# Phenotypic plasticity and rapid evolution following experimental introductions in nature[[1]](#footnote-1)

## 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 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 G1 females and 38 mature G1 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 G1 females and 45 mature G1 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 ± 1o 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 G1 generation. Each G1 brood was reared for 29 days, at which point fish could be reliably sexed (Reznick 1982). One G1 male and female were randomly selected from each family line, reared to maturity, and randomly outcrossed by family to propagate a G2 generation. The G2 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. G2 laboratory-reared fish were used to obtain H2 estimates. Twenty full sibling G2 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 H2 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 *H2* 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 H2 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 G­2progeny 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 *H2*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 regarding 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.[[2]](#footnote-2)

## Tables and figures

Table . 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 . 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 . 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; R2 represents the square of the correlation coefficient; Z = effect size scaled to units of standard deviations. | | | | | | |

Table . 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 . 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).

**../figs/Fig-2.pdf**

Figure . 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.

../figs/Fig-3.pdf

Figure . 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.

Macintosh HD:Users:emily:Dropbox:Projects:morphology:figs:Fig-4.pdf

Figure . 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.

../figs/Fig-5.pdf

Figure . 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.

../figs/Fig-6.pdf

Figure . 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.

../figs/Fig-7.pdf

Figure . 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.

## References

Adams, D. C., M. L. Collyer, E. Otarola-Castillo, and E. Sherratt. 2015. geomorph: an R package for the collection and analysis of geometric morphometric shape data.

Adams, D. C., and E. Otarola-Castillo. 2013. geomorph: an R package for the collection and analysis of geometric morphometric shape data. Methods Ecol. Evol. 4:393–399.

Agrawal, A. A. 2001. Phenotypic plasticity in the interactions and evolution of species. Science 294:321–326.

Alexander, H. J., and F. Breden. 2004. Sexual isolation and extreme morphological divergence in the Cumana guppy: a possible case of incipient speciation. J. Evol. Biol. 17:1238–1254.

Alexander, H. J., J. S. Taylor, S. Sze-Tsun Wu, and F. Breden. 2006. Parallel evolution and vicariance in the guppy (*Poecilia reticulata*) over multiple spatial and temporal scales. Evolution 60:2352–2369.

Ancel, L. W. 2000. Undermining the Baldwin expediting effect: Does phenotypic plasticity accelerate evolution? Theor. Popul. Biol. 58:307–319.

Anderson, M. J. 2001a. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26:32–46.

Anderson, M. J. 2001b. Permutation tests for univariate or multivariate analysis of variance and regression. Can. J. Fish. Aquat. Sci. 58:626–639.

Arendt, J. D., D. N. Reznick, and A. López-Sepulcre. 2014. Replicated origin of female biased adult sex ratio in introduced populations of the trinidadian guppy (*Poecilia reticulata*). Evolution 68:2343–2356.

Barrett, R. D. H., and D. Schluter. 2008. Adaptation from standing genetic variation. Trends Ecol. Evol. 23:38–44.

Bassar, R. D., M. C. Marshall, A. Lopez-Sepulcre, E. Zandona, S. K. Auer, J. Travis, C. M. Pringle, A. S. Flecker, S. A. Thomas, D. F. Fraser, and D. N. Reznick. 2010. Local adaptation in Trinidadian guppies alters ecosystem processes. Proc. Natl. Acad. Sci. 107:3616–3621.

Bookstein, F. L. 1997. Morphometric Tools for Landmark Data: Geometry and Biology. Cambridge University Press.

Burns, J. G., P. Di Nardo, and F. H. Rodd. 2009. The role of predation in variation in body shape in guppies *Poecilia reticulata*: a comparison of field and common garden phenotypes. J. Fish Biol. 75:1144–1157.

Calsbeek, R., and D. J. Irschick. 2007. The quick and the dead: correlational selection on morphology, performance, and habitat use in island lizards. Evolution 61:2493–2503.

Collyer, M. L., D. J. Sekora, and D. C. Adams. 2015. A method for analysis of phenotypic change for phenotypes described by high-dimensional data. Heredity 115:357–365.

Conner, J. K., and D. L. Hartl. 2004. A primer of ecological genetics. Sinauer Associates, Sunderland, Mass.

Crispo, E. 2008. Modifying effects of phenotypic plasticity on interactions among natural selection, adaptation and gene flow. J. Evol. Biol. 21:1460–1469.

Crispo, E. 2007. The Baldwin effect and genetic assimilation: revisiting two mechanisms of evolutionary change mediated by phenotypic plasticity. Evolution 61:2469–2479.

Day, T., J. Pritchard, and D. Schluter. 1994. A Comparison of Two Sticklebacks. Evolution 48:1723–1734.

Dryden, I. L., and K. V. Mardia. 1998. Statistical Shape Analysis. 1 edition. Wiley, Chichester ; New York.

Dussault, G. V., and D. L. Kramer. 1981. Food and feeding behavior of the guppy, Poecilia reticulata (Pisces: Poeciliidae). Can. J. Zool. 59:684–701.

Endler, J. A. 1995. Multiple-trait coevolution and environmental gradients in guppies. Trends Ecol. Evol. 10:22–29.

Endler, J. A. 1980. Natural selection on color patterns in Poecilia reticulata. Evolution 34:76–91.

Falconer, D. S. 1981. Introduction to Quantitative Genetics. 2nd ed. Longman, New York.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. Longman, Essex, England.

Fisher, R. A. 1930. The Genetical Theory of Natural Selection: A Complete Variorum Edition. Oxford University Press.

Ghalambor, C. K., K. L. Hoke, E. W. Ruell, E. K. Fischer, D. N. Reznick, and K. A. Hughes. 2015. Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. Nature 525:372–375.

Ghalambor, C. K., J. K. McKay, S. P. Carroll, and D. N. Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct. Ecol. 21:394–407.

Ghalambor, C. K., D. N. Reznick, and J. A. Walker. 2004. Constraints on adaptive evolution: the functional trade‐off between reproduction and fast‐start swimming performance in the Trinidadian guppy (*Poecilia reticulata*). Am. Nat. 164:38–50.

Gienapp, P., C. Teplitsky, J. S. Alho, J. A. Mills, and J. Merila. 2008. Climate change and evolution: disentangling environmental and genetic responses. Mol. Ecol. 17:167–178.

Godin, J. J., and S. E. Briggs. 1996. Female mate choice under predation risk in the guppy. Anim. Behav. 51:117–130.

Goodall, C. 1991. Procrustes methods in the statistical analysis of shape. J. R. Stat. Soc. Ser. B Methodol. 53:285–339.

Gordon, S. P., D. Reznick, J. D. Arendt, A. Roughton, M. N. Ontiveros Hernandez, P. Bentzen, and A. López-Sepulcre. 2015. Selection analysis on the rapid evolution of a secondary sexual trait. Proc. R. Soc. B Biol. Sci. 282:20151244.

Grether, G. F. 2005. Environmental change, phenotypic plasticity, and genetic compensation. Am. Nat. 166:E115–E123.

Hadfield, J. D. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. J. Stat. Softw. 33:1–22.

Handelsman, C. A., E. D. Broder, C. M. Dalton, E. W. Ruell, C. A. Myrick, D. N. Reznick, and C. K. Ghalambor. 2013. Predator-induced phenotypic plasticity in metabolism and rate of growth: Rapid adaptation to a novel environment. Integr. Comp. Biol. 53:975–988.

Handelsman, C. A., E. W. Ruell, J. Torres-Dowdall, and C. K. Ghalambor. 2014. Phenotypic plasticity changes correlations of traits following experimental introductions of Trinidadian guppies (*Poecilia reticulata*). Integr. Comp. Biol. 54:794–804.

Hendry, A. P., M. L. Kelly, M. T. Kinnison, and D. Reznick. 2006. Parallel evolution of the sexes? Effects of predation and habitat features on the size and shape of wild guppies. J. Evol. Biol. 19:741–754.

Herrel, A., K. Huyghe, B. Vanhooydonck, T. Backeljau, K. Breugelmans, I. Grbac, R. Van Damme, and D. J. Irschick. 2008. Rapid large-scale evolutionary divergence in morphology and performance associated with exploitation of a different dietary resource. Proc. Natl. Acad. Sci. 105:4792–4795.

Irschick, D. J., and T. Garland Jr. 2001. Integrating function and ecology in studies of adaptation: investigations of locomotor capacity as a model system. Annu. Rev. Ecol. Syst. 32:367–396.

Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, A. Hoang, P. Gibert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. Am. Nat. 157:245–261.

Kruuk, L. E. B., T. H. Clutton-Brock, J. Slate, J. M. Pemberton, S. Brotherstone, and F. E. Guinness. 2000. Heritability of fitness in a wild mammal population. Proc. Natl. Acad. Sci. 97:698–703.

Laland, K., T. Uller, M. Feldman, K. Sterelny, G. B. Müller, A. Moczek, E. Jablonka, J. Odling-Smee, G. A. Wray, H. E. Hoekstra, D. J. Futuyma, R. E. Lenski, T. F. C. Mackay, D. Schluter, and J. E. Strassmann. 2014. Does evolutionary theory need a rethink? Nature 514:161–164.

Lande, R. 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. J. Evol. Biol. 22:1435–1446.

Langerhans, R. B., and T. J. Dewitt. 2004. Shared and unique features of evolutionary diversification. Am. Nat. 164:335–349.

Langerhans, R. B., and D. N. Reznick. 2009. Ecology and evolution of swimming performance in fishes: predicting evolution with biomechanics. Pp. 200–248 *in* P. Domenici and B. G. Kapoor, eds. Fish locomotion: an etho-ecological perspective.

Layman, C. A., R. B. Langerhans, and T. J. Dewitt. 2003. Habitat-associated morphological divergence in two Neotropical fish species. Biol. J. Linn. Soc. 80:689–698.

Levin, D. A. 1988. Plasticity, canalization and evolutionary stasis in plants. Pp. 35–45 *in* A. J. Davy, M. J. Hutchings, and A. R. Watkinson, eds. Plant Population Biology. Blackwell, Oxford, England.

Losos, J. B. 1990. A phylogenetic analysis of character displacement in Caribbean Anolis lizards. Evolution 44:558–569.

Losos, J. B. 1998. Contingency and Determinism in Replicated Adaptive Radiations of Island Lizards. Science 279:2115–2118.

Losos, J. B., T. W. Schoener, and D. a Spiller. 2004. Predator-induced behaviour shifts and natural selection in field-experimental lizard populations. Nature 432:505–508.

Losos, J. B., K. I. Warheit, and T. W. Schoener. 1997. Adaptive differentiation following experimental island colonization in Anolis lizards. Nature 387:70–73.

Magurran, A. E. 2005. Evoutionary Ecology: The Trinidadian Guppy. Oxford University Press, Oxford.

Morrissey, M. B., L. E. B. Kruuk, and A. J. Wilson. 2010. The danger of applying the breeder’s equation in observational studies of natural populations. J. Evol. Biol. 23:2277–2288.

Orr, H. A. 1998. The population genetics of adaptation: The distribution of factors fixed during adaptive evolution. Evolution 52:935–949.

Paenke, I., B. Sendhoff, and T. J. Kawecki. 2007. Influence of plasticity and learning on evolution under directional selection. Am. Nat. 170:E47–E58.

Parsons, K. J., and B. W. Robinson. 2006. Replicated evolution of integrated plastic responses during early adaptive divergence. Evolution 60:801–813.

Pemberton, J. M. 2010. Evolution of quantitative traits in the wild: mind the ecology. Philos. Trans. R. Soc. B Biol. Sci. 365:2431–2438.

Pigliucci, M. 2003. Phenotypic integration: studying the ecology and evolution of complex phenotypes. Ecol. Lett. 6:265–272.

Pigliucci, M. 2001. Phenotypic Plasticity: Beyond nature and nurture. Johns Hopkins University Press, Baltimore.

Pigliucci, M., and G. B. Müller. 2010. Evolution - the Extended Synthesis. The MIT Press, Cambridge, Mass.

Price, T. D., A. Qvarnström, and D. E. Irwin. 2003. The role of phenotypic plasticity in driving genetic evolution. Proc. R. Soc. B Biol. Sci. 270:1433–1440.

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reznick, D. 1982. The impact of predation on life history evolution in Trinidadian guppies: genetic basis of observed life history patterns. Evolution 36:1236–1250.

Reznick, D., and H. Bryga. 1987. Life-history evolution in guppies (*Poecilia reticulata*): 1. Phenotypic and genetic changes in an introduction experiment. Evolution 41:1370–1385.

Reznick, D., and J. A. Endler. 1982. The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). Evolution 36:160–177.

Reznick, D. N., and H. A. Bryga. 1996. Life-history evolution in guppies (Poecilia reticulata: Poeciliidae). V. Genetic basis of parallelism in life histories. Am. Nat. 147:339–359.

Reznick, D. N., H. Bryga, and J. A. Endler. 1990. Experimentally induced life-history evolution in a natural population. Nature 346:357–359.

Reznick, D. N., and C. K. Ghalambor. 2001. The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. Genetica 112-113:183–98.

Reznick, D. N., F. H. Shaw, F. H. Rodd, and R. G. Shaw. 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). Science 275:1934.

Reznick, D. N., and J. Travis. 1996. The empirical study of adaptation in natural populations. Pp. 243–290 *in* M. R. Rose and G. V. Lauder, eds. Adaptation. Academic Press, San Diego.

Robinson, B. W., and K. J. Parsons. 2002. Changing times, spaces, and faces: tests and implications of adaptive morphological plasticity in the fishes of northern postglacial lakes. Can. J. Fish. Aquat. Sci. 59:1819–1833.

Robinson, B. W., and D. S. Wilson. 1995. Experimentally induced morphological diversity in Trinidadian guppies (*Poecilia reticulata*). Copeia 294–305.

Rohlf, F. J. 2015. The tps series of software. Hystrix Ital. J. Mammal. 26.

Rohlf, F. J., and L. F. Marcus. 1993. A Revolution in Morphometrics. Trends Ecol. Evol. 8:129–132.

Rohlf, F. J., and D. E. Slice. 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. Syst. Zool. 40–59.

Schluter, D., and J. D. McPhail. 1992. Ecological character displacement and speciation in sticklebacks. Am. Nat. 140:85.

Seghers, B. H. 1974. Schooling behavior in the guppy (*Poecilia reticulata*): an evolutionary response to predation. Evolution 28:486–489.

Siepielski, A. M., J. D. DiBattista, and S. M. Carlson. 2009. It’s about time: the temporal dynamics of phenotypic selection in the wild. Ecol. Lett. 12:1261–1276.

Svanbäck, R., and P. Eklöv. 2002. Effects of habitat and food resources on morphology and ontogenetic growth trajectories in perch. Oecologia 131:61–70.

Templeton, C. N., and W. M. Shriner. 2004. Multiple selection pressures influence Trinidadian guppy (*Poecilia reticulata*) antipredator behavior. Behav. Ecol. 15:673–678.

Teplitsky, C., J. A. Mills, J. S. Alho, J. W. Yarrall, and J. Merila. 2008. Bergmann’s rule and climate change revisited: Disentangling environmental and genetic responses in a wild bird population. Proc. Natl. Acad. Sci. 105:13492–13496.

Thompson, J. N. 1998. Rapid evolution as an ecological process. Trends Ecol. Evol. 13:329–332.

Torres-Dowdall, J., C. A. Handelsman, D. N. Reznick, and C. K. Ghalambor. 2012. Local adaptation and the evolution of phenotypic plasticity in Trinidadian guppies (*Poecilia reticulata*). Evolution 66:3432–3443.

Travis, J. 1994. Evaluating the adaptive role of morphological plasticity. Pp. 99–122 *in* P. C. Wainwright and S. M. Reilly, eds. Ecological Morphology: Integrative Organismal Biology. University of Chicago Press.

Travis J, Reznick D, Bassar RD, López-Sepulcre A, Ferriere R, Coulson T. 2014. Do Eco-Evo Feedbacks Help Us Understand Nature? Answers From Studies of the Trinidadian Guppy. In: Advances in Ecological Research. Vol. 50. Elsevier. p. 1–40.

Walker, J. A., C. K. Ghalambor, O. L. Griset, D. Mckenney, and D. Reznick. 2005. Do faster starts increase the probability of evading predators? Funct. Ecol. 19:808–815.

Webb, P. W. 1978. Fast-start performance and body form in seven species of teleost fish. J. Exp. Biol. 74:211.

West-Eberhard, M. J. 2003. Developmental Plasticity and Evolution. Oxford University Press, Oxford, England.

West-Eberhard, M. J. 2005. Phenotypic accommodation: adaptive innovation due to developmental plasticity. J. Exp. Zoolog. B Mol. Dev. Evol. 304B:610–618.

Williams, G. C. 1966. Adaptation and Nantural Selection. Princeton University Press, Princeton.

Wilson, A. J., D. Réale, M. N. Clements, M. M. Morrissey, E. Postma, C. A. Walling, L. E. B. Kruuk, and D. H. Nussey. 2010. An ecologist’s guide to the animal model. J. Anim. Ecol. 79:13–26.

Wright, S. 1931. Evolution in Mendelian Populations. Genetics 16:97–159.

Zandonà, E., S. K. Auer, S. S. Kilham, J. L. Howard, A. Lopez-Sepulcre, M. P. O’Connor, R. D. Bassar, A. Osorio, C. M. Pringle, and D. N. Reznick. 2011. Diet quality and prey selectivity correlate with life histories and predation regime in Trinidadian guppies. Funct. Ecol. 25:964–973.

Zelditch, M. L., D. L. Swiderski, H. D. Sheets, and W. L. Fink. 2004. Geometric Morphometrics for Biologists: A Primer. Academic Press.

1. Co-authored by: Emily W. Ruell (Dept. of Biology, Colorado State University), Julian Torres Dowdall (Dept. of Biology, University of Konstanz), Andrés Lopez-Sepulcre (Laboratoire Ecologie & Evolution, École Normale Supérieure, Paris, France), Sarah W. Fitzpatrick (Dept. of Biology, Colorado State University), Julia K. Feuerbacher (Dept. of Biology, Colorado State University), Lisa M. Angeloni (Dept. of Biology, Colorado State University), David N. Reznick (University of California, Riverside), Cameron K. Ghalambor (Dept. of Biology & Graduate Degree Program in Ecology, Colorado State University) [↑](#footnote-ref-1)
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. [↑](#footnote-ref-2)