they were mated with. Then, the females were paired in the introduction site with 38 new males. Paired random crosses were employed to prevent biased mating that may arise from female mate choice. Additionally, previous laboratory experiments (Reznick 1982; Reznick and Bryga 1987;

Torres-Dowdall et al. 2012; Handelsman et al. 2013) regularly produce viable progeny from paired crosses and have observed low failure rates in such crosses. In March 2009, this protocol was replicated in the upper reaches of the Caigual and Taylor tributaries of the Guanapo River (hereafter Intro–3 and Intro–4, respectively), but 45 males and females were introduced into each site.

The four introduced populations were established in 100–180 m reaches of these first-order tributaries. Waterfalls bound the upper and lower limits of each reach and were artificially enhanced (if necessary) to prevent emigration and the populations established above the streams receiving introductions and to prevent immigration from downstream populations. Natural waterfalls that served as barriers were enhanced with sandbags to bar upstream migration of guppies. However, flash floods during the wet seasons did lead to the loss of some individuals downstream. Waterfalls serving as upstream-barriers were enhanced in two reaches (Intro–2 and Intro–3) and a downstream-barrier was enhanced in Intro–4. Additionally, the canopy of the riparian forest was experimentally thinned (opened) in one stream of each pair, six months prior to the introductions (Kohler et al. 2012). Canopy-thinning increased light levels relative to the undisturbed (closed) canopies of each paired reach (as part of a separate experiment) (Kohler et al. 2012). We did not find any significant effects of canopy-thinning and therefore did not consider it in our analyses (data not shown).

Mature males (n = 67) from the ancestral population were captured, anesthetized in MS-222 (0.85 mg ml^{-1}; ethyl 3–aminobenzoate methane sulfonic acid salt) (Sigma-Aldrich, St Louis, MO) buffered with sodium bicarbonate, and photographed (see below) in January of 2008 prior to the introductions. The pairs of experimental populations were sampled three months after they were established (May of 2008 for Intro–1 and Intro–2 and May of 2009 for Intro–3 and Intro–4)

to assess first generation recruits. Under laboratory conditions, the ancestral population had an inter-brood interval of 25 days and males matured within 54 days (Handelsman unpublished data). Therefore, first generation recruits were expected to be mature, but there had not yet been sufficient time for a second brood to mature. Thus, our sampling design is intended to capture the initial plastic changes associated with developing in a low-predation stream. We collected and photographed all mature males (i.e., first generation recruits) from each population (Intro–1: n = 208, Intro–2: n = 302, Intro–3: n = 194, Intro–4: n = 286). Females were excluded because, as livebearers, their body-shape changes throughout gestation and can complicate interpretations of shape.

Analysis of body-shape

We analyzed variation in lateral body-shape with geometric morphometrics (Rohlf and Marcus 1993; Zelditch et al. 2004). We used eight homologous landmarks and six semi-landmarks (Bookstein 1997) acquired from digital images to characterize the lateral body-shape of adult male guppies. Lateral photographs of the left side of each fish were taken with Nikon D60 digital SLR cameras equipped with Nikkor 50-mm macro lenses (Nikon Inc., Melville, NY, USA) mounted on tripods. The height of the tripod was adjusted to yield an 8-cm field of view that was determined sufficient to eliminate any parallax within the lens area occupied by a guppy. To standardize the position of fish and to expose homologous landmarks, a fine-tipped artist's paintbrush was wetted and used to straighten the specimen and spread the median fins. A ruler was placed in each picture to show scale. Landmarks were digitized with TPSDig2 (Rohlf 2013). We isolated geometric shape by removing variation due to size, position, and orientation, with a Generalized Procrustes Superimposition (Rohlf and Slice 1990; Goodall 1991; Dryden

and Mardia 1998) using the geomorph package in R (Adams and Otarola-Castillo 2013). Procrustes distance was used to optimize the position of semi-landmarks with the geomorph package in R (Adams and Otarola-Castillo 2013). Specifically, semi-landmarks were slid along tangent lines and optimized by minimizing the Procrustes distance between adjacent landmarks (Bookstein 1997; Rohlf 2010). The superimposed coordinates (Procrustes Coordinates) were used in all further analysis of shape.

We used a principal component analysis (PCA) to reduce dimensionality of the data and define shape-variables. The PCA was performed on the covariance matrix of the Procrustes coordinates and the resulting principal components were used as shape-variables to calculate the **P** matrices. The PCA was performed in program MorphoJ (Klingenberg 2011). The **P**–matrix was calculated from all 24 principal components.

Body-size was measured as centroid-size, the square root of the sum of the squared distances from the centroid to each landmark, where the centroid is the mean Cartesian coordinates of each specimen. One high-quality photograph per adult male guppy was analyzed for body-shape and used to represent that individual in morphometric analyses. We regressed centroid-size on body-shape using multivariate regression and tested for significance using a permutation test with 10,000 randomizations in program MorphoJ (Klingenberg 2011). Body-size was positively correlated with lateral shape of the body ($P < 0.001$) but explained only 6.6% of the variation. Comparisons of the **P** matrices (see below) run on the raw data and the residuals from the multivariate regression produced identical results. Below, we only report results from raw data.

Phenotypic plasticity

We looked for plastic changes in body-shape by comparing the ancestral population to each experimental population. Because we evaluated first-generation recruits in the experimental populations, phenotypic differences should reflect developmental plasticity in body-shape. Discriminant function analysis (DFA) was used to compare the body-shape of the ancestral population to each experimental population. Significant differences in body-shape were assessed with cross-validated correct assignment of individuals and permutation tests in program MorphoJ (Klingenberg 2011). Permutation tests were run for 10,000 iterations and p-values were adjusted for multiple tests with Holm's sequential Bonferroni correction (Holm 1979).

P-matrix comparisons

Following Roff et al. (2012), we employed several complementary statistical methods to compare **P** matrices among the ancestral population and the experimental populations. Specifically, we used the jump-up approach to the Flury method (Phillips and Arnold 1999; Roff and Mousseau 2005), modified Mantel test (Goodnight and Schwartz 1997), Bartlett's test (Goodnight and Schwartz 1997), Jackknife-MANOVA test (Roff 2002), and the jackknife-eigenvalue test (Kirkpatrick 2009; Roff et al. 2012). The principal components generated from rotating the Procrustes coordinates in MorphoJ (see above) (Klingenberg 2011) were used as traits to produce and compare **P** matrices. However, the Jackknife-MANOVA requires a full rank model. Given our sample sizes, the models became rank deficient if more than 22 principal components were included. Thus, we ran all models with the first 22 principal components. These 22 Principal components captured 99.97% of the sample variance.

Results

Phenotypic plasticity

The first-generation recruits in all four experimental populations showed divergence in body-shape in response to being moved from high-predation to low-predation streams (Fig. 1.1). Phenotypic divergence in the introduced populations is likely due to phenotypic plasticity, given that the mean trait values changed while the phenotypic variance was relatively stable (Fig. 1.2; Tables 1.1 and 1.2), and because sampling bias, founder effects, or selection favoring certain phenotypes should reduce phenotypic variance in conjunction with shifting the mean trait values. Moreover, the plastic response produced parallel changes in all four populations (Figs. 1.1 and 1.2). Specifically, the eye underwent a dorsal and posterior shift, the caudal peduncle was elongated, and the insertion of the anal fin underwent an anterior and ventral shift that resulted in a deeper body.

*Comparisons of **P**-matrices*

We used five methods to contrast the structure of the **P** matrices between the ancestral populations and the four experimental populations. All methods produced congruent results and suggest that the **P** matrix in the experimental populations diverged from the ancestral population (Table 1.3). Specifically, these comparisons tested the null hypotheses that the **P** matrices are proportional (Table 1.3; Flury method and modified Mantel test), contain equal elements (Table 1.3; Flury method and Jackknife-MANOVA), share common principal components (Table 1.3; Flury method), are of equal size (Table 1.3; Bartlett's test), and have equal eigenvalues (e.g., total variance (Table 1.3; Jackknife-eigenvalue). In all tests, we rejected the null hypotheses of

matrix equality and found support for repeated divergence between the four experimental populations and their source population (Table 1.3).

Discussion

Adaptive evolution is a function of the strength of natural selection and the genetic architecture of the underlying traits targeted by selection (Lande 1979; Lande and Arnold 1983; Roff 1997). Because genetic effects (e.g., pleiotropy and linkage) can place constraints on whether phenotypic divergence parallels multivariate selection gradients, the structure of **G** and **P** are important determinants of a population's response to selection. Indeed, the importance of genetic correlations is perhaps exemplified in agricultural breeding programs aiming to maximize yield when the environment is constant, and selection is strong (Moose et al. 2004; Powell and Norman 2006). In natural populations, however, changes in the environment act both as a source of selection and as a trigger for developmental plasticity that can alter genetic and phenotypic correlations. We found that the translocation of guppies that had evolved under conditions of high predation to four replicate low-predation habitats resulted in parallel plastic changes in body-shape and in the underlying pattern of trait-correlations (Fig. 1.1; Tables 1.2, 1.3). Thus, the same genetic background develops a predictable change in body-shape (Fig. 1.1), and pattern of trait-correlations (Table 1.3) simply by developing in a new environment. Such results challenge the frequent assumption of stability of the structure of phenotypic covariance and have important implications for the ability of **G** and **P** to forecast patterns of phenotypic divergence. We discuss these implications in more detail below.

Correlations among quantitative traits and the traits themselves can be sensitive to environmental variation during ontogeny (Sgrò and Hoffmann 2004). The observation that

patterns of genetic correlation are dependent on the environmental context in which they are measured suggests that the ability to infer genetic constraints on evolutionary responses may be difficult to generalize when only taking measurements in a single environment (Sgrò and Hoffmann 2004). For example, comparisons of the same genetic background in different environments reveals that the direction of genetic correlations can change in response to temporal stability of the habitat (Newman 1988a; Newman 1988b; Newman 1989) and of the abundance of resources (Service and Rose 1985; Gebhardt and Stearns 1988). Complex phenotypes also are plastic in response to changing environmental conditions, and the body-morphology of fishes can be particularly sensitive to environmental conditions. For example, Parsons and Robinson (2006) compared body-shape of ancestral and derived ecomorphs of the pumkinseed sunfish (*Lepomis gibbosus*) and found that correlated patterns of phenotypic plasticity had evolved in the novel environment. Similarly, in common garden experiments, Ghalambor et al. (in revision) found parallel patterns of plasticity in body-shape of Trinidadian guppies from high-predation and low-predation locales that were contingent upon combinations of water velocity and perceived risk of predation that mimicked natural habitats of guppies. While these examples of correlated plastic responses provide compelling evidence that phenotypic plasticity can produce parallel shifts in multiple aspects of the phenotype, it remains unclear whether plasticity played a role in altering the trajectory of phenotypic divergence that would have been predicted given the structure of trait-correlations in the ancestral population. Thus, more monitoring of populations that have recently colonized new environments are needed, if we are to evaluate the role of plasticity during divergence of correlated traits.

Trinidadian guppy populations have repeatedly diverged in a suite of life-history, behavioral, and morphological characters in what is regarded as a classic example of rapid

adaptive evolution (Reznick and Bryga 1987; Endler 1995; Magurran 2005), but the role of plasticity for single or multiple traits in the evolutionary process remains unclear. In previous translocation experiments, experimental populations of guppies have been shown to exhibit rapid patterns of parallel phenotypic divergence (Reznick et al. 1990; Reznick et al. 1997), and while phenotypic plasticity may play a role in rapid evolution (Torres-Dowdall et al. 2012; Handelsman et al. 2013), no studies to date have quantified plastic changes in natural populations. Here, we show that body-shape of field-collected individuals that founded the populations and their first-generation recruits exhibited a deepening of the body and a dorsal shift in position of the eye relative to the mouth (Fig. 1.1; Table 1.2) that is consistent with patterns of divergence between native high-predation and low-predation populations of guppies (Alexander et al. 2006). These plastic responses are assumed to be adaptive given that they are in the same direction as those observed in native populations subject to low predation (Ghalambor et al. in revision). However, we also found an elongation of the caudal peduncle that contrasts with the expected direction of divergence (Alexander et al. 2006), suggesting that the initial patterns of plasticity following colonization of a new environment may also include non-adaptive responses (Ghalambor et al. 2007). An initially non-adaptive plastic response to a new environment should impose strong selection on a trait (Ghalambor et al. 2007; Handelsman et al. 2013); thus, subsequent work can test the prediction that the caudal peduncle should evolve more quickly than do other traits.

In addition to overall plastic changes in body-morphology, we found the covariance structure of body-morphology differed between the ancestral genotype and all four experimental populations. Specifically, we found that when compared to the ancestral genotype, elements of **P** in the experimental populations were not equal (Table 1.3; Flury Hierarchy, Jackknife–

MANOVA), had unequal eigenvalues (i.e., total variance) (Table 1.3; Jackknife–eigenvalue), were unequal in size (Table 1.3; Bartlett's test), were not proportional (Table 1.3; Flury Hierarchy, Modified Mantel test), and did not share common principal components (Table 1.3; Flury Hierarchy). Thus, translocation of the ancestral genotype (high predation) into low-predation streams changes the correlations between traits and may therefore influence the evolutionary trajectory of these populations to the new selection pressures they experience. Had we examined the pattern of correlations among traits in the ancestral population to infer the evolutionary response to selection, we would have drawn different conclusions on how integration biases phenotypic divergence.

Phenotypic trait correlations making up **P** are thought to arise from genetic correlations that result in phenotypically integrated organisms. The underlying shared developmental processes that give rise to this integration may therefore be the mechanism by which constraints or trade-offs influence the evolution of complex phenotypes (Ghalambor et al. 2003; Merilä and Björklund 2004). For example, highly predated populations of guppies have longer caudal peduncles and a more ventral position of the eye relative to guppies subject to low predation (Ghalambor et al. in revision). These correlated components of shape are thought to be adaptive to rapidly flowing stream currents (Ghalambor et al. in revision) and greater utilization of the surface of the water column (Torres-Dowdall et al. 2012). Thus, our result that caudal peduncles are both deeper and longer in the experimental populations represents a novel combination of traits not observed in naturally occurring low-predation or high-predation populations of guppies. The developmental mechanism responsible for this novel phenotype is not known, but likely involves changes in gene expression in response to the low-predation environment (Gunter et al. 2013). The critical question is whether this new combination of traits is a long-term constraint

imposed by plasticity. Monitoring these introduced populations through time will shed light on whether these attributes of shape will become decoupled, as is observed in naturally occurring low-predation populations, or whether they will impose a lasting constraint on the direction of the evolution of body shape.

In conclusion, while there is evidence that correlations of traits can be stable through time (Spitze et al. 1991; Shaw et al. 1995; Roff and Mousseau 1999; Bégin and Roff 2003; Game and Caley 2006; Pitchers et al. 2013), we found **P** can exhibit plasticity and change immediately following the colonization of a novel environment. Most previous work has focused on comparisons of populations of conspecifics across environments or comparisons of taxonomically distant groups long after they diverged. Here, we show that phenotypic plasticity that resulted from translocating the same high-predation genotypes into four low-predation streams induced both adaptive and non-adaptive changes in body-shape and reshuffled correlations of traits, thereby changing the combination of traits that were exposed to selection. These findings suggest our ability to make inference about patterns of divergence based on correlations of traits in extant populations may be limited if novel environments not only induce plasticity in traits, but also change the correlations among those traits.[2]

[2] This research was approved by the Colorado State University Institutional Animal Care and Use Committee (protocols # 12-3269A and 09-1348A). This research was supported by the National Science Foundation Faculty Early Career Development grant DEB-0846175 (Cameron Ghalambor) and the National Science Foundation Frontiers in Integrative Biological Research grant EF-0623632 to David Reznick.

Tables and figures

Table 1.1 Population means and standard deviations (SD) for each principal component.

	N	PC1	SD	PC2	SD	PC3	SD	PC4	SD
Ancestor	67	-0.0327	0.0123	0.0078	0.0088	-0.0087	0.0070	-0.0137	0.0077
Intro–1	208	-0.0040	0.0180	0.0033	0.0122	0.0031	0.0091	-0.0023	0.0072
Intro–2	302	-0.0034	0.0172	0.0057	0.0120	0.0013	0.0089	-0.0009	0.0065
Intro–3	194	0.0109	0.0140	-0.0071	0.0144	0.0013	0.0094	0.0012	0.0079
Intro–4	286	0.0068	0.0192	-0.0055	0.0143	-0.0025	0.0098	0.0050	0.0073

	N	PC5	SD	PC6	SD	PC7	SD	PC8	SD
Ancestor	67	-0.0043	0.0069	-0.0059	0.0082	0.0023	0.0053	0.0005	0.0059
Intro–1	208	-0.0027	0.0064	0.0011	0.0053	-0.0002	0.0050	-0.0016	0.0044
Intro–2	302	-0.0007	0.0066	0.0007	0.0059	-0.0002	0.0054	0.0006	0.0045
Intro–3	194	0.0022	0.0063	0.0016	0.0055	-0.0016	0.0065	-0.0008	0.0046
Intro–4	286	0.0023	0.0066	-0.0013	0.0055	0.0009	0.0056	0.0009	0.0045

	N	PC9	SD	PC10	SD	PC11	SD	PC12	SD
Ancestor	67	-0.0013	0.0044	0.0013	0.0057	0.0023	0.0042	-0.0010	0.0033
Intro–1	208	0.0007	0.0034	-0.0014	0.0039	-0.0008	0.0034	0.0004	0.0031
Intro–2	302	0.0002	0.0034	0.0006	0.0036	-0.0004	0.0031	-0.0002	0.0030
Intro–3	194	-0.0022	0.0041	-0.0011	0.0035	0.0013	0.0031	0.0001	0.0034
Intro–4	286	0.0010	0.0040	0.0008	0.0035	-0.0004	0.0032	0.0002	0.0030

	N	PC13	SD	PC14	SD	PC15	SD	PC16	SD
Ancestor	67	0.0014	0.0031	0.0012	0.0028	-0.0001	0.0024	0.0000	0.0021
Intro–1	208	-0.0006	0.0025	-0.0007	0.0024	0.0000	0.0020	0.0001	0.0018
Intro–2	302	-0.0008	0.0024	0.0002	0.0022	0.0001	0.0022	0.0002	0.0018
Intro–3	194	0.0008	0.0024	-0.0002	0.0024	0.0005	0.0023	-0.0002	0.0020
Intro–4	286	0.0004	0.0025	0.0002	0.0024	-0.0004	0.0021	-0.0002	0.0019

	N	PC17	SD	PC18	SD	PC19	SD	PC20	SD
Ancestor	67	0.0001	0.0016	-0.0008	0.0020	0.0000	0.0013	0.0003	0.0013
Intro–1	208	0.0000	0.0014	0.0003	0.0012	-0.0002	0.0010	0.0000	0.0007
Intro–2	302	-0.0001	0.0014	0.0001	0.0011	0.0001	0.0011	-0.0002	0.0008
Intro–3	194	0.0000	0.0015	-0.0001	0.0015	0.0000	0.0011	0.0001	0.0010
Intro–4	286	0.0001	0.0014	0.0000	0.0014	0.0000	0.0012	0.0001	0.0008

	N	PC21	SD	PC22	SD	PC23	SD	PC24	SD
Ancestor	67	0.0001	0.0002	-0.0001	0.0003	0.0000	0.0001	0.0000	0.0001
Intro–1	208	0.0000	0.0002	0.0000	0.0002	0.0000	0.0001	0.0000	0.0001
Intro–2	302	0.0000	0.0002	0.0000	0.0002	0.0000	0.0001	0.0000	0.0001
Intro–3	194	0.0000	0.0002	0.0000	0.0002	0.0000	0.0001	0.0000	0.0001
Intro–4	286	0.0000	0.0004	0.0000	0.0002	0.0000	0.0002	0.0000	0.0001

Table 1.2 Discriminant function analysis testing for plastic changes in body shape between the ancestral population and each experimental population.

	Procrustes Distance	Cross-validated classification	$P^{\#}$
Ancestor—Intro-1	0.035	99.7%	<0.001*
Ancestor—Intro-2	0.035	99.5%	<0.001*
Ancestor—Intro-3	0.051	100%	<0.001*
Ancestor—Intro-4	0.047	99.7%	<0.001*

[#] *P* values for permutation tests with 10,000 permutation runs
[*] Significant after Holm's sequential Bonferroni correction

Table 1.3 Summary of pairwise comparisons of **P** matrices.

	Flury Hierarchy			**Modified Mantel Test**		**Bartlett's Test**			**Jackknife-MANOVA**			**Jackknife-eigenvalues**		
	Equal matrices (*P*)	Proportional matrices (*P*)	CPC[#] (*P*)	Obs. M	*P*	χ^2	df	*P*	Wilk's λ	df	*P*	Wilk's λ	df	*P*
Ancestor—Intro-1	0.003	<0.001	<0.001	0.852	<0.001	499.8	171	<0.001	0.168	1, 273	<0.001	0.796	1, 273	<0.001
Ancestor—Intro-2	0.040	<0.001	<0.001	0.865	<0.001	568.6	171	<0.001	0.245	1, 367	<0.001	0.779	1, 367	<0.001
Ancestor—Intro-3	0.002	<0.001	<0.001	0.814	<0.001	445.2	171	<0.001	0.195	1, 259	<0.001	0.782	1, 259	<0.001
Ancestor—Intro-4	<0.001	<0.001	<0.001	0.844	<0.001	627.8	171	<0.001	0.236	1, 351	<0.001	0.807	1, 351	<0.001

[#]Common principal component

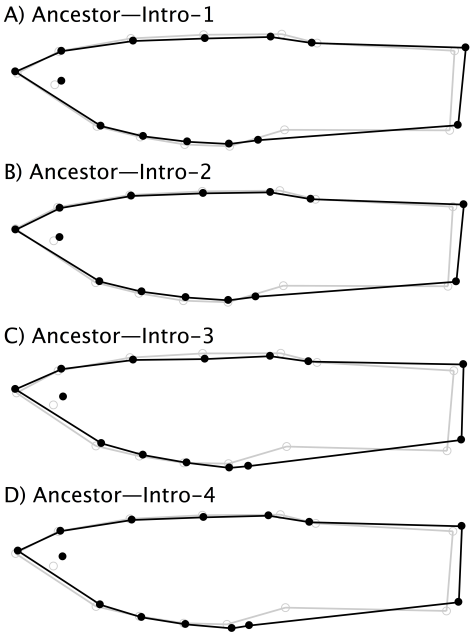

Figure 1.1 Deformation grids depicting the discriminant function that describes changes in shape between the ancestral population from a high-predation stream (gray outline) and A) Intro–1, B) Intro–2, C) Intro–3, and D) Intro–4 (black outlines). Separate discriminant-function analyses were run for each pairwise comparison. Note the parallel patterns of plasticity in position of the eye, depth of the body along the ventral surface, and depth of the caudal peduncle.

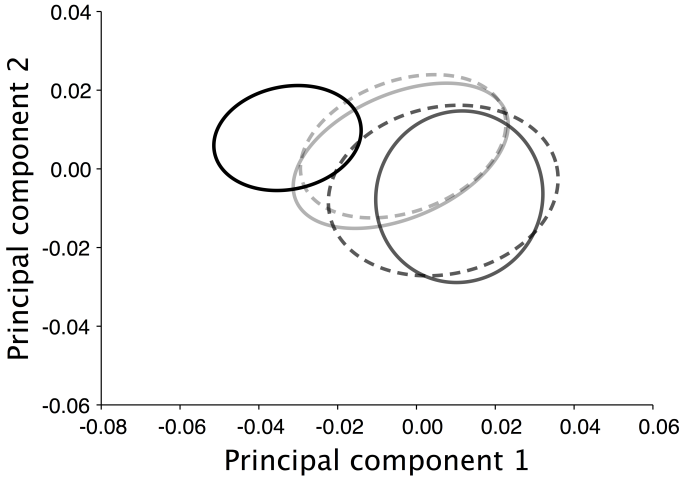

Figure 1.2 Bivariate plot of correlations between principal component 1 (40.8% of the sample variance) and principal component 2 (21.0% of the sample variance). Moving descendants of the ancestral population (black ellipse) into four low-predation streams (Intro–1, light gray ellipse; Intro–2, light gray dashed ellipse; Intro–3, dark gray ellipse; Intro–4, dark gray dashed ellipse) led to phenoytypic changes seen as the shift in morphospace by all four introduced populations, and changes the structure of phenotypic correlations (i.e., the shape and size of the ellipses). Ellipses represent 2 standard deviations from the population mean.

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Chapter 2. Phenotypic plasticity and rapid evolution following experimental introductions in nature[3]

Introduction

The colonization of novel environments often leads to rapid adaptive evolution (Endler 1980; Losos 1990, 1998; Losos et al. 1997; Thompson 1998; Reznick and Ghalambor 2001). However, the mechanisms that facilitate or constrain adaptive evolution during the initial stages of population divergence are poorly understood. Abundant standing genetic variation is the primary mechanism thought to facilitate evolutionary responses to divergent selection (reviewed in Barrett and Schluter 2008), but because natural selection acts on phenotypes, not genotypes, environmentally induced phenotypic variation can constrain evolutionary responses (Day et al. 1994; Agrawal 2001; Gienapp et al. 2008; Teplitsky et al. 2008). The most common form of environmentally induced variation is phenotypic plasticity, which is the ability of a genotype to produce a range of phenotypes in response to different environments (Travis 1994; Pigliucci 2001; West-Eberhard 2003). Yet, traditional models of adaptive evolution ignore a mechanistic role for phenotypic plasticity (Fisher 1930; Wright 1931; Falconer 1981; Orr 1998), because it has been assumed to represent non-heritable phenotypic variation that shields heritable genetic variation from the effects of selection, and therefore, constrains evolution (Wright 1931; Williams 1966; Falconer 1981; Levin 1988). Recently, however, plasticity has been recognized as an important process that can influence contemporary evolution and has been prominently

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incorporated into the proposed "Extended Evolutionary Synthesis" (Pigliucci and Müller 2010; Laland et al. 2014). Yet, the role of plasticity in evolutionary change is complex and can take different roles. However, most theory incorporating a role for plasticity in adaptive evolution is based on whether the environment induces plastic responses that are closer or farther away from local optima (Ancel 2000; Price et al. 2003; Ghalambor et al. 2007; Paenke et al. 2007; Crispo 2008).

When plasticity is adaptive (i.e., the environment induces phenotypes that are closer to local optima), theory predicts two alternative outcomes. First, if plasticity results in phenotypes that approximate a local optima (i.e., complete or "perfect" plasticity), stabilizing selection is expected to inhibit evolutionary divergence (Ancel 2000; Price et al. 2003; Ghalambor et al. 2007; Paenke et al. 2007). Alternatively, plasticity could be adaptive but incompletely shift the mean phenotype toward local optima. In this scenario, directional selection is expected to facilitate adaptive evolution by further shifting the mean trait value toward a new adaptive peak (Price et al. 2003; Ghalambor et al. 2007; Paenke et al. 2007). However, when novel environments induce non-adaptive plasticity (i.e., phenotypes that lie further away from local optima relative to an ancestral phenotype), phenotypic plasticity is predicted to increase the strength of directional selection, because selection must overcome the mismatch between the phenotype and the environment (Price et al. 2003; Grether 2005; Ghalambor et al. 2007; Paenke et al. 2007). Because non-adaptive plasticity reduces fitness and increases the probability of extinction, less theoretical and empirical work has been focused on its evolutionary implications. Although, recent work suggests that non-adaptive plasticity may actually accelerate an evolutionary response compared to adaptive plasticity, given that the phenotype–environment mismatch should increase the strength of selection (Handelsman et al. 2013; Ghalambor et al.

2015). Yet, a remaining challenge is how to move beyond plasticity in individual traits, and understand how multiple integrated traits respond to new environments through plasticity and evolution (Pigliucci 2003; Parsons and Robinson 2006).

Empirical studies reveal that adaptation to new environments typically involves whole suites of traits, and trade-offs among traits are common (Reznick and Travis 1996; Reznick and Ghalambor 2001). Thus, the differential influence of a novel environment on multiple traits produces an integrative response that ultimately determines the rate of adaptive divergence. This is particularly important for morphological traits that must function in a coordinated manner to determine whole organism performance (Irschick and Garland Jr 2001; Losos et al. 2004; Calsbeek and Irschick 2007; Herrel et al. 2008). Thus, understanding the dual role of the environment as a selective pressure and a source of both phenotypic and genetic variation requires examining multiple traits that contribute to adaptive evolution in new environments, but few studies have attempted to take such a multivariate approach (Parsons and Robinson 2006).

Natural populations of Trinidadian guppies (*Poecilia reticulata*) are found throughout the tropical streams of Trinidad's Northern Range Mountains and provide a model system for studying rapid evolutionary change in multiple integrated traits (Reznick et al. 1997). Guppies that occupy larger lowland rivers and streams experience relatively high predation risk from a suite of larger piscivorous fishes. In contrast, guppies in small headwater streams and tributaries experience significantly lower risk of predation compared to the downstream locales, because barrier waterfalls exclude the larger piscivorous predators and guppy populations co-occur with only a small gape-limited killifish *Rivulus hartii* (Reznick 1982; Reznick and Endler 1982; Reznick and Bryga 1996). Differences in predation and other environmental covariates, such as food resources or stream velocity, are correlated with adaptive divergence in life history

(Reznick and Endler 1982), behavior (Seghers 1974; Endler 1995; Godin and Briggs 1996; Templeton and Shriner 2004), and morphology (Layman et al. 2003; Langerhans and Dewitt 2004; Alexander et al. 2006; Hendry et al. 2006; Burns et al. 2009).

Here, we experimentally translocated guppies from a high predation lowland site into four replicate low predation upstream tributaries, which did not previously contain guppies. Although prior studies also transplanted guppies from high predation streams into low predation streams and found rapid evolution in the aforementioned traits (Reznick and Bryga 1987, 1996; Reznick et al. 1990, 1997), these studies have not captured the initial phenotypic changes that arise immediately following colonization. We specifically explored this early period of divergence in order to evaluate how plasticity contributes to the evolution of an integrated multivariate phenotype: body size and shape.

Methods

Establishment and sampling of experimental populations

We established four experimental populations by transplanting the progeny of guppies from one high predation (HP) locality in the lower Guanapo River in the Northern Range of Mountains in Trinidad, West Indies into four small upstream tributaries, which are above a series of barrier waterfalls that have excluded all species of fish except *R. hartii*. These populations were established in 100–180 meter upstream reaches of small, first-order streams or tributaries of the Guanapo River. The killifish *Rivulus hartii* was the only fish species present prior to the introductions. Each experimental reach was located above a barrier waterfall that blocked immigration from downstream guppy populations. Additionally, the upper limit of each reach was bound by a waterfall to prevent emigration and establishment of populations above the

introduction streams. In March 2008, the Lower Lalaja and Upper Lalaja (hereafter, Intro1 and Intro2, respectively) were each stocked with 38 gravid G_1 females and 38 mature G_1 males. To minimize the potential for founder effects and equalize genetic diversity in each stream, the males and females were crossed and introduced into alternate streams. Each fish was given a unique subcutaneous marking of visible implant elastomer (NorthWest Marine Technology, Shaw Island, WA, USA). In March 2009, additional populations were established in the Caigual and Taylor tributaries (hereafter, Intro3 and Intro4, respectively) but the streams were stocked with 45 gravid G_1 females and 45 mature G_1 males. Previous work highlights specific details of the introduction protocols and locations of each stream (Handelsman et al. 2013, 2014; Arendt et al. 2014; Travis et al. 2014; Gordon et al. 2015).

Each month, all guppies above 14mm standard length were collected from each experimental reach and transported to the laboratory. All sampled adult male fish were identified, weighed for mass, and photographed for standard length and morphometric analysis (see Table 2.1 for sample sizes). All new population recruits were tagged with a unique mark (see above). Lateral photographs of the left side were taken with Nikon D60 digital SLR cameras equipped with Nikkor 50mm macro lenses (Nikon Inc., Melville, NY, USA) mounted on tripods. Tripod height was adjusted to yield an 8-cm field of view that was determined sufficient to eliminate any parallax within the lens area occupied by a guppy. To standardize fish position and expose homologous landmarks, a fine-tipped wetted artist's paintbrush was used to straighten the specimen and spread the fins (Fig. 2.1). A ruler was placed in each picture to set a scale in each image. All fish were returned to the experimental streams at their collection site (e.g., nearest pool or riffle) each month after being processed (Travis et al. 2014).

Sampling of native populations

In March 2008, 25–30 wild caught adult males were sampled and photographed from the HP source population (hereafter, source) and two native low predation populations that occurred downstream of the introduction reaches in the Caigual and Taylor tributaries (hereafter, LP1 and LP2). Wild caught adult males were also sampled and photographed from a third native low predation population in the Tumbason tributary in the Guanapo River (hereafter, LP3) in March 2012. Thus, body shape evolution could be compared to the ancestral HP source population, and to multiple reference LP populations, which provide a target for how the introduction populations should evolve.

Common garden assays

After one year (3–4 generations), 25 juvenile females and 25 juvenile males were collected from each of the four introduced populations (see Table 2.1 for final sample sizes). In addition, 25–30 wild caught males and females were collected from the source, LP1, and LP2 populations in 2008, and the LP3 population in 2012, and brought to the laboratory at Colorado State University to undergo common garden life history assays following Reznick (1982). Fish were reared for two generations under common garden conditions to control for maternal and environmental effects. Wild caught guppies were held in 1.5 L recirculating tanks (Aquatic Habitats, Apopka, FL, USA) connected to a custom made recirculating system and maintained on a 12-hr light cycle at 27 ± 1^{o} C. Fish were reared on standardized food levels adjusted weekly for age and number of individuals per tank (a.m. – Tetramin® tropical fish flakes, Spectrum Brands, Inc., Cincinnati, Ohio, USA; p.m. – brine shrimp nauplii (Artemia)). Food quantity was comparable to the high food level administered by Reznick (1982).

At maturity, each wild caught female was randomly crossed with a unique male to generate a G_1 generation. Each G_1 brood was reared for 29 days, at which point fish could be reliably sexed (Reznick 1982). One G_1 male and female were randomly selected from each family line, reared to maturity, and randomly outcrossed by family to propagate a G_2 generation. The G_2 generation underwent the same rearing protocol, and males were photographed (see above) on the day of sexual maturity for morphometric analysis.

Characterization of body shape and size

We used geometric morphometric methods to assess variation in body shape (Rohlf and Slice 1990; Rohlf and Marcus 1993; Zelditch et al. 2004). One high-quality photograph per adult male guppy was analyzed for body shape and used to represent that individual in morphometric analyses. Specifically, we used a landmark configuration of 8 homologous landmarks and 6 sliding semi-landmarks (Bookstein 1997) to characterize the lateral morphology of guppies (Fig. 2.1). This configuration contains 28 variables (Cartesian coordinates) describing the body shape of each specimen. Landmarks were digitized on digital images of the left lateral aspect of each specimen as described by Handelsman et al. (Handelsman et al. 2014) with TPSDig2 (Rohlf 2015). We aligned the landmark configurations and removed variation due to orientation, position, and scale with generalized Procrustes analysis (Rohlf and Slice 1990; Goodall 1991; Dryden and Mardia 1998). The Procrustes residuals (aligned coordinates) were used in all statistical analyses and shape changes were visualized by projecting the Procrustes residuals from the field and lab samples onto their respective principal components (PC). Body size was measured as centroid size, the square root of the sum of the squared distances from the centroid to each landmark, where the centroid is the mean Cartesian coordinates of each specimen.

Generalized Procrustes analysis, rotation of Procrustes residuals onto their principal components, and calculations of centroid size were performed in R (R Core Team 2015) using the geomorph package (Adams and Otarola-Castillo 2013; Adams et al. 2015).

Statistical analyses

Geometric morphometric datasets frequently contain more variables than independent observations, making the use of parametric multivariate models problematic. However, recently developed nonparametric methods utilize multivariate distances (Anderson 2001a) and permutation tests (Anderson 2001b) that are particularly useful for high-dimensional data (Collyer et al. 2015). We tested for differences in body shape and size in the common garden experiments by fitting nonparametric multivariate analysis of variance models (npMANOVA) with the reduced residual permutation procedure (RRPP) (Collyer et al. 2015) to the Procrustes residuals. We tested for evolution and plasticity by modeling body shape as a function of population, rearing treatment, and the population x rearing treatment interaction, using the natural log of centroid size as a covariate. npMANOVA included 10,000 random permutations of RRPP (Collyer et al. 2015). *Post-hoc* comparisons of pairwise means were generated from the same RRPP permutations (Collyer et al. 2015). npMANOVA with RRPP was perfomed with the geomorph package in R (Adams and Otarola-Castillo 2013; R Core Team 2015).

Heritability of body shape and size

We tested for the presence of heritable variation in body shape and size in the source population by estimating broad-sense heritability (H^2) with full-siblings. Full-sibling analysis can yield upwardly-biased estimates of H^2 because estimates include dominance and maternal effects

(Falconer and Mackay 1996; Conner and Hartl 2004), however, we attempted to minimize these maternal effects by rearing the progeny of wild caught individuals through two generations in a common environment. In addition, we were less concerned with a precise estimate of H^2, and more interested in whether significant heritable variation was present, as this is the raw material for selection. G_2 laboratory-reared fish were used to obtain H^2 estimates. Twenty full sibling G_2 families (n = 4 brothers per family) descendant from the source population were used to partition phenotypic variance in the source population. We fit an animal model with the MCMCglmm package for R (Hadfield 2010; Wilson et al. 2010) to decompose variation in body shape and size into additive genetic variance and residual effects (Table 2.2). Principal components with non-zero eigenvalues (18 axes) were used to describe shape, and centroid size was used as the metric of body size. Animal models for shape and size were run for 500,000 iterations with a 100,000 iteration burn-in period and a thinning interval of 100 iterations. We employed weak proper priors after confirming that the choice of prior had little effect on H^2 estimates. Heritability estimates are reported as the posterior mode of the trait variance divided by the sum of the posterior modes of the trait variance and residual variance (Wilson et al. 2010; Table 2.2). Highest posterior densities (95%) of heritability estimates are also reported (Wilson et al. 2010; Table 2.2).

Results

Potential for evolution of body shape and size

To estimate H^2 of body size and shape from full-siblings, we used centroid size and the first 18 principal components of the Procrustes coordinates characterizing body shape.

Heritability of body size was 0.71 and H^2 of the principal components describing body shape ranged from 0.49–0.54 (Table 2.2).

Changes in body size and shape in native and experimental populations over time

To establish the initial pattern of body shape in the ancestral population, and the expected direction of change in the LP environment, we characterized body shape in the HP source population and three native low predation populations. We found field populations of naturally occurring low predation populations were significantly larger than the source population (Fig. 2.2). Native low predation populations also showed divergent body shapes compared to the source population: LP1 and LP3 exhibited a smaller head, deepening of the anterior aspect of the body, and a larger caudal peduncle than the source population (Fig. 2.3). The LP2 population also exhibited a deeper body but this was coupled with a more upturned mouth than the source population (Fig. 2.4).

To characterize the pattern of phenotypic change in the wild, we evaluated the introduced populations each month for 12 months after they were established. Body size increased in all four experimental populations within the first month and remained larger than the source population for the following 12 months (Figs. 2.2A, 2.2B). Body shape diverged away from the phenotype of the source population and beyond the native low predation populations (Figs. 2.3A, 2.3B, 2.4B). Thus, experimental populations increased body size, developed a deeper abdomen, a larger caudal peduncle, and a more upturned mouth (Intro3 and Intro4 only) relative to the ancestral source population.

Common garden assays of body shape and size

To test the genetic basis of changes in body shape and patterns of plasticity, we examined how body shape varied across the different populations when raised under common garden conditions that mimicked the presence or absence of predator chemical cue in the environment. We found that the differences in body size observed in nature (Figs. 2.2A, 2.2B) were not maintained in the G_2 progeny reared in the laboratory. Body size was, however, sensitive to the rearing environment ($P < 0.001$; Table 2.3), and similar to the pattern in the field: guppies from all populations tended to be larger when predator chemical cue was absent from the environment (Figs. 2.5A, 2.5B).

Body shape was different between the source and native low predation populations between the two common garden rearing environments ($P < 0.001$; Tables 2.3, 2.4). Notably, the way in which body shape differed in nature was partially conserved in the laboratory. In the common garden assays, native low predation guppies retained deeper bodies, but exhibited shorter caudal peduncles than the source population (Figs. 2.6A, 2.6B). Moreover, native low predation populations had a more downturned mouth than the source population.

Three of the four introduction populations (Intro1, Intro2, and Intro4) were significantly different from the source population when raised in the common garden (Table 2.4). Generally, divergence resembled a shift away from the source and toward the phenotypes of the native low predation populations, with a deeper body, larger head, and shorter caudal peduncle in the laboratory (Figs. 2.6A, 2.6B), suggesting rapid adaptive evolution. However, Intro1 and Intro2 diverged into novel phenotypic space relative to both the source and low predation populations, producing an upturned mouth in addition to a deeper abdomen (Fig. 2.7A).

We also tested whether adult body shape was sensitive to the presence of predator chemical cue during ontogeny. The source and native low predation populations were not plastic and developed the same body shape when reared in the presence and absence of predator chemical cue ($P > 0.05$; Table 2.4). In contrast, Intro1, Intro2, and Intro3 were plastic ($P < 0.001$; Table 2.4) and developed a body shape more similar to the source population when reared in the presence of predator chemical cue (ancestral-like environment). Intro4 also showed a tendency to resemble the source phenotype when reared with predator chemical cue, but the pairwise comparison between Intro4 reared with and without predator cue was not statistically significant ($P = 0.068$; Table 2.4).

Discussion

To assess the nature and timing of phenotypic change following the colonization of a novel environment, we quantified male body size and shape in four guppy populations each month following their introduction to a low predation environment and contrasted them with the ancestral high predation source population and three naturally occurring low predation populations within the same drainage. Comparisons to the source population provided insight into the magnitude of divergence, whereas comparisons to the native, and presumably locally adapted, low predation populations provided insight into whether the direction of change was adaptive. Following the experimental introductions, all four introduced populations exhibited rapid phenotypic divergence in male body size and shape from the ancestral population. In the field, we observed an immediate shift in the phenotype of the introduced populations away from the source, suggesting plasticity was responsible for divergence rather than recruitment of offspring with differing body sizes and shapes (see below). Body size increased relative to the

source population, and either approached or matched the native low predation populations (Figs. 2.2A, 2.2B). Relatively large body size at maturity is characteristic of low predation populations of guppies, and reflects adaptation in response to natural and sexual selection favoring larger males in low predation environments (Reznick 1982; Reznick and Endler 1982; Magurran 2005). Our finding that male body size increased in the introduced populations is consistent with previous translocation experiments that found an increase in body size in low predation environments (Reznick et al. 1990, 1997).

In the months following the introductions, body shape also rapidly diverged from the source phenotype, and, in some cases, beyond the expected phenotype that was seen in the native low predation populations (Figs. 2.3A, 2.3B). All introduced populations exhibited smaller heads, deeper bodies, and larger caudal peduncles. These morphological patterns have been previously found in guppies and other small prey fish that experience low levels of predation (Alexander and Breden 2004; Langerhans and Dewitt 2004). However, two of the introduction populations, Intro3 and Intro4, produced a seemingly novel phenotype, with a more upturned mouth orientation than observed in any of the native populations (Fig. 2.4B).

Body morphology appears to be a strong indicator of locally adapted ecotypes in fish populations, with different body regions likely responsive to different sources of selection. For example, head shape may be associated with foraging ecology (Robinson and Wilson 1995), whereas the shape of the caudal peduncle may vary in response to stream flow or the evasion of predators (Webb 1978; Langerhans and Reznick 2009). Body depth also increases in response to structural complexity of habitats and benthic foraging in sticklebacks (*Gasterosteus aculeatus*) (Schluter and McPhail 1992) and perch (*Perca fluviatilis*) (Svanbäck and Eklöv 2002). In addition, guppies from high and low predation populations differ in food preference (Bassar et al.

2010; Zandonà et al. 2011) and swimming performance (Ghalambor et al. 2004; Walker et al. 2005). Low predation guppy populations navigate more complex habitats and exploit a diverse, and often benthic, food base that should favor deeper bodies with a terminal or ventral orientation of the mouth. Thus, the phenotypic divergence away from the high predation body shape, and towards the low predation phenotype likely reflects adaptive changes. Yet, body shape in fish is also known to be highly plastic in response to different environmental cues (reviewed in Robinson and Parsons 2002), and the rapid changes observed in the introduction populations suggests the same.

To test if the observed phenotypic differences between the natural and introduction populations had a genetic basis, we used laboratory common garden breeding experiments. Because the four experimental populations were evaluated one year (3–4 generations) after the introductions occurred, any observed differences were interpreted as evidence for evolutionary divergence. In contrast to our field observations that low predation and introduced guppies matured at a larger size than the source population, all populations tended to mature at a common centroid size when reared under common garden conditions (Figs. 2.5A, 2.5B). Notably, this pattern was driven by native low predation and introduced populations maturing at smaller sizes in the laboratory than in the wild, while the source population matured at a similar size in both environments (Figs 2.2A, 2.2B, 2.5A, 2.5B). However, body size was sensitive to the presence of predator chemical cue in the rearing environment. All populations except LP2 matured at a smaller size when the cue was present versus when it was not (Figs. 2.5A, 2.5B).

Predation pressure is known to favor a smaller size at maturity in Trinidadian guppies (Reznick and Bryga 1996). Further, natural experiments have shown that guppies typically mature at a larger body size when predation risk is reduced (Reznick 1982; Reznick and Bryga

1987; Reznick et al. 1990, 1997). While our findings are consistent with previous work showing that the perception of predation risk correlates with size at maturity, we did not find evidence for a genetic basis underlying differences in size at maturity between the source, the three native low predation, and the four introduced populations. Despite the lack of evolutionary divergence in body size, we estimated H^2 of body size to be 0.71 (95% CI: 0.40, 0.88; Table 2.2) in the source population, so a lack of divergence was unlikely to have been constrained by a lack of genetic variance. Instead, the results are consistent with view that adaptive plasticity weakens the strength of selection and that environmentally induced phenotypic variation can mask underlying heritable variation from selection.

Unlike body size, which converged among populations in the laboratory, differences in body shape in the field did appear to have a genetic basis. Native low predation guppies had deeper bodies than the high predation source in the field (Figs. 2.3A, 2.3B) and under common garden conditions (Figs. 2.6A, 2.6B; Table 2.4). This result is consistent with previous contrasts of high and low predation guppy populations (e.g., Alexander and Breden 2004) and other small prey species (Langerhans and Dewitt 2004). Similarly, after only 3–4 generations removed from their major predators, the introduced populations showed evidence of genetic divergence from the source population. However, the introduction populations were still distinct from the native low predation populations ($P < 0.001$ for all pairwise comparisons; Table 2.4), suggesting they have yet to achieve the locally adapted phenotype. In addition, two populations, Intro1 and Intro2, diverged, in part, into a novel phenotypic space (Fig. 2.7A). They exhibited a substantially upturned mouth coupled with a deepening of the abdomen (Fig. 2.7A). This change may reflect an alternate strategy to evolve a deeper body, similar to LP2, Intro3, and Intro4 in the

field (Fig. 2.4B), and/or indicate an underlying constraint due to a change in trait correlations (Handelsman et al. 2014).

None of the native guppy populations (source, LP1, LP2, LP3) were plastic with regard to body shape when reared with or without predator chemical cue (Table 2.4). However, three of the introduction populations, Intro1, Intro2, and Intro3, were plastic in response to the two rearing environments. Intro 4 showed a similar trend, but was not statistically significantly different between rearing environments. Specifically, the introduction populations demonstrated an "anchor effect" in that they developed body shapes similar to the high predation source population when reared in the presence of predator chemical cue, but diverged toward the low predation phenotype when reared in the absence of the cue (Figs. 2.6, 2.7). Thus, similar to our previous findings for head morphology (Torres-Dowdall et al. 2012), the introduction populations evolved plasticity from a non-plastic ancestral genotype.

These results provide empirical evidence for a potentially important role of phenotypic plasticity in the establishment and subsequent evolution of populations occupying new environments. First, body size and shape exhibited plasticity in response to a new environment. Some of the plasticity appeared adaptive, given the phenotypic changes either matched or were in the direction of naturally occurring low predation populations (Figs. 2.5–2.7). Plasticity also produced novel phenotypes in two experimental populations (Intro1 and Intro2, Fig. 2.7B) that fell outside the range of natural variation observed in native populations. Specifically, the combination of a deeper body (expected) with an upturned mount (unexpected) represents a unique combination of traits that likely is a transient phenotype on the way to the more typical low predation body shape. Thus, while adaptive plasticity may have contributed to helping the experimental populations persist in the new environment, the novel phenotypic changes may

represent another pathway toward a low predation phenotype. Alternatively, the novel combination of traits may reflect a shift in the underlying genetic correlations between these traits due a founder effect or drift (Handelsman et al. 2014). Tracking these populations in the future will allow evaluating how stable these trait correlations are over time. Nevertheless, despite exhibiting plasticity that appears to be both adaptive and non-adaptive in direction, there is still strong evidence for rapid and adaptive evolution of body shape that is replicated across the introduction populations. Similarly, patterns of gene expression in two of the introduced populations (Intro1 and Intro2) also showed evidence of evolutionary divergence from the source population and toward the low predation populations, but only when plasticity produced novel or non-adaptive expression profiles (Ghalambor et al. 2015). These findings suggest that both adaptive and non-adaptive plasticity can have roles in adaptive evolution (Price et al. 2003; Ghalambor et al. 2007; Handelsman et al. 2013). The former is likely to increase fitness by better pairing the phenotype to the environment, while the latter should increase the strength of selection, and thus, the pace of divergence. Further, each pair of introductions (2008, 2009) was sampled from the same source population and exhibited similar diversity across neutral genetic markers (Reznick unpublished), so it is unlikely that these results are simply due to founder effects or genetic drift.

Previous studies have also documented rapid evolution in natural populations (Reznick et al. 1997), quantified the mode and strength of natural selection (Kingsolver et al. 2001; Siepielski et al. 2009), and statistically partitioned genetic from environmental responses to selection (Kruuk et al. 2000; Morrissey et al. 2010; Pemberton 2010). Here, we provide empirical evidence that adaptive plasticity in a trait (e.g., body size) might slow evolutionary divergence, while a non-plastic trait (body shape) can evolve plasticity during the initial stages of

evolutionary divergence in response to directional selection. These patterns are consistent with the idea that traits exhibiting near complete or perfect plasticity do not diverge, because the plastic responses are likely to be under stabilizing rather than directional selection (Price et al. 2003). In contrast, there is relatively strong divergent selection on traits where plasticity produces incomplete or novel phenotypes.

Although we cannot definitively say which component of the environment drove these evolutionary and plastic changes in the introduced populations, changes in head morphology were likely associated with changes in food resources and foraging behavior in the low predation streams; guppies from high predation environments feed selectively on high quality invertebrate prey, while those from low predation environments feed indiscriminately on detritus, algae, and invertebrates (Dussault and Kramer 1981; Zandonà et al. 2011). Furthermore, the differences in head shape among high and low predation guppies correspond to those between limnetic and benthic sticklebacks, which share similar differences in foraging and food preferences (Schluter and McPhail 1992). Shape changes in the body depth and the caudal peduncle could have either been due to the release from predators, the reduced water velocity in these small streams (Webb 1978), or utilization of more complex habitat (Dussault and Kramer 1981; Svanbäck and Eklöv 2002). Naturally-occurring low predation guppies exhibit larger body size than high predation guppies (Reznick and Endler 1982), and prior translocations have confirmed that a larger body size is favored in low predation environments (Reznick et al. 1997). Thus, many of the plastic and evolutionary changes we detected here correspond to expectations for adaptive change based on native low predation populations of guppies and other fish species.

Over time, monitoring of these populations will reveal whether the directional changes in body shape persist in the face of temporally varying selection pressures (Siepielski et al. 2009),

and whether the highly plastic trait, body size, ultimately diverges, possibly via genetic assimilation (Price et al. 2003; Grether 2005; West-Eberhard 2005; Ghalambor et al. 2007; Lande 2009). In light of our findings, it seems unlikely that adaptive divergence is easily predicted from standing genetic variation measured in an ancestral environment. Phenotypic plasticity alters the distribution of phenotypes exposed to selection in new environments, and can have dramatic effects on the strength and direction of divergent selection. Thus, plasticity can play an important role in the pace of contemporary evolution and the probability that traits evolve.[4]

[4] This research was approved by the Colorado State University Institutional Animal Care and Use Committee (protocols # 12-3269A and 09-1348A). This research was supported by the National Science Foundation Faculty Early Career Development grant DEB-0846175 (Cameron Ghalambor) and the National Science Foundation Frontiers in Integrative Biological Research grant EF-0623632 to David Reznick.

Tables and figures

Table 2.1 Population sample sizes of male guppies used to evaluate body size and shape from field observations and laboratory common garden assays. Laboratory sample sizes reflect the number of individuals per treatment.

Sample Sizes for Populations from the field	
Native Population	**n**
Source	67
LP1	21
LP2	33
LP3	19

	Number of Months from Time of Introduction											
Introduction Population	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Intro1	5	5	26	49	41	42	39	57	55	68	58	56
Intro2	8	9	44	52	24	20	22	35	33	36	41	57
Intro3	52	37	34	29	31	44	47	43	48	54	52	57
Intro4	45	25	48	76	62	24	19	21	32	40	46	56

Sample Sizes for Populations from the Laboratory	
Native Population	**n**
Source	23
LP1	21
LP2	15
LP3	13
Introduction population	**n**
Intro1	19
Intro2	22
Intro3	13
Intro4	12

Table 2.2 Broad-sense heritability (H^2) of body shape (Principal Components (PC) 1-18, which together explain greater than 99% of the variance) and body size (centroid size) in the native high predation source population.

Trait	H^2 (95% highest posterior density)
PC1	0.5199 (0.4181, 0.6313)
PC2	0.5443 (0.4278, 0.6339)
PC3	0.5284 (0.4309, 0.6442)
PC4	0.5156 (0.4290, 0.6427)
PC5	0.5366 (0.4272, 0.6501)
PC6	0.4978 (0.4221, 0.6414)
PC7	0.5411 (0.4332, 0.6453)
PC8	0.5020 (0.4241, 0.6384)
PC9	0.5200 (0.4297, 0.6511)
PC10	0.5360 (0.4265, 0.6461)
PC11	0.5474 (0.4334, 0.6426)
PC12	0.5442 (0.4312, 0.6416)
PC13	0.5540 (0.4460, 0.6557)
PC14	0.5189 (0.4222, 0.6555)
PC15	0.5265 (0.4400, 0.6667)
PC16	0.5302 (0.4244, 0.6372)
PC17	0.5216 (0.4301, 0.6369)
PC18	0.5484 (0.4132, 0.6371)
Centroid size	0.7064 (0.4001, 0.8820)

Table 2.3 Results of the nonparametric multivariate analysis of variance model (npMANOVA) testing for variation in body shape between populations and laboratory common garden rearing environments (with and without predator cue). The model was run with 10,000 random permutations using the reduced residual permutation procedure (RRPP).

	d.f.	**SS**	**MS**	R^2	**Z**	***P*-value**
Log(CS)	1	0.0120	0.0120	0.058	13.307	**<0.001**
Population	7	0.0628	0.0090	0.304	12.221	**<0.001**
Treatment	1	0.0046	0.0046	0.022	8.390	**<0.001**
Log(CS):Population	7	0.0035	0.0005	0.017	1.053	0.322
Log(CS):Treatment	1	0.0004	0.0004	0.002	0.829	0.442
Population:Treatment	7	0.0054	0.0008	0.026	1.688	**0.003**
Log(CS):Population:Treatment	7	0.0030	0.0004	0.015	0.979	0.458
Error	244	0.1150	0.0005			

CS represents centroid size; Treatment represents the two rearing environments in the laboratory; d.f. represents degree of freedom; SS represents sums of squares; MS represents mean squares; R^2 represents the square of the correlation coefficient; Z = effect size scaled to units of standard deviations.

Table 2.4 Pairwise Procrustes distances (above diagnal) and *P*-values (below diagnal). Pairwise comparisons were based on npMANOVA in Table 2.3.

		Source		**Intro1**		**Intro2**		**Intro3**		**Intro4**		**LP1**		**LP2**		**LP3**	
		w/ Cue	**w/out Cue**	**w/ Cue**	**w/out Cue**	**w/ Cue**	**w/out Cue**	**w/ Cue**	**w/out Cue**	**w/ Cue**	**w/out Cue**	**w/ Cue**	**w/out Cue**	**w/ Cue**	**w/out Cue**	**w/ Cue**	**w/out Cue**
Source	**w/ Cue**		0.009	0.014	0.023	0.012	0.020	0.013	0.011	0.015	0.022	0.029	0.031	0.026	0.030	0.034	0.036
	w/out Cue	0.239		0.014	0.017	0.014	0.014	0.020	0.010	0.017	0.020	0.027	0.027	0.024	0.025	0.032	0.032
Intro1	**w/ Cue**	**0.021**	**0.027**		0.016	0.008	0.013	0.022	0.018	0.023	0.029	0.032	0.032	0.029	0.032	0.037	0.037
	w/out Cue	**<0.001**	**0.003**	**0.011**		0.018	0.009	0.033	0.022	0.026	0.026	0.026	0.022	0.023	0.021	0.031	0.029
Intro2	**w/ Cue**	0.056	**0.022**	0.473	**0.004**		0.015	0.020	0.017	0.019	0.027	0.029	0.030	0.025	0.030	0.034	0.034
	w/out Cue	**<0.001**	**0.012**	**0.040**	0.295	**0.015**		0.030	0.021	0.025	0.028	0.030	0.027	0.026	0.026	0.034	0.033
Intro3	**w/ Cue**	0.081	**0.003**	**0.001**	**<0.001**	**0.001**	**<0.001**		0.018	0.017	0.027	0.038	0.040	0.033	0.038	0.039	0.042
	w/out Cue	0.213	0.251	**0.007**	**0.001**	**0.017**	**0.001**	**0.026**		0.015	0.015	0.026	0.027	0.022	0.025	0.027	0.029
Intro4	**w/ Cue**	**0.039**	**0.017**	**0.001**	**<0.001**	**0.002**	**<0.001**	**0.034**	0.112		0.016	0.027	0.027	0.018	0.024	0.027	0.029
	w/out Cue	**0.001**	**0.002**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.092	0.068		0.023	0.022	0.018	0.018	0.020	0.023
LP1	**w/ Cue**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**		0.010	0.015	0.017	0.026	0.024
	w/out Cue	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	0.201		0.014	0.010	0.024	0.020
LP2	**w/ Cue**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**0.014**	**0.017**	**0.030**	**0.037**		0.012	0.020	0.020
	w/out Cue	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**0.016**	**0.021**	0.259	0.188		0.018	0.017
LP3	**w/ Cue**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.005**	**<0.001**	**<0.001**	**0.005**	**0.017**		0.011
	w/out Cue	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.002**	**<0.001**	**0.002**	**0.008**	**0.032**	0.375	

P-values less than 0.05 are in bold. "w/ cue" represents values for laboratory fish reared with predator cue. "w/out cue" represents values for laboratory fish reared without predator cue.

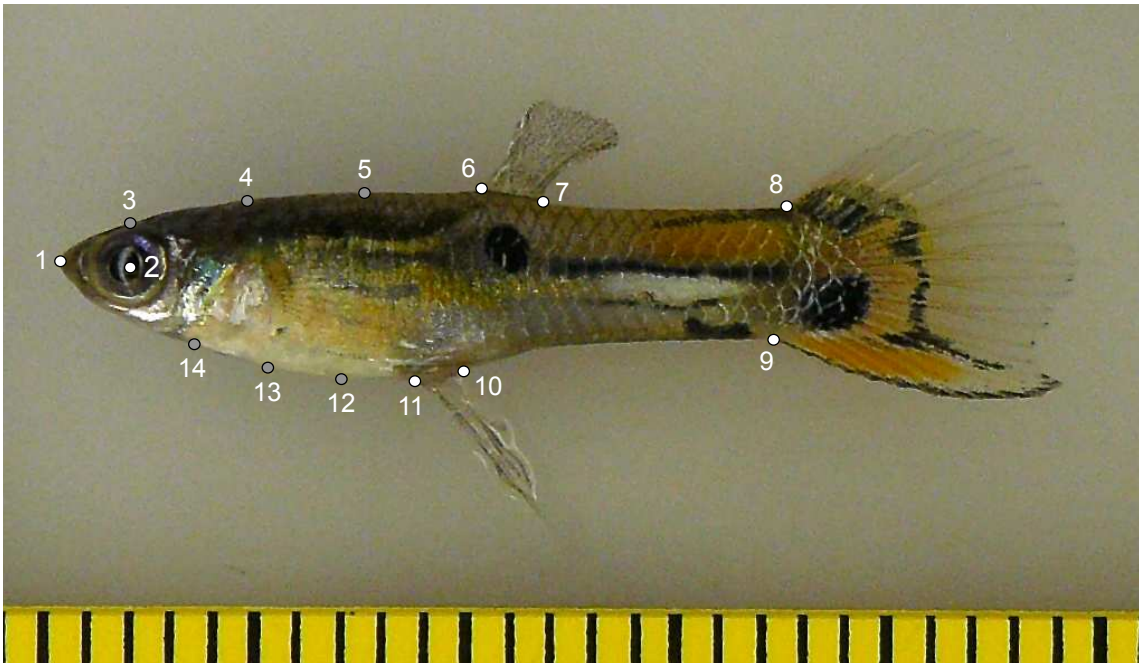

Figure 2.1 Example photograph of the left lateral aspect of an adult male guppy with fins spread. Landmarks used for geometric morphometric analysis are labeled (1–14). Homologous landmarks are represented by white circles (numbered 1, 2, 6–11) and semi-landmarks are identified by gray circles (3–5, 12–14).

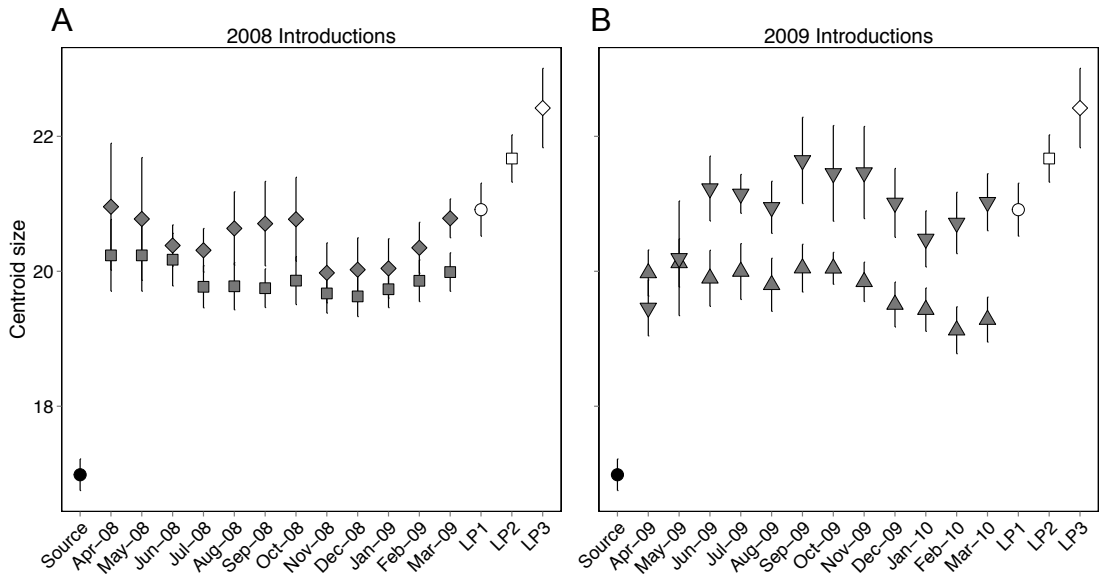

Figure 2.2 Changes in body size (represented by centroid size) of experimentally introduced populations in the wild over the 12 months post-introduction: A) Intro-1 (gray squares) and Intro-2 (gray diamonds) in 2008, and B) Intro-3 (upright gray triangles) and Intro-4 (upside-down gray triangles) in 2009. Body size of the native high predation source population (black circles) from 2008 and the native low predation populations: LP1 (open circles) from 2008, LP2 (open squares) from 2008, and LP3 (open diamond) from 2012 are shown in both panels for comparison.

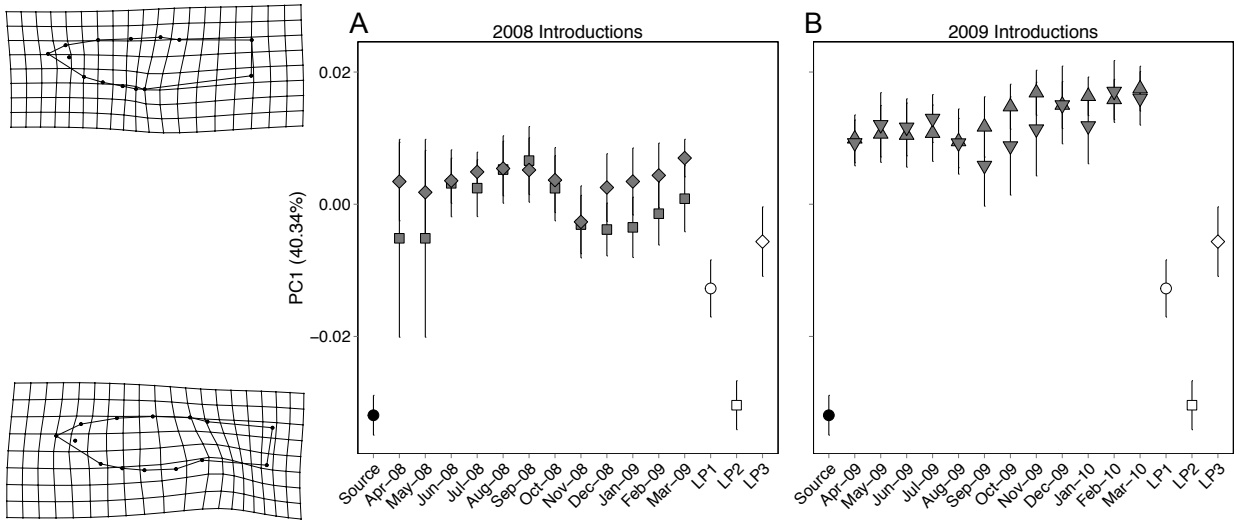

Figure 2.3 Changes in body shape (represented by the first Principal Component (PC1), which explains 40.34% of the variance) of experimentally introduced populations in the wild over the 12 months post-introduction: A) Intro-1 (gray squares) and Intro-2 (gray diamonds) in 2008, and B) Intro-3 (upright gray triangles) and Intro-4 (upside-down gray triangles) in 2009. Body shape of the native high predation source population (black circles) from 2008 and the native low predation populations: LP1 (open circles) from 2008, LP2 (open squares) from 2008, and LP3 (open diamond) from 2012 are shown in both panels for comparison.

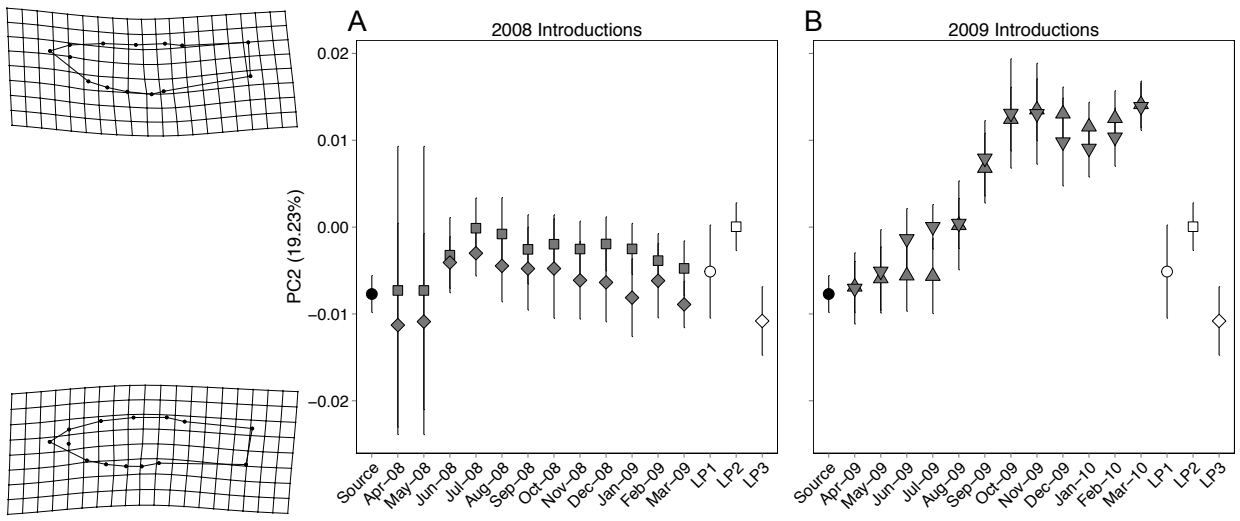

Figure 2.4 Changes in body shape (represented by the second Principal Component (PC2), which explains 19.23% of the variance) of experimentally introduced populations in the wild over the 12 months post-introduction: A) Intro-1 (gray squares) and Intro-2 (gray diamonds) in 2008, and B) Intro-3 (upright gray triangles) and Intro-4 (upside-down gray triangles) in 2009. Body shape of the native high predation source population (black circles) from 2008 and the native low predation populations: LP1 (open circles) from 2008, LP2 (open squares) from 2008, and LP3 (open diamond) from 2012 are shown in both panels for comparison.

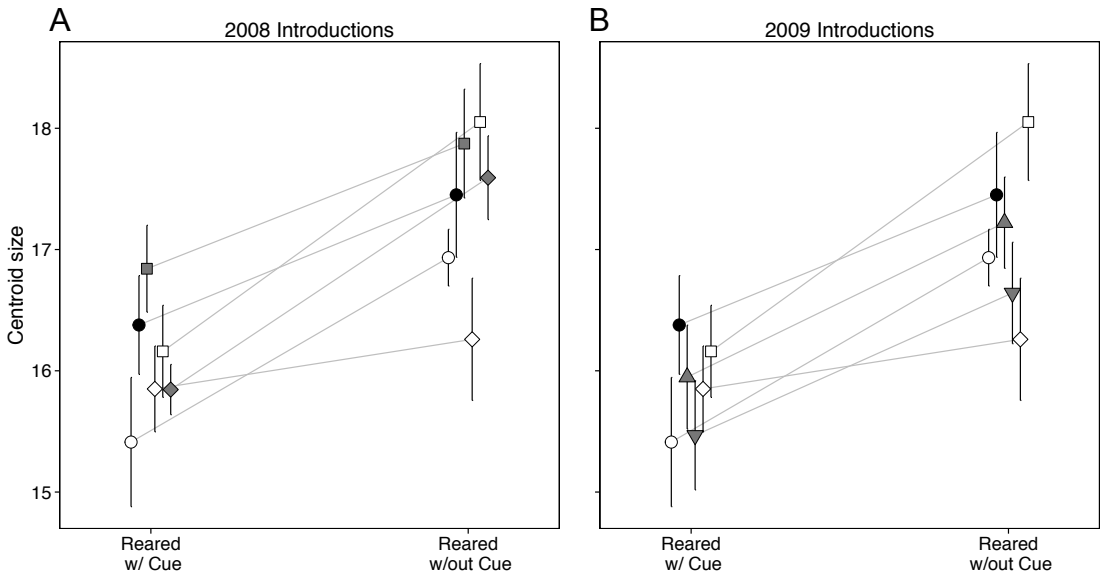

Figure 2.5 Differences in body size (represented by centroid size) in laboratory common garden assays, comparing experimentally introduced populations 12 months post-introduction: A) Intro-1 (gray squares) and Intro-2 (gray diamonds) in 2008, and B) Intro-3 (upright gray triangles) and Intro-4 (upside-down gray triangles) in 2009. Centroid size of the native high predation source population (black circles) from 2008 and the native low predation populations: LP1 (open circles) from 2008, LP2 (open squares) from 2008, and LP3 (open diamond) from 2012, in laboratory common garden assays are shown in both panels for reference. Reaction norms are shown for full siblings reared with or without the presence of predator chemical cue.

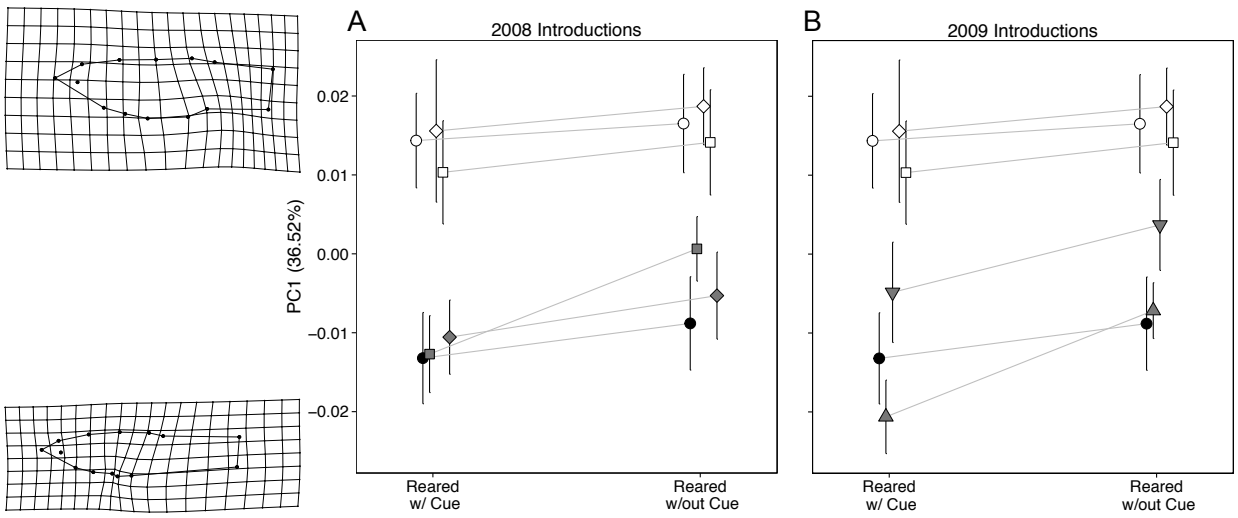

Figure 2.6 Differences in body shape (represented by the first Principal Component (PC1), which explains 36.52% of the variance) in laboratory common garden assays, comparing experimentally introduced populations 12 months post-introduction: A) Intro-1 (gray squares) and Intro-2 (gray diamonds) in 2008, and B) Intro-3 (upright gray triangles) and Intro-4 (upside-down gray triangles) in 2009. PC scores of the native high predation source population (black circles) from 2008 and the native low predation populations: LP1 (open circles) from 2008, LP2 (open squares) from 2008, and LP3 (open diamond) from 2012, in laboratory common garden assays are shown in both panels for reference. Reaction norms are shown for full siblings reared with or without the presence of predator chemical cue.

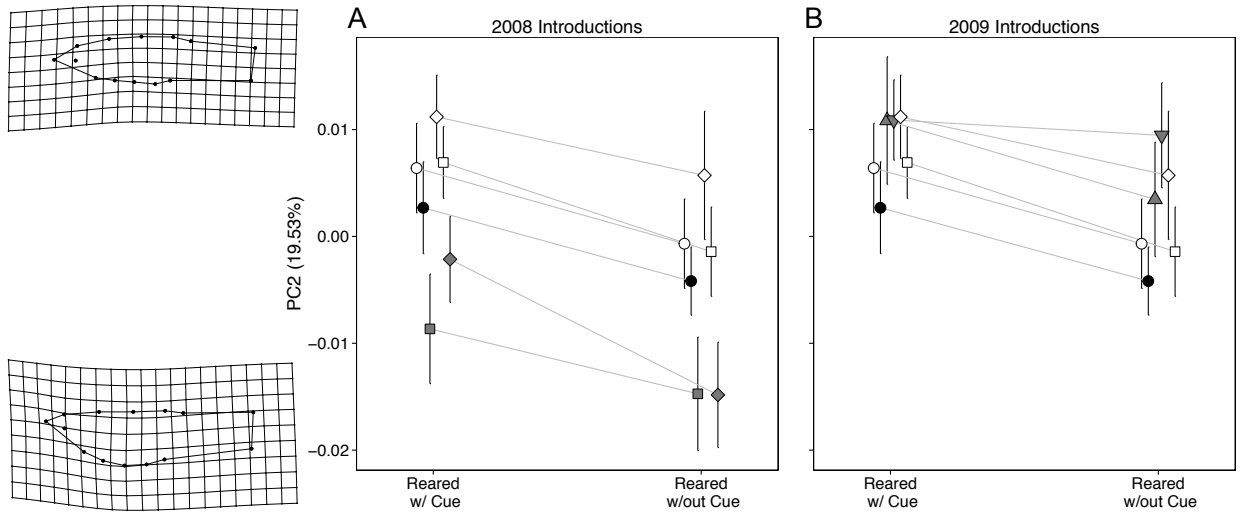

Figure 2.7 Differences in body shape (represented by the second Principal Component (PC2), which explains 19.53% of the variance) in laboratory common garden assays, comparing experimentally introduced populations 12 months post-introduction: A) Intro-1 (gray squares) and Intro-2 (gray diamonds) in 2008, and B) Intro-3 (upright gray triangles) and Intro-4 (upside-down gray triangles) in 2009. PC scores of the native high predation source population (black circles) from 2008 and the native low predation populations: LP1 (open circles) from 2008, LP2 (open squares) from 2008, and LP3 (open diamond) from 2012, in laboratory common garden assays are shown in both panels for reference. Reaction norms are shown for full siblings reared with or without the presence of predator chemical cue.

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Chapter 3. The time course of rapid evolution in male life histories: divergence of trait means and their plasticity in experimental populations of Trinidadian guppies[5]

Introduction

A fundamental problem in evolutionary biology is how phenotypically plastic traits evolve in response to natural selection (Schlichting and Pigliucci 1998; West-Eberhard 2003; Ghalambor et al. 2007; Paenke et al. 2007; Lande 2009). The evolution of plastic traits in natural populations imposes several conceptual challenges that derive from the problem of the environment acting as both a source of natural selection and as the cue that induces phenotypic plasticity (Scheiner 1993; Schlichting and Pigliucci 1998). Because the environment alters the expression and distribution of phenotypic variation, predicting evolutionary responses to selection is a major challenge in natural populations (Kruuk et al. 2003; Wilson et al. 2006; Morrissey et al. 2010; Wilson et al. 2010; Merilä and Hendry 2014). Indeed, environmentally induced or non-heritable phenotypic variation has traditionally been thought to shield genotypes from selection and slow evolutionary responses (Grant 1977; Falconer 1981; Levin 1988; Price et al. 2003; Ghalambor et al. 2007). However, recent theoretical and empirical studies have emphasized that how the environment alters the distribution of phenotypes relative to the direction of selection will influence whether plasticity constrains or facilitates evolutionary responses (e.g., Falconer 1990; Huey et al. 2003; Grether et al. 2005; Ghalambor et al. 2007; Conover et al. 2009; Handelsman et al. 2013). For example, when the environment alters the distribution of phenotypes towards a local optimum (i.e., adaptive plasticity), directional

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selection in that environment should be reduced (Ancel 2000: 200; Huey et al. 2003; Price et al. 2003; Paenke et al. 2007; Ghalambor et al. 2015). Nevertheless, such adaptive plasticity may play an important initial step in the process of evolutionary differentiation by allowing populations to persist in new environments and providing an opportunity for selection to act (Robinson and Dukas 1999; Price et al. 2003; Lande 2009; Wund 2012). Yet, plastic responses do not necessarily remain static over time, and can also evolve in response to selection (Via and Lande 1985; Gavrilets and Scheiner 1993; Lande 2009; Torres-Dowdall et al. 2012; Lande 2014). Indeed, empirical studies have demonstrated that reaction norms can evolve relatively rapidly in response to selection (Murren et al. 2014). Thus, because the patterns of environmentally induced and heritable variation may not remain stable, the potential for the joint evolution of both traits and their plasticity poses a fundamental challenge when attempting to predict how populations will evolve in response to selection. However, few studies have documented the joint or independent evolution of traits and their plasticity during the early stages of population divergence.

A powerful framework for testing how selection acts on phenotypically plastic traits is to track genetic and plastic changes following the colonization of a new environment (Reznick and Ghalambor 2001; Ghalambor et al. 2007). Such an approach allows for direct comparisons of how trait means and their plasticity diverge between the ancestral source population and descendant derived populations, and for testing specific predictions on how phenotypically plastic traits evolve (Gotthard and Nylin 1995; Ghalambor et al. 2007; Wund 2012). For example, if the ancestral population exhibits adaptive plasticity, such that the new environment shifts the mean phenotype towards the local optimum, the potential for an evolutionary response should depend on the proximity of the induced phenotype to the new optimum; the closer to the

optimum the weaker directional selection should be (Ancel 2000; Price et al. 2003; Paenke et al. 2007). In contrast, plasticity could also result in a non-adaptive mismatch between the phenotype and the environment, resulting in stronger directional selection and rapid evolution (Grether et al. 2005; Ghalambor et al. 2007; Conover et al. 2009; Handelsman et al. 2013; Ghalambor et al. 2015). The relative importance of these processes likely depends on the stability of the environment, physiological constraints, and the amount of genetic variation for a given trait across environments (Levins 1968; Arnold 1983; Lande and Arnold 1983; Schlichting and Pigliucci 1998).

Life history traits are well suited for testing how selection acts on plastic traits, given that they are closely tied to fitness, known to rapidly evolve, and exhibit plasticity to a diversity of environmental conditions (Stearns and Koella 1986; Reznick 1990; Price and Schluter 1991; Fox et al. 1999; Martin and Leberg 2011; Torres-Dowdall et al. 2012). Furthermore, life history theory provides a strong conceptual framework for predicting how traits such as the age and size at maturity should evolve in response to changes in mortality risk (Gadgil and Bossert 1970; Law 1979; Roff 1992; Stearns 1992; Charlesworth 1994). The Trinidadian guppy (*Poecilia reticulata*) is a classic example of how life history traits evolve in response to changes in extrinsic mortality risk (Haskins et al. 1961; Seghers 1973; Seghers 1974a; Seghers 1974b; Reznick 1982; Reznick and Endler 1982). High predation (HP) populations of guppies occur in lowland streams where they coexist with a suite of larger piscivorous fishes and experience high extrinsic mortality. Upstream, fish communities become less diverse because natural barriers gradually exclude predators and low predation (LP) populations of guppies inhabit smaller tributary and headwater streams where they co-occur with only a singe fish species, *Rivulus hartii* (Endler 1978; Reznick and Endler 1982; Torres Dowdall et al. 2012). In concordance with predictions from life history

theory, HP male guppies typically grow faster and mature earlier and at a smaller size than males from LP populations (Reznick 1982; Reznick 1990; Reznick et al. 1990; Arendt and Reznick 2005; Magurran 2005; Handelsman et al. 2013). In contrast, smaller males are less competitive for mates in LP environments because females typically prefer to mate with larger males (Reynolds and Gross 1992; Magellan et al. 2005). Male guppies also exhibit asymptotic growth trajectories following sexual maturity and can reach larger body sizes by delaying sexual maturation and prolonging resource allocation to somatic growth. Previous work has shown a genetic basis to these differences and the capacity for rapid evolutionary change in life histories following experimental introductions in nature (reviewed in Reznick and Bryga 1987; Reznick et al. 1990; Reznick et al. 1997).

In the current study, we take advantage of the capacity for rapid evolution in Trinidadian guppies by experimentally introducing guppies from a HP environment into four replicate LP streams that previously contained *R. hartii* and lacked guppies. Two of these populations were experimentally introduced in 2008 and two were introduced in 2009. In each year, one of the introduction streams had its canopy thinned to allow for more primary productivity (Kohler et al. 2012; Travis et al. 2014). We conducted annual common garden experiments to test if the time course of evolutionary divergence between the HP source and the LP descendant populations, differed between the canopy treatments. These experiments simulated two important environmental differences between the source population's environment (i.e., the ancestral environment; higher predation risk and higher food availability) and a typical upstream environment (i.e., the derived environment; lower predation risk and lower food availability). We used this experimental approach to evaluate whether or not males in the four experimentally introduced populations showed evidence of adaptive evolutionary divergence. Specifically, we

tested if trait means shifted away from the ancestral HP condition and toward an LP life history, and if the rate of adaptation was related to the canopy treatment. We also tested whether plasticity in response to predator cues and food level was adaptive or non-adaptive, and if patterns of plasticity constrained or facilitated adaptive evolution. Comparison of ancestral and derived reaction norms also allowed us to compare if adaptation to the LP environment resulted in the evolution of plasticity (Torres-Dowdall et al. 2012; Handelsman et al. 2013).

Methods

Natural and experimental populations of guppies

Four experimental populations of guppies were established as paired introductions in 2008 and 2009 in the upper Guanapo River drainage in the Northern Range Mountains of Trinidad, West Indies (for detailed descriptions of the introductions see Handelsman et al. 2013; Travis et al. 2014). Briefly, we translocated the progeny of HP guppies from a single source population in the lower Guanapo River into four upstream reaches. Natural and enhanced barriers bound each reach and prevented immigration of native guppies into the experimental reaches. The experimental reaches previously lacked natural guppy populations but otherwise had habitat attributes similar to other LP streams in Trinidad. We also thinned the forest canopy in two of the experimental streams (Kohler et al. 2012; Travis et al. 2014). As a result, light availability and primary productivity increased (Kohler et al. 2012), allowing us to evaluate the role of resource availability independent of predation risk. Canopy thinning increased the abundance of alpha chlorophyll (Kohler et al. 2012) in the environment and introduced populations reached higher densities compared to the two streams that were not thinned (Figure 1.7 in Travis et al. 2014).

Sampling of guppies for laboratory experiments

The HP source population (hereafter, source) was sampled from a downstream site where guppies coexist with a suite of predator species, including a major predator on guppies, the pike cichlid (*Crenicichla* spp.; Gilliam et al. 1993; Torres Dowdall et al. 2012). Between 25 and 30 wild caught females and 25–30 wild caught males were sampled from the source population in spring 2008, 2010, 2011, and 2012. Guppies were also sampled annually from the four experimental populations. The populations introduced in 2008 were established in the Lower LaLaja and Upper LaLaja tributaries of the Guanapo River (hereafter, Intro1 and Intro2, respectively). The populations introduced in 2009 were established in the Caigual and Taylor tributaries of the Guanapo River (hereafter, Intro3 and Intro4, respectively). Fifty juveniles were collected from Intro1 and Intro2 in 2009 (3–4 generations after being introduced), 2010 (6–8 generations), 2011 (9–12 generations), and 2012 (12–16 generations). Fifty wild caught juveniles were sampled from Intro3 and Intro4 in 2010 (3–4 generations after being introduced), 2011 (6–8 generations), and 2012 (9–12 generations).

Common garden rearing protocol

To minimize maternal and other environmental effects, we reared all wild-caught guppies for two generations in custom made recirculating systems under common garden lab conditions as described in Torres-Dowdall et al. (2012); Handelsman et al. (2013); Ruell et al. (2013). Wild caught females were randomly outcrossed with unique males to produce first generation (G1) laboratory-born individuals. G1 individuals were reared to maturity and randomly outcrossed to produce full-sibling broods of second-generation (G2) laboratory-born individuals.

We used a full-sibling split-brood design to expose G2 families to chemical cues from a predator and different levels of food resources. Within 24 hours of birth, G2 broods were randomly split between 1.5-liter tanks (2–10 full siblings per tank) that differed in exposure to chemical cues from a pike cichlid predator (reared with or without predator cues) and food levels (reared on high or low food). Siblings reared with predator cues were reared in recirculating units that housed a pike cichlid within the sump that supplied water to the tanks. Predator cues included both predator kairomones and guppy alarm pheromones that are released when the predator consumed two guppies daily. Guppies reared without predator cues were housed in identical recirculating units without predators in the water supply. Guppies reared on high food were fed quantities approaching *ad libitum* (a.m. – Tetramin® tropical fish flakes, Spectrum Brands, Inc., Cincinnati, Ohio, USA; p.m. – brine shrimp nauplii, *Artemia* spp.) and were comparable to "high" food levels administered by Reznick (1982). Guppies reared on low food were fed half the daily food allotments of guppies in the high food treatment. In both food treatments, food levels were adjusted weekly for age and number of individuals per tank.
At 29 days, G2 juveniles were anesthetized in tricaine methanesulfonate (MS-222), Sigma-Aldrich, St. Louis, Missouri, USA) and sexed. At the age of 4 weeks, juvenile males can be differentiated from females based on the presence/absence of melanophores in a triangular patch that appears on their ventral abdomen, which is present only in females (Reznick 1982). Once sexed, 1–5 males per family per rearing treatment were housed individually and reared under constant conditions until they reached sexual maturity.

Somatic growth rate

Somatic growth rate must be measured prior to maturity because male guppies shift resources away from somatic growth in favor of reproductive tissue, such that their asymptotic growth all but ceases shortly after maturity (Reznick 1982; Reznick 1990). After being separated by sex, G2 males were carefully dried of all free surface water and weighed for wet mass (mg) at 29 days. Males were weighed again at 43 days, which was prior to the onset of metamorphosis of the anal fin (energy allocation to the intromittent organ) in all individuals. Thus, male somatic growth rates were measured over a 14-day period when they were housed and fed individually. Growth rate was calculated as the change in wet mass per unit time (mg per day).

Age and size at maturity

Males are considered to be sexually mature when the apical hood grows even with the tip of their gonopodium (Reznick 1982; Reznick 1990). Males were checked weekly for the first appearance of the apical hood. Subsequently, they were checked daily until the day they reached maturity. Males were then anesthetized, spread laterally along a white background alongside a metric ruler, and digitally photographed using either a Panasonic® DMC FZ8 digital camera (Panasonic Corporation of North America, Secaucus, NJ, USA) or a Canon EOS Rebel XSi SLR digital camera (Canon U.S.A., Inc., Melville, NY, USA). Male standard lengths were then measured from photographs using ImageJ v.1.44 (Abramoff et al. 2004).

Data analysis

We tested for evolutionary divergence and phenotypic plasticity in three male life history traits (age at maturity, growth rate, and size at maturity) with Bayesian generalized linear mixed-

effects models (GLMMs) in the R package MCMCglmm (Hadfield 2010). First, using repeated measures GLMMs, we modeled the populations' origin (HP source, experimentally introduced), population (source, Intro1, Intro2, Intro3, Intro4), and rearing environment (high food, low food, with predator cue, without predator cue) as fixed effects. The family identity (ID) of full siblings and time the population was sampled (year) were modeled as random effects.

Then we ran *post-hoc* GLMMs for each of the three life history traits each year to assess the direction and magnitude of phenotypic divergence through time. Population, food level, and predator cue treatments were modeled as fixed effects and family ID was modeled as a random effect.

Finally, we tested if and how plasticity in the introduced populations diverged from the source genotype. There were no significant interactions between population x food treatment, population x predator cue treatment, or population x food treatment x predator cue treatment (*data not shown*). Because all populations exhibited common slopes across treatments, we pooled the introduced populations and tested for differences in plasticity each year between the source and the introduced populations with GLMMs. Again, we modeled population type (source, introduction) as a fixed effect and family ID as a random effect. There were also no significant effects of the canopy thinning experiment on the introduction populations (data not shown), thus this term was dropped from all subsequent models.

Repeated measures GLMMs and annual GLMMs were run for 1,300,000 iterations with a thinning interval of 100 and a burn-in period of 300,000 iterations to estimate the posterior distribution and minimize autocorrelation. The Markov-chain was sampled 10,000 times to estimate the variances of the fixed and random effects. GLMMs modeling plasticity each year converged faster and were run for 100,000 iterations with a thinning interval of 50 and a burn-in

period of 30,000, resulting in the Markov-chain being sampled 1,400 times to estimate the variance components in the model. Parameter-expanded priors were used in all models (Gelman 2006; Hadfield 2010). Plots of all posterior distributions were visually inspected to confirm that each model properly converged and for autocorrelation. We also calculated autocorrelation and found it to be less than 0.06 in all models. Plots of all variances were visually inspected and approximated normal distributions. All analyses were performed in R version 3.2 (R Core Team 2015).

Results

Evolution of male life history traits

We tested for evolution in age at maturity, growth rate, and size at maturity across time using repeated measures GLMMs for each trait. We predicted all introduction populations should evolve a delayed age at maturity, slower growth, and a larger size at maturity. For age at maturity, all introduced populations delayed age at maturity relative to the source population (Table 3.1). For growth rate, three of the four introduced populations had slower growth rates than the source (Table 3.1). Finally, an overall pattern of increased size at maturity was observed in all four introduced populations by the third and fourth year of the study (Fig 3.1C).

To better understand the time course of evolutionary divergence, we modeled annual differences among populations for each trait with GLMMs. Age at maturity in the introduced populations was similar to the source in 2009 and 2010 (Fig. 3.1A). Beginning in 2011, all four introduced populations started delaying maturity relative to the source and continued to diverge in 2012. Growth rate in the introduced populations appeared to be relatively stable over time (Fig. 3.1B). However, in 2010 and 2011, growth rates did slow in the introduced populations

relative to the source, but most 95% credible intervals overlapped zero with the exception of Intro4 in 2010 and Intro3 in 2011. In 2012, all four introduced populations again had very similar growth rates to the source population. Size at maturity in Intro1 and Intro2 had not yet diverged from the source in 2009 (Fig. 3.1C). In 2010, one of the four introduced populations (Intro4) matured at a smaller size than the source (Table 3.1, Fig. 3.1C). The other three introduced populations exhibited a similar trend to mature at a smaller size but the 95% credible intervals included zero (Table 3.1; Fig. 3.1C). By 2011 all four introduced populations matured at a larger size than the source population, with the 95% credible intervals for Intro1, Intro3, and Intro4 not overlapping zero. In 2012, all four introduced populations appeared to retain a larger size at maturity relative to the source population, with the 95% credible intervals for Intro1 and Intro2 not overlapping zero.

Plasticity in male life history traits

All three traits were plastic in response to the food treatment in the source and introduced populations in the repeated measures models (Table 3.1). However, the reaction norms (slopes between treatment levels; population x treatment interaction term) were not different between the introduced and source populations, indicating that plasticity had not evolved. All guppies reared on the low food level delayed age at maturity (Table 3.1, Fig. 3.2A–D), had a slower growth rate (Table 3.1, Figs. 3.3A–D), and matured at a smaller size (Table 3.1, Figs. 3.4A–D).

Growth rate and size at maturity were also plastic in response to predator cues in the rearing environment (Table 3.1). Again, there was no evidence for the evolution of plasticity as reaction norms were not different between the introduced and source populations. Guppies

reared with predator cues did not delay age at maturity, despite growing slower. Thus, they matured at a smaller size.

Discussion

The environment can act as both a source of natural selection and phenotypic plasticity (Scheiner 1993; Schlichting and Pigliucci 1998). Theory predicts that plasticity can either slow or facilitate adaptive evolution (Falconer 1990; Schlichting and Pigliucci 1998; Huey et al. 2003; Ghalambor et al. 2007; Conover et al. 2009), but few empirical studies have quantified how this dual role of the environment influences the early stages of adaptive evolutionary divergence in natural populations. We examined the plasticity of male life history traits and the rate at which they evolved. We found that, although plasticity was generally adaptive in response to variation in food levels and predator cues, male life history traits rapidly evolved in an adaptive pattern that is consistent with how these traits diverge in wild populations (Endler 1978; Reznick 1982; Reznick and Endler 1982; Reznick et al. 1996). Reznick et al. (1997) previously found life history traits in introduced populations to evolve within four years in males and seven years in females. After examining experimental introductions at a finer time scale, we found that these traits can begin to diverge within 1–2 years in male guppies. Yet, despite previous results showing plasticity evolves as a by-product of life history evolution (Torres-Dowdall et al. 2012), we found no detectable changes in the slope of the reaction norms between the source and introduction populations. This study was unique in that we were able to establish and follow replicate experimental introductions in the wild, in a system where the nature of adaptive evolution of life histories has been well studied. Below we discuss these results in more detail.

Time course of evolutionary divergence

Surprisingly, the time since a population was introduced was not the primary determinant of the magnitude of divergence. Rather, we found parallel patterns of divergence among all four introduced populations despite the fact that the two paired introductions commenced one year apart. Specifically, although the two populations introduced in 2008 (Intro1 and Intro2) did not appear to have diverged for any of the traits one year after their introduction (sampled in 2009), the two populations introduced in 2009 (Intro3 and Intro4) had started to diverge one year following their introduction (sampled in 2010), and in synchrony with Intro1 and Intro2 (Figs. 3.1A–C), which had been in the novel environment for two years. This suggests that something about the environmental conditions during the second year of the study, and not elapsed time, was the primary driver of the changes we observed. Previous longitudinal studies have found that the magnitude and direction of natural selection oscillates through time (Siepielski et al. 2009). For example, patterns of evolutionary change in the Galapagos finches *Geospiza fortis* and *G. scandens* reflect fluctuations in the environment rather than a linear pattern of divergence over 30 years (Grant and Grant 2002). While we do not know what aspect of the environment caused both populations to diverge in parallel between 2009 and 2010, population density may be important factor. Population growth rates of HP guppies are higher than LP guppies at low but not at high population densities (Bassar et al. 2013), suggesting that the HP phenotype is sensitive to density-dependent feedback. Our experimental populations exhibited a marked increase in densities beginning in late 2009 (see Figure 1.7 in Travis et al. 2014). Thus, density-dependent effects may have contributed to the onset of divergence in all four streams.

Patterns of evolution and plasticity

We also quantified phenotypic plasticity in life history traits, both in response to food availability and the presence of predator cues, and compared the introduced populations to the high predation source population. All traits were plastic in response to food availability (Figs. 3.2–3.4). Previous work on guppies has shown these traits to be plastic in response to food levels, and the results found here are qualitatively similar to those previously reported (Reznick and Bryga 1987; Reznick 1990). Growth rate and size at maturity, but not age at maturity, were plastic in response to olfactory predator cues during development (Figs. 3.2–3.4). Previous work has also found predator cues can induce plasticity in growth rate (Handelsman et al. 2013). However, while previous studies have documented ancestral and derived patterns of plasticity in guppies by comparing low and high predation populations and inferred how plasticity may have contributed to adaptive divergence (e.g., Reznick and Bryga 1987; Reznick 1990; Dugatkin and Godin 1992; Huizinga et al. 2009; Torres-Dowdall et al. 2012), these data capture the time course of changes in plasticity. Nevertheless, we found that plasticity did not vary between the introduced populations and the source population during the time frame of the study.

The similarity in plasticity among the HP source population and the introduced populations is likely indicative of the strength of selection on life history traits. Age at maturity, growth rate, and size at maturity are intrinsically tied to fitness due to the implicit trade-offs associated with growth and reproduction in environments that vary with regard to resource availability and extrinsic mortality risk (Gadgil and Bossert 1970; Law 1979; Roff 1992; Stearns 1992; Charlesworth 1994). For instance, environments with high adult extrinsic mortality should select for individuals that mature earlier, smaller, and increase energy allocation to reproduction (Gadgil and Bossert 1970; Law 1979; Roff 1992; Stearns 1992; Charlesworth 1994). In contrast,

environments that have low extrinsic mortality and higher adult survival confer a fitness advantage to individuals that grow slower, mature later, and reproduce less frequently. Thus, these traits should coevolve and be constrained along an age-size trajectory rather than diverge independently (Stearns and Koella 1986). We trimmed the forest canopy to produce variation in resource abundance in an environment with low extrinsic mortality in adults expecting that resource limitation would increase the rate of evolution and/or favor divergence in reaction norms, but aside from increased population size and density (Travis 1994), we did not detect a canopy effect on the rate of divergence. Below, we discuss how age at maturity, growth rate, and size at maturity responded when guppies were translocated from an environment with high mortality risk to a novel environment with high adult survivorship.

Previous work by Reznick et al. (1997) has shown that male guppies delay maturity within four years of being moved from high resource, high predation environments to environments with lower resources and lower predation. In the current study, we found a similar pattern where age at maturity was progressively delayed through time (Fig. 3.1A). We also found that age at maturity was not plastic in response to an environment that simulated a higher risk of predation. Ruell et al. (2013) showed that there was no difference in age at maturity between full siblings reared with and without predator cues in one of these introduced populations (Intro2) in 2009, although those reared with predator cues did delay the development of color patterns. Here we show that this pattern holds in three additional introduced populations and in the high predation source population over 3–4 years time (Figs. 3.2E–H). However, age at maturity was sensitive to food abundance and all populations exhibited a delay in maturation when food resources were restricted, similar to the findings of Reznick et al. (1990). All four introduced populations diverged from the source in age at maturity but not in plasticity.

Somatic growth rate would be expected to be faster in populations that experience high extrinsic mortality and/or where resource availability and quality are high (Stearns 1992; Charlesworth 1994). In natural guppy populations and within the drainage studied here, native high predation populations have faster growth rates than native low predation populations (Reznick et al. 2001; Bassar et al. 2013; Handelsman et al. 2013). Although we did find that the certain introduced populations evolved slower growth rates in the second and third year of the experiment (Table 3.1; Fig. 3.1B; also see Handelsman et al. 2013), there was no net divergence in growth rate relative to the source population by the end of the study period. Growth rate was plastic in response to both food abundance and the presence of predator cue but invariant between the source and introduced populations (Figs. 3.3A–E). Consistent with previous work (Reznick 1990; Krause and Liesenjohann 2012), lower food levels suppressed growth rate as we would expect in response to resource limitation. However, in contrast to patterns in nature, the presence of predator cue in the laboratory led to slower growth rate indicating a non-adaptive plastic response.

Initially, guppies reared in the presence of predator cue grew slower than those reared without predator cue (Fig. 3.3E). Slowed growth in response to predator cues has also been found in female and male guppies exposed to both olfactory and visual predator cues (Krause and Liesenjohann 2012). Although we would have expected to observe faster growth rates when predation risk was perceived to be high to facilitate faster maturation, this may have been offset by behavioral plasticity in response to predator cues (Torres-Dowdall et al. 2012). For instance, guppies reared with predator cues likely consumed fewer available resources than their siblings reared without predator cues due to behavioral plasticity in foraging behavior in response to the perceived predation risk. Guppies reared with predator cues typically remain near the surface of

the water rather than foraging near the bottom of the tank (Krause and Liesenjohann 2012; Torres-Dowdall et al. 2012). Also, reduced growth could have been caused by a predator-induced chronic stress response. Over the duration of this study, we also observed a decline in the plastic response in growth to predator cues in both the introduced populations and the source population (Figs. 3.3F–H). We do not know the underlying cause of this global change in phenotypes but it may be due to changes in the Guanapo drainage in the field over our sampling period or a laboratory effect. Increased activity in local quarries contributed to sediment buildup and turbidity in the habitat of the HP source population (C. Ghalambor personal observation) and disease, particularly mycobacteria infection, increased over the course of the experiment (E. Ruell personal observation). Nonetheless, our findings that the experimental populations diverged from the source population were qualitatively consistent with field and lab contrasts of HP versus LP populations from a neighboring drainage (Aripo River drainage; data not shown).

Size at maturity is often the manifestation of trade offs in growth rate and age at maturity in response to predation risk, other forms of extrinsic mortality, or intraspecific competition. As populations are released from predation risk, size at maturity is expected to increase if growth rate increases, age at maturity is delayed, or if sexual selection favors larger males (Reynolds and Gross 1992; Magellan et al. 2005). We found that the introduced populations evolved to mature at a larger size than the source population in the third and fourth year post introduction, but, initially, divergence was toward a smaller size at maturity (Fig. 3.1C). Given that these four streams were colonized by the descendants of a high predation source population, individuals that mature earlier and at a smaller size were likely to have higher relative fitness because they were able to reproduce before individuals that delayed maturity. Therefore, we might expect to see size at maturity initially decrease until a competing selective pressure (e.g., intraspecific

competition) led to slower growth rate (Bassar et al. 2013) or conferred higher fitness to larger individuals that were better competitors or preferred by females (Magellan et al. 2005). For example, in the absence of predation risk, females should show a preference to mate with larger males, and thus, reversal in the direction of divergence leading to the evolution of a larger body size at maturity may reflect sexual selection (Reynolds and Gross 1992; Magellan et al. 2005).

Similar to growth rate, size at maturity was also plastic in response to both food abundance (Figs. 3.4A–D) and the presence of predator cue (Figs. 3.4E–H). Guppies reared on low food levels and with predator cue were smaller at maturity than their siblings. A smaller size at maturity when reared in the presence of predator cue could reflect a trade-off in the allocation of resources toward sexual organs versus a larger body size. Additionally, maturing at a smaller size could be a byproduct of slower growth in presence of predator cue, but these traits became decoupled over the course of the experiment (Figs. 3.3A–D, 3.4A–D). Again, there was no difference in the amount of plasticity between the introduced populations and the source population over time and divergence in the trait means proceeded without divergence in phenotypic plasticity.

Conclusions

Traditional models of adaptive evolution have not incorporated an explicit role for phenotypic plasticity (e.g., Schlichting and Pigliucci 1998; Barrett and Schluter 2008). Recently, it has been proposed that plasticity may directly affect the strength of selection and the pace of contemporary evolution (Price et al. 2003; Ghalambor et al. 2007; Handelsman et al. 2014). Our results imply that plasticity, although present, does not diverge between the ancestral and the derived populations and does not hinder rapid evolutionary divergence in three life history traits.

However, these findings may not be ubiquitous given that previous work suggests that plasticity can evolve as a byproduct of adaptive evolution, particularly when a population is diverging from a non-plastic ancestor (Via and Lande 1985; Torres-Dowdall et al. 2012; Handelsman et al. 2013). Moreover, the correlation among traits can change across environments and potentially affect the direction and pace of evolution by altering how traits coevolve (Handelsman et al. 2014). Indeed, these three life history traits influence the multivariate life history phenotype of male guppies. All three traits are simultaneously exposed to selection, and there may be multiple solutions to producing an adaptive phenotype. Thus, we might expect variation in these traits to be maintained in diverging populations, especially during the initial stages of evolutionary divergence in a novel environment.[6]

[6] This research was approved by the Colorado State University Institutional Animal Care and Use Committee (protocols # 12-3269A and 09-1348A). This research was supported by the National Science Foundation Faculty Early Career Development grant DEB-0846175 (Cameron Ghalambor) and the National Science Foundation Frontiers in Integrative Biological Research grant EF-0623632 to David Reznick.

Tables and Figures

Table 3.1 Estimates from Bayesian univariate generalized linear mixed models for male age at maturity (days), growth rate (mg/day), and size at maturity (mm) comparing the high predation source population (source) to the four introduction populations (Intro1, Intro2, Intro3, and Intro4; modeled as fixed effects), and examining the fixed effects of rearing environment: reared on high or low food levels and reared with and without predator cue, and the their interaction. Family ID and time (year) were modeled as random effects. Upper and lower credible intervals (CrI) were estimated from the 95% highest posterior densities associated with each effect.

Models	Posterior mode	Lower CrI	Upper CrI
Age at maturity (days)			
Fixed effects			
Intercept (Source & high food & w/o cue)	59.363	46.289	72.572
Intro1 (Δ between Intro1 & Source)	5.624*	3.357	8.141
Intro2 (Δ between Intro2 & Source)	3.207*	0.940	5.634
Intro3 (Δ between Intro3 & Source)	3.699*	1.676	6.624
Intro4 (Δ between Intro4 & Source)	6.394*	3.875	8.784
Low food (Δ between low food & high food)	9.451*	8.456	10.807
w/ cue (Δ between w/ cue & w/o cue)	-0.375	-1.453	0.847
Low food:w/ cue	0.412	-1.492	2.435
Random effects			
Family variance	29.336	20.241	43.953
Time (year) variance	79.592	22.167	639.075
Residual variance	42.247	32.847	52.583
Growth rate (mg/day)			
Fixed effects			
Intercept (Source & high food & w/o cue)	1.517	0.916	2.111
Intro1 (Δ between Intro1 & Source)	-0.114*	-0.207	-0.022
Intro2 (Δ between Intro2 & Source)	-0.155*	-0.243	-0.060
Intro3 (Δ between Intro3 & Source)	-0.050	-0.140	0.051
Intro4 (Δ between Intro4 & Source)	-0.169*	-0.261	-0.069
Low food (Δ between low food & high food)	-0.343*	-0.406	-0.289
w/ cue (Δ between w/ cue & w/o cue)	-0.291*	-0.342	-0.230
Low food:w/ cue	-0.047	-0.139	0.052
Random effects			
Family variance	0.027	0.011	0.045
Time (year) variance	0.126	0.027	1.508
Residual variance	0.126	0.105	0.142
Size at maturity (mm)			
Fixed effects			
Intercept (Source & high food & w/o cue)	15.189	14.379	15.884
Intro1 (Δ between Intro1 & Source)	0.203	-0.013	0.472
Intro2 (Δ between Intro2 & Source)	0.014	-0.192	0.288
Intro3 (Δ between Intro3 & Source)	0.113	-0.144	0.367
Intro4 (Δ between Intro4 & Source)	0.382*	0.104	0.610
Low food (Δ between low food & high food)	-0.537*	-0.640	-0.449
w/ cue (Δ between w/ cue & w/o cue)	-0.674*	-0.763	-0.582
Low food:w/ cue	-0.005	-0.162	0.146
Random effects			
Family variance	0.459	0.353	0.587
Time (year) variance	0.207	0.033	2.335
Residual variance	0.131	0.054	0.206

Δ = 'difference'

* = the 95% CrI of a fixed effect did not overlap zero, indicating statistical significance

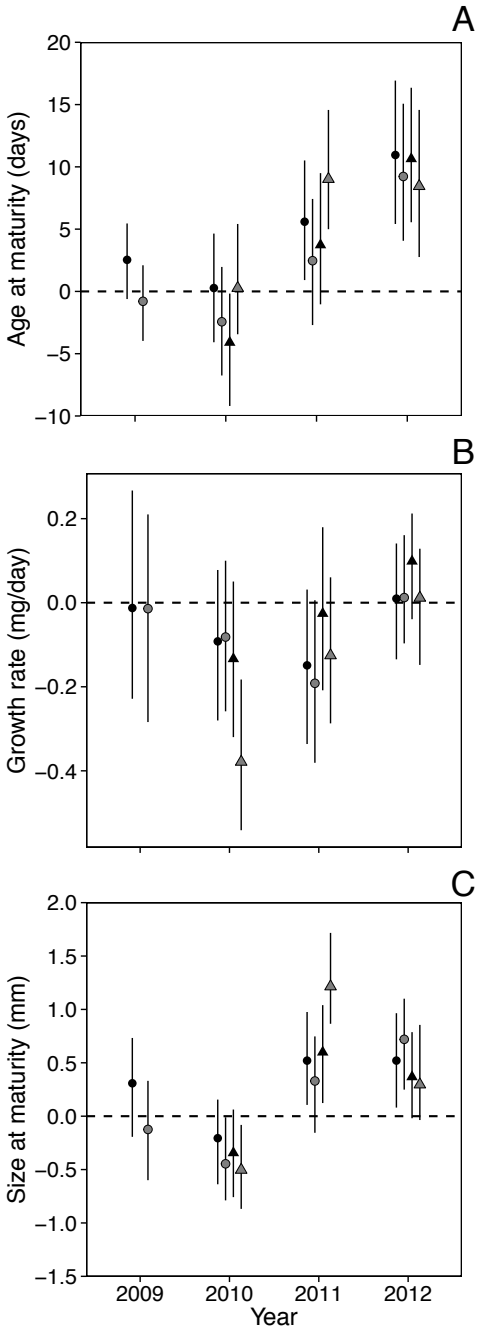

Figure 3.1 Representation of the evolution over time of (A) age at maturity (days), (B) growth rate (mg/day), and (C) size at maturity (mm) in the four introduction populations (Intro1 = black circle, Intro2 = gray circle, Intro3 = black triangle, Intro4 = gray triangle) relative to the high predation source population (dashed line at zero on the y-intercept) over time. Shown are estimates of the posterior mode and 95% credible intervals of the fixed effect of each introduction population (i.e., the difference between each introduction population and the source population) from Bayesian univariate generalized linear mixed models for each of the three traits for each year. Family ID was modeled as a random effect in each model.

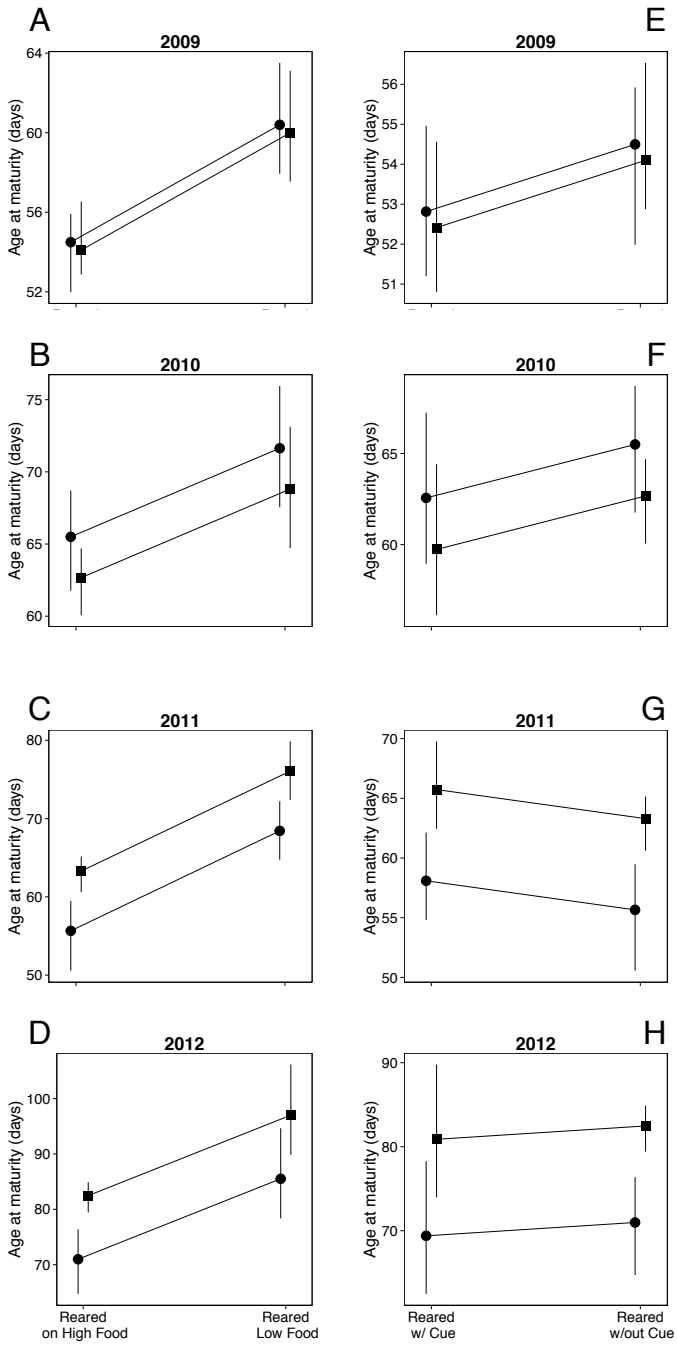

Figure 3.2 Representation of the plasticity of age at maturity (days) in response to being reared at different food levels (reared on high or low food) and reared with or without predator cue, comparing the high predation source population (black circle) to the introduction populations combined (black square) over time. Shown are estimates of the posterior mode and 95% credible intervals of the fixed effects of food treatment (i.e., the difference between fish reared on low food and high food) and predator cue (i.e., the difference between fish reared with predator cue and fish reared without predator cue) from Bayesian univariate generalized linear mixed models for each year. Family ID was modeled as a random effect in each model.

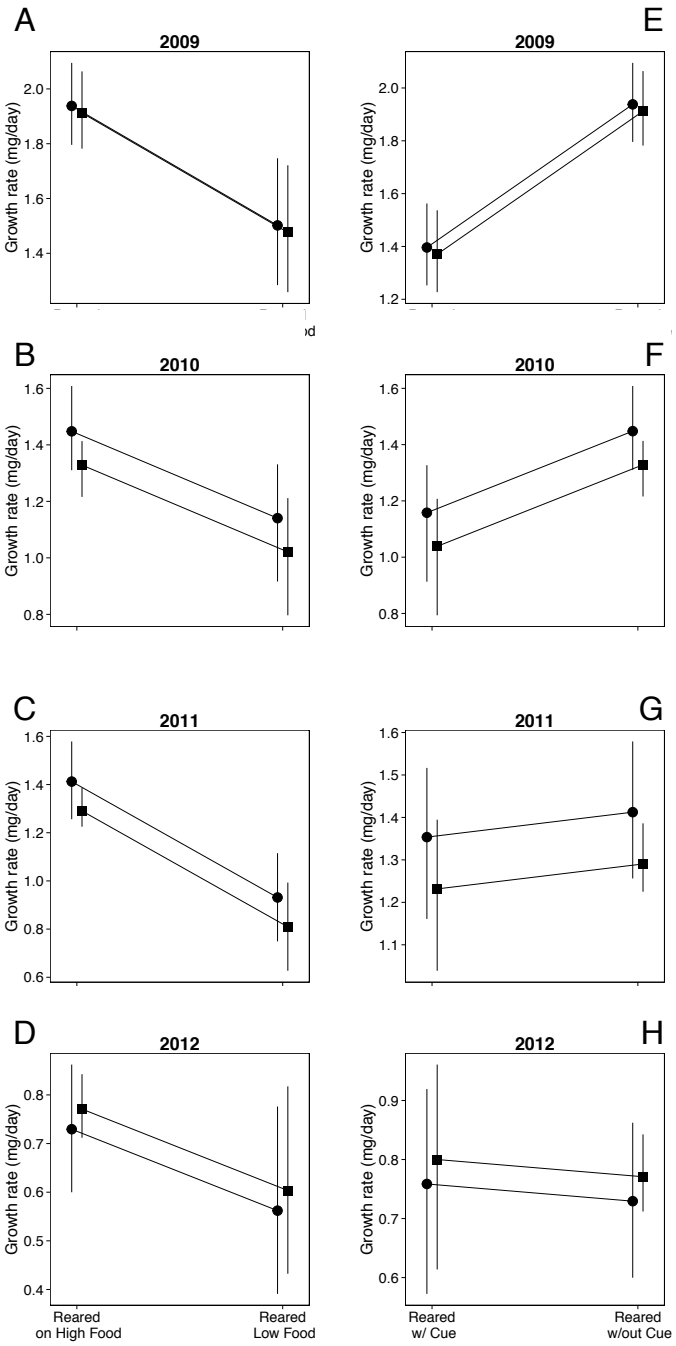

Figure 3.3 Representation of the plasticity of growth rate (mg/day) in response to being reared at different food levels (reared on high or low food) and reared with or without predator cue, comparing the high predation source population (black circle) to the introduction populations combined (black square) over time. Shown are estimates of the posterior mode and 95% credible intervals of the fixed effects of food treatment (i.e., the difference between fish reared on low food and high food) and predator cue (i.e., the difference between fish reared with predator cue and fish reared without predator cue) from Bayesian univariate generalized linear mixed models for each year. Family ID was modeled as a random effect in each model.

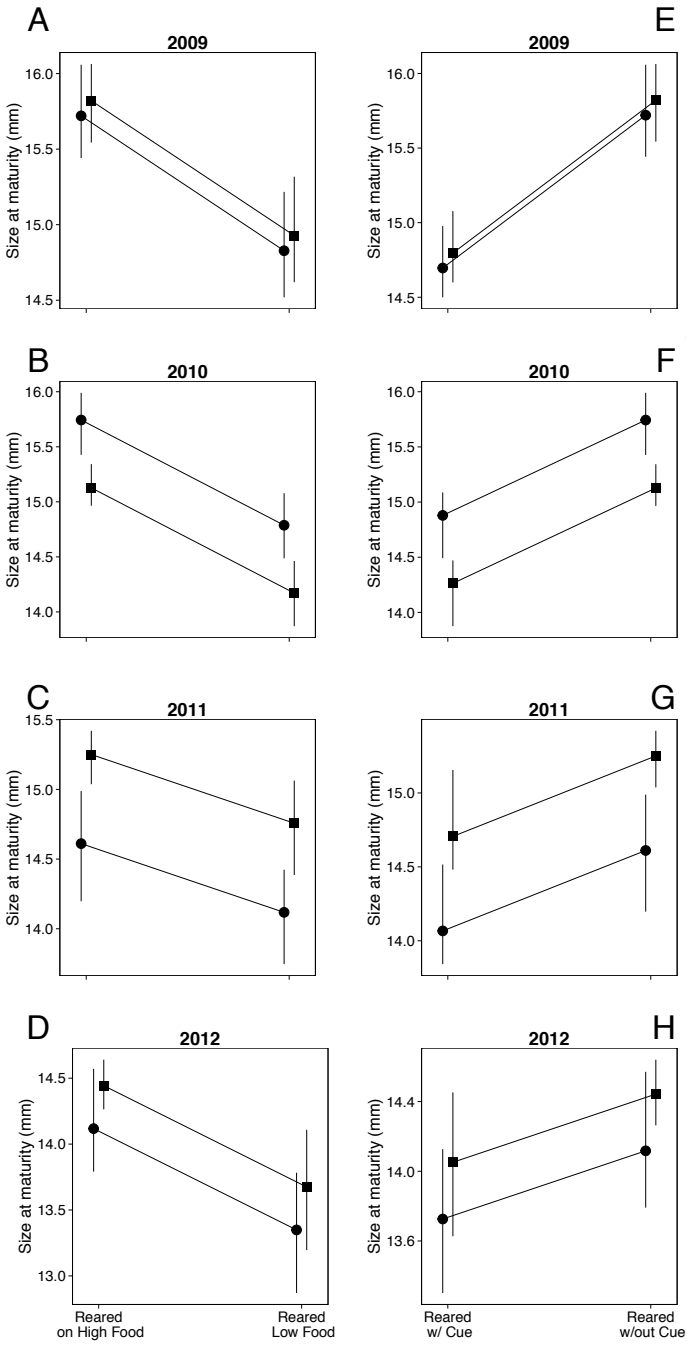

Figure 3.4 Representation of the plasticity of size at maturity (mm) in response to being reared at different food levels (reared on high or low food) and reared with or without predator cue, comparing the high predation source population (black circle) to the introduction populations combined (black square) over time. Shown are estimates of the posterior mode and 95% credible intervals of the fixed effects of food treatment (i.e., the difference between fish reared on low food and high food) and predator cue (i.e., the difference between fish reared with predator cue and fish reared without predator cue) from Bayesian univariate generalized linear mixed models for each year. Family ID was modeled as a random effect in each model.

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Chapter 4. Predator-induced phenotypic plasticity in metabolism and rate of growth: rapid adaptation to a novel environment[7]

Introduction

During range expansions or colonizations of novel environments, populations may experience directional selection pressures that result in contemporary adaptation (Reznick and Ghalambor 2001). Traditional evolutionary models predict that directional selection on standing genetic variation will move the mean phenotype toward a new adaptive peak (Lande 1979; Lande and Arnold 1983; Barrett and Schluter 2008). However, many traits are differentially expressed across environments, such that novel environments can also induce phenotypic plasticity. Given that phenotypic plasticity results in predictable phenotypic responses across environments, and populations can harbor genetic variation in the form of genotype-environment interactions (GxE) (Falconer 1981), selection can act on plastic traits and produce adaptive responses (Schmalhausen 1949; Bradshaw 1965). Thus, novel environments can simultaneously impose directional selection and induce plasticity, yet traditional models and empirical studies of adaptive evolution tend to ignore an explicit role for plasticity.

How might selection and plasticity jointly shape adaptive evolution? Quantifying plastic responses within the native or historic range of environments of a population can provide insight into the ancestral patterns of plasticity (Schlichting and Pigliucci 1998; Conover et al. 2009), and be compared to derived patterns of plasticity when a population moves into a new environment (Ghalambor et al. 2007; Conover et al. 2009). When the local optimum in the new environment

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is known, insight can be gained into whether the pattern of plasticity in the ancestral population exhibits adaptive or non-adaptive plasticity. For example, threespine sticklebacks (*Gasterosteus aculeatus*) have colonized freshwater lakes and evolved different trophic morphologies in limnetic and benthic habitats that in part reflect adaptation to different diets (Schluter 1993). However, trophic morphology is also plastic, such that when sticklebacks are forced to feed on the preferred food of a limnetic or benthic ecotype, they develop a trophic morphology that makes them phenotypically more similar to these respective ecotypes (e.g., Day et al. 1994; Day and McPhail 1996; Wund et al. 2008). Thus, the plastic response to feeding on a particular diet appears adaptive as it is in the same direction of evolutionary change. When the environment induces plastic changes that are in the same direction favored by selection, it is termed synergistic (Falconer 1990) and can result in cogradient variation in which there is a positive covariance between genetic and environmental influences on the phenotype (Conover et al. 2009). In contrast, many non-adaptive plastic responses that often are induced by stress, or poor quality environments that result in physiological limitations (e.g., temperature, limited nutrients, predators) result in antagonistic selection (Falconer 1990) because the environment induces a plastic response that is opposite the direction of selection. Such antagonistic selection in turn can lead to the evolution of countergradient variation, where there is a negative covariance between genetic and environmental influences on the phenotype (Levins 1968; Conover et al. 2009). For instance, lower temperatures and low availability of food often favor the evolution of faster growth in many ectotherms, but the plastic response to these environments results in slower growth (Berven 1982a; Berven 1982b; Conover and Present 1990; Conover and Schultz 1995; Finstad et al. 2004; Conover et al. 2009). In such cases of countergradient variation, selection must overcome the plastic response, which often leads to cryptic adaptive evolution (Grether

2005; Conover et al. 2009). The implications of such adaptive and non-adaptive plasticity for adaptive evolution remains a largely unexplored area, but are likely to be important during the early stages of adaptive divergence when directional selection is strong.

Plasticity and evolution of energy budgets in novel environments

Constraints and trade-offs from allocating finite energy budgets form central tenets of life-history theory (Stearns 1992). For instance, organisms allocate energy stores between reproduction and somatic growth only after meeting the energy requirements of self-maintenance, or resting metabolism (Myrick 2011). Thus, the evolution of a relatively high or low cost of self-maintenance may reflect variation in the availability of resources and the energy costs associated with their acquisition, and we would expect individuals to exhibit plasticity in response to environmental conditions that alter either the availability or the acquisition of resources (e.g., risk of predation, availability of food, temperature).

Resting metabolic rate (RMR), the minimum energy expenditure achieved in a post-absorptive (i.e. digestive) state (reviewed by Burton et al. 2011), represents the energetic cost of self-maintenance. RMR is considered to be central to evolutionary and ecological physiology because it dictates the residual resources available for competing physiological functions (e.g., activity, growth, reproduction) (Garland and Carter 1994; McNab 2002; Ricklefs and Wikelski 2002). However, there are competing predictions on how evolution shapes the cost of self-maintenance (reviewed by Burton et al. 2011). The *compensation hypothesis* views RMR as a direct cost of maintenance and, therefore, predicts that organisms with a low relative RMR can increase their allocation of energy to growth and reproduction, and have higher relative fitness (Gadgil and Bossert 1970; Nilsson 2002; Boratyński and Koteja 2010). In contrast, the *increased*

intake hypothesis predicts that individuals with a high relative RMR will have higher relative fitness due to their propensity for greater intake of energy and thus turnover (Nilsson 2002; Boratyński and Koteja 2010). In the latter case, higher RMR is due to increases in organ mass (Daan et al. 1990; Konarzewski and Diamond 1995; Speakman and McQueenie 1996; reviewed by Blackmer et al. 2005) and facilitates greater maximum metabolic rates that can sustain higher levels of processing and absorption of energy (McNab 1980; Thompson 1992). Although both hypotheses have some empirical support, many studies have produced conflicting or ambiguous results (Blackmer et al. 2005; Boratyński and Koteja 2010; summarized in Table 4.1 in Burton et al. 2011).

In an effort to address how environmental variation influences the relationship between RMR and fitness, and reconcile the two aforementioned hypotheses, Burton et al. (2011) proposed the *context-dependent hypothesis*. The context-dependent hypothesis proposes different environmental conditions that would favor either high or low RMR. For example, higher RMRs may positively correlate with fitness under favorable environmental conditions, but prove disadvantageous in unstable or poor environments in which the cost of self-maintenance may become cumbersome (Burton et al. 2011). Thus, plastic or evolutionary responses that lower RMR could serve to buffer or safeguard individuals in variable environments.

The cost of self-maintenance should also be correlated with other traits related to fitness, such as survival, growth, and reproduction (Stearns 1992; Ricklefs and Wikelski 2002). Despite some ambiguity in the relationship between RMR and fitness, growth rates can be susceptible to variation in RMR, and can in turn affect fitness (Arendt 1997; Metcalfe and Monaghan 2003; Auer et al. 2010). Growth rate may also be decoupled from RMR and evolve independently in response to different environmental conditions. For example, faster growth and early maturity

should be favored in environments with high extrinsic mortality despite any costs that may be incurred (Arendt 1997; Metcalfe and Monaghan 2003; Auer et al. 2010). Conversely, when extrinsic mortality is low, lower growth rates may be favorable; allowing individuals to mature in better condition than can be achieved with faster growth rates. Collectively, these hypotheses and perspectives argue for a potentially important link between fitness and metabolism, as well as the potential for plasticity in metabolic rates. Nevertheless, few studies have simultaneously evaluated plasticity and evolution in metabolic rates along with potentially correlated traits such as growth rate. We take such an approach here.

Trinidadian guppies as a model system

In natural populations of Trinidadian guppies (*Poecilia reticulata*), suites of life-history, behavioral, and morphological traits exhibit differences across populations that are consistent with adaptive responses to differences in extrinsic mortality among environments (Endler 1995; Reznick and Bryga 1996; Magurran 2005). We tested for evolutionary divergence and phenotypic plasticity in RMR and growth rate in populations of Trinidadian guppies (hereafter, guppies). Specifically, we used a combination of field-transplant experiments and laboratory common gardens to measure plastic and evolved responses. Guppies are particularly suited to study the evolution and maintenance of RMR due to the contrasting environments in which natural populations occur. Guppies in high-predation locales co-occur with a suite of piscivorous predators and have high extrinsic mortality. In contrast low-predation habitats are associated with lower extrinsic mortality, but the quality of resources can be poor (Reznick and Endler 1982; Reznick et al. 2001; Zandonà et al. 2011; El-Sabaawi et al. 2012). Under laboratory conditions, we reared male guppies in the presence or absence of chemical cues from a predator

to mimic these contrasting environments and examined whether populations showed evidence for evolutionary divergence and developmental plasticity in rates of metabolism and growth. We measured these traits in a natural populations subjected to high predation and to low predation and four experimentally low-predation populations that were established with individuals from the high-predation population.

Methods

Sampling of natural and experimental populations of guppies

We sampled six populations of guppies within the Guanapo River drainage in the Northern Range Mountains of Trinidad, West Indies. The first population, hereafter referred to as HP, is a native population subject to high predation in the Guanapo river drainage that contains a variety of predator species, including the common predator on guppies, the pike cichlid Crenicichla frenata (Gilliam et al. 1993; Torres Dowdall et al. 2012) The second population, hereafter referred to as LP, represented a native low-predation population from the same drainage and was sampled from the Tumbason tributary of the Guanapo river where guppies co-exist with only one other species, a killifish (*Rivulus hartii*). Rivulus hartii are gape-limited omnivores that prey primarily on juvenile guppies (Mattingly and Butler 1994). Thirty adult females and 30 adult males were sampled from the HP population in March of 2008 and from the LP population in March of 2012. Fifty juveniles (likely between 4-6 weeks old given their size class) were also sampled from the HP population in March 2010. The remaining four populations were descendants of high-predation individuals from the HP population that had been experimentally introduced into four low-predation streams (within the Guanapo river drainage) that previously lacked guppies. The four introduced populations were established in 100-180m

reaches of small, first-order tributaries that contained only *R. hartii*. Waterfalls bound the upper and lower limits of each reach and were artificially enhanced (if necessary) to prevent emigration and the establishment of populations above the streams receiving introductions and immigration from downstream populations, respectively. Upstream barrier waterfalls were enhanced in two reaches and a downstream barrier was enhanced in one reach (see below).

Paired introductions were conducted across two consecutive years. In March 2008, HP guppies were introduced into the Lower La Laja and Upper La Laja tributaries of the Guanapo drainage (hereafter, Intro1 and Intro2, respectively). Each stream was stocked with 38 gravid females and 38 mature males. To minimize the potential for founder effects and equalize genetic diversity in each stream, males and females from each random cross were introduced into alternate streams with the consequence that the introduced females carried sperm stored from the 38 males that they were mated with. Then, the females were paired in the introduction site with 38 new males. In March 2009, this protocol was replicated in the Caigual and Taylor tributaries (hereafter, Intro3 and Intro4, respectively), but 45 males and females were introduced into each site. The riparian forest canopy was experimentally thinned (opened) in one stream of each pair, six months prior to the introductions (Kohler et al. 2012). Canopy thinning increased light levels relative to the undisturbed (closed) canopies of the paired reach (as part of a separate experiment) (Kohler et al. 2012). Collectively, the four introduced populations are called "introduced populations" hereafter. We didn't find any significant effects of canopy thinning (data not shown). Forty juveniles were collected from each introduced population two years (6–8 generations) after their establishment: in March 2010 from the Intro1 and Intro2 populations, and in March 2011 from the Intro3 and Intro4 populations. These juveniles were reared to adulthood in the laboratory, and then mated to produce laboratory lines.

To minimize maternal and other environmental effects, we reared all wild-caught guppies for two generations under common garden laboratory conditions (modified from Reznick 1982)in 1.5-liter tanks (Aquatic Habitats, Apopka, FL, USA) connected to a custom-made recirculating system and maintained on a 12-hr light cycle at 27 ± 1^{o} C. Fish were reared on standardized food levels adjusted weekly for age and number of individuals per tank (a.m. – Tetramin® tropical fish flakes, Spectrum Brands, Inc., Cincinnati, Ohio, USA; p.m. – brine shrimp nauplii Artemia spp.). The quantity of food offered daily approximated ad libitum and was comparable to the high level of food administered by Reznick (1982).

To establish a G1 generation, when wild adults were sampled (see above), gravid females were housed individually until parturition. Each G1 brood was housed separately. Females that did not give birth within about 30-35 days of capture were randomly crossed with a wild-caught male. No two females were crossed with the same male. When juveniles were sampled from the wild (see above), guppies were anesthetized in buffered MS-222 (0.85mg ml^{-1}; ethyl 3-aminobenzoate methane sulphonic acid salt) (Sigma-Aldrich, St. Louis, Missouri, USA) and separated by sex. Juvenile females (28–56 days) can be identified by the presence of melanophores in a triangular patch that appears on their ventral abdomens, which is absent in males (Reznick 1982). After reaching maturity, each wild-caught female was crossed with a single male to produce the G1 generation. Males are considered to be sexually mature when the apical hood grows even with the tip of their gonopodium; females usually mature within ±1–2 days of males (Reznick 1990; Auer et al. 2010). The protocol was replicated on the G1 generation to produce the second (G2) generation.

Within 24 hours of birth, G2 full-sibling broods were randomly assigned to two 1.5-liter tanks (2-10 full siblings per tank) that differed in exposure to chemical cues from a predator

(reared with or without cues from a predator) using a split-brood design. Siblings reared with cues from predators were reared in recirculating units that housed a pike cichlid within the sump that supplied water to the tanks (Torres-Dowdall et al. 2012; Ruell et al. 2013). Cues from predators included both kairomones and alarm pheromones, or chemical signals released from pike cichlids consuming two guppies daily. Guppies reared without cues from predators were housed in identical recirculating units without predators in the water supply. G2 juveniles were anesthetized and sexed at 29 days (see above), and one male per family per rearing treatment was randomly selected and reared individually under the same conditions thereafter.

Rates of somatic growth

Somatic growth must be measured prior to maturity because male guppies shift resources away from growth and towards reproduction (male guppies exhibit asymptotic growth that ceases shortly after maturity). After being separated by sex, G2 males were carefully dried of all free surface water and weighed for wet mass (g) at 29 days. Males were weighed again at 43 days. This time period reflects juvenile growth prior to the onset of metamorphosis of the anal fin (investing in the intromittent organ). The initiation of metamorphosis of the anal fin was observed to commence at 50 days in our fastest growing HP population (Reznick 1990; Reznick and Bryga 1996; E. Ruell personal observation). Thus, males' somatic growth rates were measured over a 14-day period when they were housed and fed individually, and prior to the metamorphosis of their anal fins. Growth rate was calculated as the change in mass per unit time (g day^{-1}) as follows:

$$\frac{mass_{final} - mass_{initial}}{time}$$

where $mass_{final}$ is wet mass at 43 days, $mass_{initial}$ is wet mass at 29 days, and time is the 14-day growth period.

Resting metabolic rate

Male guppies were fasted for 24 hours prior to measurement of their resting metabolic rate. Fish reared with predator cues were transferred to tanks without predator cues 24 hours prior to any respiratory measurements. Each fish was then placed in a jar-type static respirometer (Fig. 10.1 in Cech 1990) using water without predator cues, and acclimated for a minimum of 60 minutes (range = 60–70 minutes). Water current was permitted to flow through each respirometer during acclimation. Respirometers were on shelves that were covered by an opaque blind throughout the experiment to minimize additional stress to the fish that could elevate O_2 consumption. A blank respirometer was measured along with each group of fish to correct for microbial respiration.

Oxygen concentrations of water samples were measured with a SI130 Microcathode Oxygen Electrode housed in a MC100 Microcell using a Strathkelvin 928 6-channel O_2 Interface connected to a PC running Strathkelvin 928 Oxygen System software (Strathkelvin Instruments Ltd., Glasgow, UK). Prior to all measurements, the O_2 electrode was calibrated with saturated water sampled from the aerated water supply (100% saturation) and anoxic water (achieved by dissolving a small amount of anhydrous sodium sulfite in the same water; 0% saturation) and adjusted for temperature and barometric pressure. Following the acclimation period, an initial measurement of O_2 concentration (mg O_2 l^{-1}) was taken. After approximately 90 minutes (range = 90–100 minutes), a second water sample was measured for O_2 concentration. $\dot{M}O_2$ (mg O_2 h^{-1}) was calculated for each individual in as follows:

$$\dot{M}O_2 = \frac{(cO_{2\,initial} - cO_{2\,final})V}{T}$$

where $\dot{M}O_2$ is measured O_2 consumption, cO_2 initial is the initial O_2 concentration, cO_2 final is the ending O_2 concentration, V is volume is the respirometer volume (l), and T represents the time duration between initial and final O_2 sampling. Wet mass (g) of the fish was measured immediately following the measurement of cO_2 final. Mass independent $\dot{M}O_2$ was calculated by dividing the raw data by wet mass raised to the mass exponent. The mass exponent was determined from the slope of a linear regression of log transformed $\dot{M}O_2$ on log transformed wet mass (Innes and Wells 1985; Cech 1990). The mass exponent was 0.508 (SE = 0.1045, $P <$ 0.001). All further analyses of $\dot{M}O_2$ were performed on the mass independent data.

Statistical analyses

We used linear mixed effects models (lme) to analyze the effects of genetic background and cues from a predator on RMR and growth rate. Variation among families in intercept was modeled as a random effect. RMR and growth rate were used as response variables and modeled with Gaussian error. Residual plots were used to visually determine if model assumptions of normality and homoscedasticity were met. A log transformation was applied to the RMR data to adjust for deviation from homoscedasticity. All other data conformed to model assumptions. All lme models were performed in R (R core team 2012) with the lme4 package (Bates et al. 2012). We tested for significance of fixed effects with Markov chain Monte Carlo sampling of the posterior distribution of the parameters using the languageR package (Baayen 2011).

The full RMR model was not significantly different between populations or treatments (Table 4.2). However, there was a significant interaction between the Intro3 population and experimental treatment ($P = 0.024$; Table 4.2). This pattern was driven by the tendency for RMR

values to converge in all populations when reared with the predator cue and the Intro3 population exhibiting a greater response to the rearing environment than we observed in the other populations. Additionally, given the combination of the number of parameters estimated by the model and our sample sizes (see Table 4.1 for sample sizes), there was insufficient power to detect population and treatment effects. Because we were primarily interested in the direction of plasticity and evolved differences between genetic backgrounds, we also fit a reduced lme that excluded the population by treatment interaction term (Table 4.2). We focus our interpretation on the reduced model while acknowledging the significant interaction present in the full model.

We also examined the effect of RMR on growth rate with an lme. RMR and treatment were modeled as fixed effects and family intercept was modeled as a random effect.

Results

Resting metabolic rate

Resting metabolic rate was higher in the derived LP population than in the ancestral HP population (Fig. 4.1; Table 4.2 reduced model). Thus, our expectation was that introduced populations should evolve a higher RMR relative to the HP population from which they were derived. Indeed, three of the four experimentally introduced populations exhibited higher RMR (Fig. 4.1). However, there was also plasticity in RMR, as most of the populations tended to increase RMR when reared without the cues from a predator (Fig. 4.1). This pattern of plasticity also influenced the patterns of divergence between populations, as there was greater divergence between the LP and introduced populations and the ancestral HP population when guppies were reared without cues from a predator (Fig. 4.1; Table 4.2). Casual observations revealed that spontaneous activity was minimal inside the respirometers and did not appear to differ between

fish reared with and without cues of a predator or among populations (C. Handelsman personal observation).

Rate of somatic growth

The LP population had the lowest growth rate and the HP population had the highest growth rate (Fig. 4.2; Table 4.3), again providing an expectation that growth rate should evolve to become slower in the introduced populations. Indeed, the Intro1 and Intro2 populations exhibited slower growth than did the HP population and the Intro4 showed the same trend (Fig. 4.2; Table 4.3). Again, as with RMR, the pattern of divergence between the experimental populations and their HP source is greater in the absence of cues from a predator (Fig. 4.2). This pattern arises because growth rate was plastic in response to the predator's cue, increasing when fish were reared without the cues. Variation among populations in this plasticity led to significant interaction terms in the model for the Intro3 and Intro4 populations (Table 4.3). These populations exhibited less plasticity in response to the rearing environment (Fig. 4.2). Thus, they are diverging toward the native LP phenotype but are less sensitive to the rearing environment than are the other four populations. Because the HP population had a faster growth rate than the LP population and the plastic response to predators' cues was to slow growth rate, genetic divergence in growth rate showed countergradient variation.

Relationship between rate of somatic growth and resting metabolic rate

RMR was not correlated with growth rate across all individuals when controlling for the rearing environment (slope = 0.0025, n = 7–24 per population, P = 0.9). Nonetheless, the HP population had the highest growth rate and the lowest RMR while the reciprocal pattern was

found in the LP population. The lack of a relationship between RMR and growth rate suggests that RMR does not constrain growth rate and that these traits can evolve independently.

Discussion

Newly established populations are likely to experience the dual effects of directional selection and plasticity in response to new environmental conditions. Physiological and life-history traits may be particularly sensitive to changes in risk of predation and in availability of food, as these features of the environment are known to induce plasticity and act as sources of selection (Lima and Dill 1990; Werner and Anholt 1993; Beckerman et al. 2007; Steiner and Van Buskirk 2009). Here we find that RMR and growth rate of guppies show a general pattern of being plastic in response to cues from predators. A native LP population from an upstream tributary of the Guanapo drainage exhibited a higher mean RMR and lower mean growth rate than did the downstream ancestral HP population under common garden conditions (Figs. 4.1, 4.2). Faster growth rates and a lower RMR in the HP population are thought to be adaptive in the face of high mortality from predators (e.g., McPeek et al. 2001; McPeek 2004). Indeed, we also found that the four experimental introductions of HP guppies into low-predation tributaries showed similar patterns of divergence within only 6–8 generations (Figs. 4.1, 4.2). These patterns of divergence and rapid evolution complement a suite of other life-history (Reznick and Endler 1982), behavioral (Seghers 1974; Endler 1995; Godin and Briggs 1996; Templeton and Shriner 2004; Torres-Dowdall et al. 2012), and morphological traits (Layman et al. 2003; Langerhans and Dewitt 2004; Alexander et al. 2006; Hendry et al. 2006; Burns et al. 2009, Ghalambor et al. in review) that have been shown between contrasting habitats with high and low predation. Furthermore, while RMR in the HP source population was not plastic in response

to cues from predators, the rapid evolution of RMR in the introduced populations also resulted in the rapid evolution of plasticity (i.e., an increase in the slope of the reaction norm, Fig. 4.1). In contrast, the HP source population exhibited rapid evolution of a lower growth rate without a change in plasticity (Fig. 4.2). However, the direction of this evolutionary change was opposite to the plastic response to being reared without the predator's cues (Fig. 4.2), suggesting a non-adaptive plastic response and antagonistic selection on growth. Below, we elaborate on how evolutionary divergence and plasticity in RMR and growth rate corresponds to different selection pressures.

Resting metabolic rate

Animals living under the risk of predation often face a trade-off between foraging to fulfill their energy needs and foregoing foraging to avoid predators (Lima and Dill 1990; Ball and Baker 1996; McPeek et al. 2001; Fraser et al. 2004). As a result, prey species can respond to the risk of predation by reducing their energy demands via lowering their metabolic rates (Ball and Baker 1996; Beckerman et al. 2007). Consistent with these patterns, we found that, within this drainage, a native LP population and the introduced populations generally exhibited higher RMRs than did the HP population from which they were derived (Fig. 4.1). The environmental effect of rearing guppies with and without predators' chemical cues on RMR generally paralleled the pattern between the ancestral HP population and the derived LP population, with RMR increasing in the absence of the predators' cues (Fig. 4.1). This represents adaptive plasticity as it is in the same direction as evolution. However, the ancestral HP population was not plastic in response to the cues (Fig. 4.1), and the evolution of plasticity for increased RMR in the absence of predators' cues followed the colonization of an LP stream by a HP population. This

divergence was much stronger in the derived environment (without cues from predators), given that most populations exhibited the ancestral phenotype when reared with the cues (Fig. 4.1), suggesting that the evolution of plasticity is driven by selection acting on RMR in the low-predation environment (see also Torres-Dowdall et al. 2012).

A general assumption in predator-prey interactions is that predators constrain the activity of the prey, which in turn reduces the prey's ability to forage (Lima and Dill 1990; Brown et al. 2006). Thus, predators could serve to lower metabolism because they either induce plastic changes in activity, or act as a selection pressure that reduces activity and associated energy demands (Werner et al. 1983; Ball and Baker 1996; McPeek et al. 2001; Brown et al. 2006; Beckerman et al. 2007). While there was certainly some spontaneous activity during our measurements of metabolic rate, we did not observe any qualitative differences between the two treatments, suggesting that guppies are plastically changing energy demands. Nevertheless, guppies are prey for larger fish throughout their range (Reznick and Endler 1982) and thus exhibit various plastic behavioral strategies in nature that reduce activity and exposure to predators. For example, guppies preferentially utilize the peripheral banks of streams, where they take shelter under vegetation and in shallow water (Seghers 1973; Reznick et al. 2001; Ghalambor et al. in review). Moreover, guppies will cease feeding at night in the presence of predators (Fraser et al. 2004). Collectively, while these behaviors may help guppies avoid predators, they also likely reduce access to food, which in turn favors lower metabolism, such that some combination of changes in metabolism and activity likely occur in nature. A prediction that arises from these results is that those individuals with higher RMRs should be more likely to engage in risk-taking behavior in order to meet their energy demands relative to those individuals with lower RMRs. In support of this, risk-taking behavior was positively correlated with RMR in

the common carp (*Cyprinus carpio*) (Huntingford et al. 2010). Similarly, deprivation of food increased risk-taking behavior in European sea bass (*Dicentrarchus labrax*) in general, but individuals with the highest RMRs increased risk-taking the most (Killen et al. 2011).

While a low metabolic rate could help offset the costs of reduced foraging in high-predation environments, other aspects of the environment may also contribute to differences in metabolism. In addition to increased predation, guppy populations in downstream environments also experience faster average velocities of water (Reznick and Endler 1982; Ghalambor et al. in review), which likely increase the energetic costs of sustained swimming. For example, Atlantic salmon parr with low RMRs outgrew conspecifics with higher RMRs when forced to forage in swiftly flowing water (Armstrong et al. 2011). Thus, selection may favor individuals with lower RMRs in high-predation environments because both they are better equipped to endure periods of food-deprivation and because they can cope with the energetic demands of higher velocities of water (Burton et al. 2011). Alternatively, competitive ability may also be positively correlated with RMRs given that RMR is positively correlated with aggression and dominance in many taxa (Biro and Stamps 2010). Guppies in low-predation environments are thought to experience high intraspecific competition for food (Reznick et al. 2001), which could favor a higher RMR. Indeed, dominance in Atlantic salmon was found to increase proportionally with the relative difference in RMR between conspecifics (Metcalfe et al. 1995). While we found that guppies have a higher RMR when reared without cues from a predator, this cue may also be associated with increased competition. However, given that feeding levels in the present study approximated *ad libitum*, it is not clear whether the perception of ample resources triggered the observed response by the guppies reared without cues from a predator, rather than the lack of risk of predation. If so, our RMR measurements for guppies reared without cues from a predator

may represent the mean RMR of each population, and the rearing environment with cues could have suppressed RMR.

Rate of somatic growth

A behavioral reduction in feeding and growth are commonly observed in animals that are under the risk of predation (e.g., Brown et al. 2006). However, a plastic reduction in growth rate could either reflect a behavioral reduction in foraging, or a physiological change that reduces growth (e.g., Beckerman et al. 2007). In contrast, life-history models and empirical evidence all predict the evolution of faster growth rates when predation and mortality are high (Stearns and Koella 1986; Fraser and Gilliam 1987; Liebold and Tessier 1991; Spitze 1991; Stearns 1992). We find evidence for both a plastic reduction in growth rate in response to cues from a predator, and the evolution of slower growth rates when the risk of predation is reduced (Fig. 4.2; Table 4.3). The ancestral HP population had a higher growth rate than did the naturally derived LP population in the common gardens. Additionally, three of the four introduced populations showed an evolutionary response, in which growth rate became slower within 6–8 generations (Fig. 4.2; Table 4.3). Growth rate was also plastic and increased in the absence of cues from a predator (Fig. 4.2). Because these fish were all kept on controlled food levels, plastic changes in growth rate occurred either because of physiological changes or of differences in rates of food consumption (Beckerman et al. 2007).

There is precedent for the adaptive evolution and optimization of growth rate and conversion efficiency. For example, work on Atlantic silversides *Menidia menidia* by David Conover and colleagues provide an informative contrast to the growth rate results presented above. Northern populations of Atlantic silversides grow faster than southern populations by

converting food to biomass with greater efficiency (Conover and Present 1990; Schultz et al. 1998). This ability to undergo rapid growth increases survival in northern populations where the growing season is short (Conover and Present 1990) but comes at the cost of predator-escape performance (Billerbeck et al. 2001; Lankford et al. 2001). Similarly, preliminary evidence suggests that guppies in high-risk environments (reared with cues of a predator) retain more of the nitrogen they consume compared to individuals reared without cues of a predator (C. Dalton unpublished data). Populations of Trinidadian guppies with high extrinsic mortality are also under selection for faster growth, earlier maturity, and elevated predator-escape abilities compared to populations with low extrinsic mortality (Reznick and Endler 1982; Ghalambor et al. 2003; Walker et al. 2005). Yet, unlike the silversides no trade-off between growth rate and swimming performance has been found in guppies, given that they exhibit faster growth and superior predator-escape performance under high-predation compared to conspecifics that experience low-predation (O'Steen et al. 2002; Ghalambor et al. 2003; Walker et al. 2005). Given the absence of cues from a predator caused an increase in metabolic rate, the concomitant increase in growth rates are likely due to increased feeding and activity. Although we did not quantify feeding or daily activity in this study, other work suggests that cues from a predator result in guppies restricting their movements to the surface of the water (Torres-Dowdall et al. 2012), which likely reduces movement and their opportunities for foraging.

What is the consequence of this plasticity for the evolution of growth rate? Because growth rates increased when fish were reared without cues from a predator, but evolved to be lower when risk of predation was reduced, these patterns reflect non-adaptive plasticity and antagonistic selection, in which the plastic response is in the opposite direction of evolutionary divergence (Falconer 1990; Grether 2005: 205; Conover et al. 2009). The presence of cues in the

rearing environment suppressed growth rate in all but the Intro1 population. We also found significant GxE in the Intro1 and Intro2 populations (Table 4.3), which reflects their lack of plasticity in growth rate relative to the other populations (Fig. 4.2). Nonetheless, the HP population exhibited the fastest growth rate while the LP population had the slowest growth rate under both rearing environments, and the growth rates of the introduced populations were intermediate or closer to the rates of the LP population. Such results suggest that there must be relatively strong directional selection for slower growth in the absence of predators, despite the plastic response to increase growth when cues from a predator are absent (Perrin and Rubin 1990; Metcalfe and Monaghan 2003; Biro et al. 2006).

Relationship between resting metabolic rate and rate of somatic growth

The cost of self-maintenance should be tightly correlated with fitness due to its influence on survival, growth, and reproduction (Stearns 1992; Ricklefs and Wikelski 2002). Within individuals, RMR has been shown to be repeatable over extended periods of time and across life-history stages (McCarthy 2000; Nespolo and Franco 2007). Thus, the constraints of RMR are likely consistent throughout an animal's lifespan. Yet, empirical studies often fail to find a significant correlation between RMR and growth rate (see Table 4.1 in Burton et al. 2011). We also did not find a correlation between individual RMR and growth rate. The lack of a correlation suggests that their evolutionary trajectories are unlikely to place direct constraints on one another. However, the relationship between RMR and growth rate may be complex. Because RMR and growth rate are products of the production and allocation of energy, the mitochondrial and nuclear genomes both contribute to these phenotypes. Moreover, recent evidence suggests that intergenomic epistasis underlies variation in metabolic phenotypes through genotype-

genotype-environment interactions (GxGxE) (Arnqvist et al. 2010). Thus, RMR and growth rate could be tied to GxGxE but testing these complex interactions is beyond the scope of the current study.

Plasticity and rapid evolution in novel environments

Finite energy budgets are thought to constrain phenotypic evolution through compulsory trade-offs in life-history traits (Stearns 1992). The ability to colonize a novel environment depends on how well phenotypes can be paired with the environment. Plasticity is undoubtedly the first response by individuals colonizing a new environment and determines the range of phenotypes selection acts on. When the environment induces adaptive plasticity the strength of selection is by definition reduced because the phenotypes shift towards the new optimum (e.g., Price et al. 2003). In contrast, when the plasticity in non-adaptive, the strength of selection is stronger because the environment incudes a greater phenotype-environment mismatch and adaptation then requires that selection overcomes the plastic response (Grether 2005; Ghalambor et al. 2007; Conover et al. 2009). Examples of adaptive plasticity, particularly in physiological traits, are rare and found primarily in morphological traits (e.g., Day et al. 1994; Day and McPhail 1996; Wund et al. 2008; reviewed by Conover et al. 2009). Yet, we showed that the HP population of guppies had lower RMR than did their naturally derived descendants in the low-predation environment. When we experimentally replicated that process in the introduced populations, we found that adaptive plasticity in RMR evolved from a non-plastic ancestor. This response shows how plasticity can evolve as a by-product of directional selection in different environments (Via and Lande 1985; Gotthard and Nylin 1995; Via et al. 1995; Czesak et al. 2006).

In contrast, non-adaptive plasticity is usually found when environmental pressures (e.g., temperature, growing season, abundance of resources) moderate physiological rate processes (Grether 2005; Conover et al. 2009). Thus, selection favors individuals better capable of developing under adverse conditions, and, when released from the environmental cue, the genotype produces elevated values of a trait and exhibits countergradient variation (e.g., Conover and Present 1990; Conover and Schultz 1995; Grether 2005). In the current study, growth rate showed non-adaptive plasticity and a countergradient response between divergent populations, with the HP population having the fastest intrinsic growth rate despite the tendency for cues from a predator to suppress growth rate. However, when experimentally transplanted into low-predation streams, the ancestral HP population must have harbored sufficient additive genetic variation for selection to overcome the phenotype-environment mismatch and the introduced populations rapidly diverged in low-predation environments.

Conclusions

We found that RMR and growth rate show genetic divergence between natural HP and LP populations of guppies. These differences appear to reflect the evolution of adaptive divergence, given that the introduced populations rapidly diverged in parallel patterns towards the phenotypes of the LP population. Both traits also exhibited a plastic response to being reared in the presence or absence of cues from a predator. RMR in the introduced populations evolved adaptive plasticity that led to cogradient variation; growth rate exhibited non-adaptive plasticity that produced countergradient variation in response to the novel environment. Although, we found evidence that both traits evolved rapidly and in the direction expected, RMR only diverged in the derived environment (absence of cues from a predator) while growth rate exhibited a

greater degree of divergence and diverged in both rearing environments. These results suggest that patterns in phenotypic plasticity and their influence on underlying genetic variance can alter the range of phenotypes exposed to selection, and likely contribute to the rate of phenotypic evolution in novel environments.[8]

[8] This work was approved by the Colorado State University Institutional Animal Care and Use Committee (protocols # 11-2799A and 11-3072A). This work was supported by the National Science Foundation Faculty Early Career Development grant [DEB-0846175 to Cameron Ghalambor and National Science Foundation Frontiers in Integrative Biological Research grant (EF-0623632 to David Reznick).

Tables and figures

Table 4.1 Sample sizes for statistical analyses of oxygen consumption and rate of somatic growth.

Population	Reared without cues from a predator	Reared with cues from a predator
Oxygen consumption		
HP	24	24
Intro1	20	20
Intro2	24	24
Intro3	9	9
Intro4	13	11
LP	7	7
Somatic growth rate		
HP	51	57
Intro1	14	15
Intro2	16	16
Intro3	18	18
Intro4	20	20
LP	11	11

Table 4.2 Statistical results for the full factorial and reduced linear mixed models (fit by REML) on RMR.

Fixed effect	Estimate (s.e.)	HPD 95% interval	pMCMC
Full factorial model			
HP (intercept)	-1.11 (0.03)	-1.16, 1.06	<0.001***
Intro1	0.01 (0.04)	-0.07, 0.08	0.89
Intro2	-0.01 (0.04)	-0.08, 0.07	0.85
Intro3	-0.02 (0.05)	-0.13, 0.07	0.64
Intro4	0.08 (0.05)	-0.01, 0.17	0.08
LP	0.08 (0.06)	-0.03, 0.18	0.17
Reared without predator's cues	-0.01 (0.04)	-0.08, 0.07	0.84
Intro1 × without predator's cues	0.08 (0.06)	-0.02, 0.20	0.13
Intro2 × without predator's cues	0.04 (0.05)	-0.06, 0.14	0.47
Intro3 × without predator's cues	0.16 (0.07)	0.01, 0.29	0.02*
Intro4 × without predator's cues	-0.002 (0.07)	-0.13, 0.12	0.97
LP x without predator's cues	0.11 (0.08)	-0.04, 0.27	0.16
Reduced model			
HP (intercept)	-1.14 (0.02)	-1.18, 1.10	<0.001***
Intro1	0.05 (0.03)	-0.01, 0.11	0.09
Intro2	0.01 (0.03)	-0.04, 0.06	0.64
Intro3	0.06 (0.04)	-0.02, 0.13	0.11
Intro4	0.08 (0.03)	0.02, 0.15	0.01*
LP	0.13 (0.04)	0.06, 0.21	0.001**
Reared without predator's cues	0.04 (0.02)	0.01, 0.08	0.03*

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$

Table 4.3 Statistical results for the full factorial linear mixed model (fit by REML) on somatic growth rate.

Fixed effect	Estimate (s.e.)	HPD 95% interval	pMCMC
HP (intercept)	1.4×10^{-3} (6.0×10^{-5})	1.3×10^{-3}, 1.5×10^{-3}	<0.001***
Intro1	-3.5×10^{-4} (1.3×10^{-4})	-6.0×10^{-4}, -1.0×10^{-4}	0.01**
Intro2	-5.5×10^{-4} (1.3×10^{-4})	-8.0×10^{-4}, -3.0×10^{-4}	<0.001***
Intro3	-1.4×10^{-4} (1.2×10^{-4})	-4.0×10^{-4}, 1.0×10^{-4}	0.21
Intro4	-2.3×10^{-4} (1.2×10^{-4})	-5.0×10^{-4}, 0.00	0.06
LP	-4.5×10^{-4} (1.5×10^{-4})	-8.0×10^{-4}, -2.0×10^{-4}	0.002**
Reared without predator's cues	5.5×10^{-4} (7.7×10^{-5})	4.0×10^{-4}, 7.0×10^{-4}	<0.001***
Intro1 × without predator's cues	-2.6×10^{-4} (1.7×10^{-4})	-6.0×10^{-4}, 1.0×10^{-4}	0.19
Intro2 × without predator's cues	1.5×10^{-5} (1.6×10^{-4})	-3.0×10^{-4}, 4.0×10^{-4}	0.89
Intro3 × without predator's cues	-3.5×10^{-4} (1.6×10^{-4})	-7.0×10^{-4}, 0.00	0.05*
Intro4 × without predator's cues	-4.5×10^{-4} (1.5×10^{-4})	-8.0×10^{-4}, -1.0×10^{-4}	0.01**
LP x without predator's cues	-4.1×10^{-4} (1.9×10^{-4})	-8.0×10^{-4}, 0.00	0.07

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$

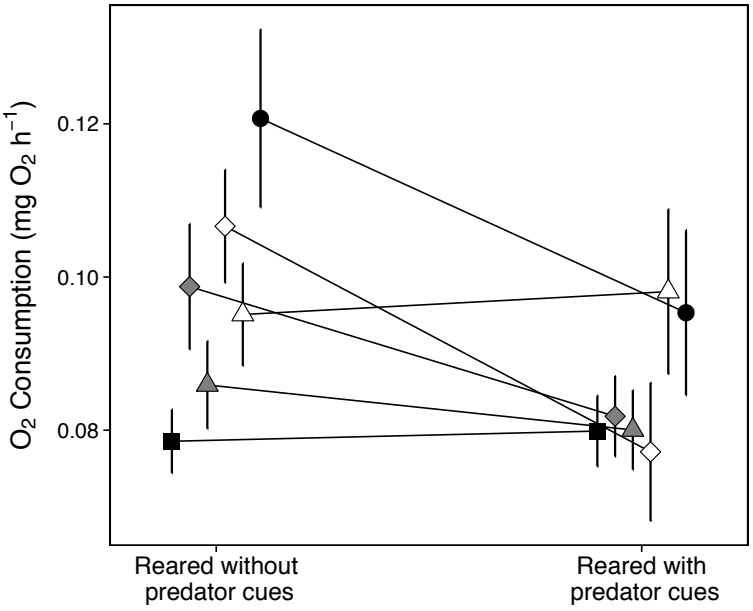

Figure 4.1 Phenotypic plasticity and evolutionary divergence in mean RMR (±SE) for the ancestral high-predation population (closed squares), derived low-predation population (closed circles), and the introduced populations (Intro1 1 = gray diamonds, Intro2 = gray triangles, Intro3 = white diamonds, and Intro4 = white triangles) reared with and without cues from a predator.

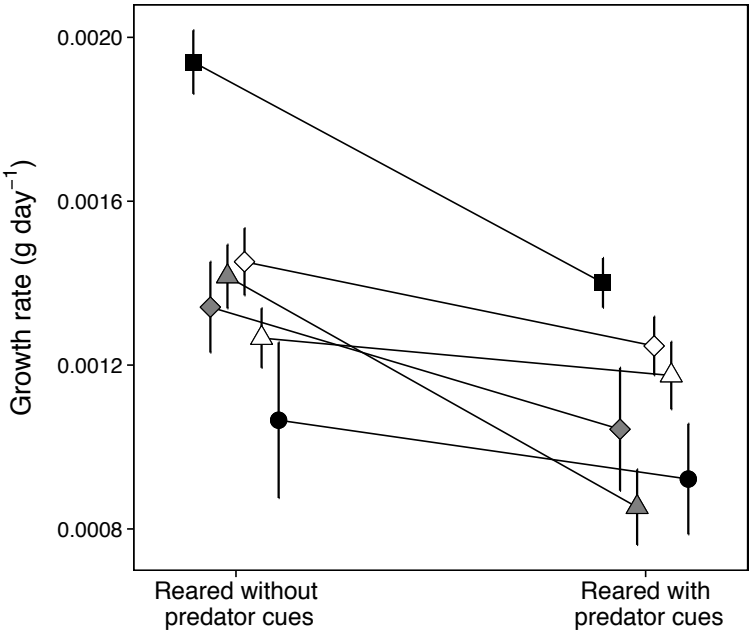

Figure 4.2 Phenotypic plasticity and evolutionary divergence in mean rate (±SE) of somatic growth for the ancestral high-predation population (closed squares), derived low-predation population (closed circles), and the introduced populations (Intro1 = gray diamonds, Intro2 = gray triangles, Intro3 = white diamonds, and Intro4 = white triangles) reared with and without cues from a predator.

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