



LOCAL ADAPTATION AND THE EVOLUTION OF PHENOTYPIC PLASTICITY IN TRINIDADIAN GUPPIES (*POECILIA RETICULATA*)

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Divergent selection pressures across environments can result in phenotypic differentiation that is due to local adaptation, phenotypic plasticity, or both. Trinidadian guppies exhibit local adaptation to the presence or absence of predators, but the degree to which predator-induced plasticity contributes to population differentiation is less clear. We conducted common garden experiments on guppies obtained from two drainages containing populations adapted to high- and low-predation environments. We reared full-siblings from all populations in treatments simulating the presumed ancestral (predator cues present) and derived (predator cues absent) conditions and measured water column use, head morphology, and size at maturity. When reared in presence of predator cues, all populations had phenotypes that were typical of a high-predation ecotype. However, when reared in the absence of predator cues, guppies from high- and low-predation regimes differed in head morphology and size at maturity; the qualitative nature of these differences corresponded to those that characterize adaptive phenotypes in high- versus low-predation environments. Thus, divergence in plasticity is due to phenotypic differences between high- and low-predation populations when reared in the absence of predator cues. These results suggest that plasticity might initially play an important role during colonization of novel environments, and then evolve as a by-product of adaptation to the derived environment.

KEY WORDS: Baldwin effect, behavior, life-history evolution, predation, reaction norm, trophic morphology.

Understanding the causes and consequences of phenotypic divergence among natural populations is a fundamental goal of evolutionary ecology. When populations occupy different environments, divergent selection pressures can result in phenotypic differentiation in traits that confer a local fitness advantage (Kawecki and Ebert 2004). Adaptive phenotypic divergence can occur through local adaptation and/or adaptive phenotypic plasticity. In the absence of any homogenizing effects of gene flow, divergent selection can lead to the evolution of locally adapted ecotypes, where resident genotypes produce phenotypes with higher relative fitness than genotypes originating from other habitats

(Kawecki and Ebert 2004). Alternatively, adaptive phenotypic plasticity in response to environmental cues can move population phenotypes toward a local optimum without any genetic differentiation (Schlichting and Pigliucci 1998; Price et al. 2003).

Empirical studies of local adaptation often use translocation or common garden experiments to test the degree to which fitness, or traits linked to fitness, persist across different environments (Gotthard and Nylin 1995; Kawecki and Ebert 2004). By measuring the plasticity or shift in trait values by a genotype across different environments (i.e., the reaction norm), these approaches can separate the genetic and environmental contributions to the

phenotype (Via et al. 1995; Kawecki and Ebert 2004; Williams et al. 2008). For example, demonstrating local adaptation typically requires that adaptive phenotypic differences observed in the field be maintained under common garden conditions (Hereford 2009). Further, any genotype \times environment interactions should confer higher relative fitness to a population in its native environment relative to individuals from other populations (Hereford 2009). Thus, significant evidence for genotype by environment interactions reveals how phenotypic plasticity has evolved and the expected phenotypes that would initially be produced by each population invading the opposite environment (Gotthard and Nylin 1995; Schlichting and Pigliucci 1998; Ghalambor et al. 2007).

To identify which features of the environment select for adaptive divergence and induce plasticity is often challenging in natural populations. Predation is often heterogeneous in its distribution and has been shown to impose strong evolutionary and plastic responses in the phenotypes of potential prey (Kerfoot and Sih 1987). For example, predator-induced mortality has been demonstrated to be a strong selective pressure capable of driving rapid evolution in a suite of antipredator traits in insects (e.g., Whitman and Agrawal 2009), fish (e.g., Webb 1986; Reznick and Ghalambor 2001; O'Steen et al. 2002), and lizards (e.g., Irschick and Losos 1998; Warner and Andrews 2002). Predation can also cause strong ecological effects, inducing plastic responses in many of the same traits under direct selection from predators (e.g., Harvell 1990; Agrawal 2001; Stoks et al. 2003; Relyea and Auld 2005). For example, predators can directly reduce fitness in prey populations through increased mortality, and indirectly by eliciting stress responses that lead to changes in behavior, reductions in foraging time budgets, and reductions in growth rate (e.g., Sih 1980; Lima and Dill 1990; Sih and Moore 1993; Werner and Anholt 1993; Brown et al. 1999; Nonacs and Blumstein 2010). Thus, spatial variation in predation risk has the potential to act as both an agent of divergent selection and an environmental cue that induces phenotypic plasticity.

Guppies (*Poecilia reticulata*) show a repeated spatial pattern of local adaptation to the presence and absence of predators throughout the Northern Range Mountains of Trinidad, where small headwater tributaries generally lack piscivorous fish and downstream rivers contain a suite of piscivorous species (Haskins et al. 1961; Seghers 1973; Endler 1995). Genetic studies of natural populations of Trinidadian guppies suggest that this spatial pattern of local adaptation has independently evolved numerous times because adjacent high- and low-predation populations within a drainage are genetically similar and highly divergent from populations in neighboring drainages (Carvalho et al. 1991; Crispo et al. 2006; Alexander et al. 2006; Suk and Neff 2009; Willing et al. 2010). Thus, guppy populations experiencing relatively high predation in larger streams appear to have repeatedly colonized and successfully established populations in upstream

tributaries where both predation pressure and resource availability are reduced (Reznick et al. 2001).

Variation in predation pressure has repeatedly led to population-level divergence in multiple guppy traits, including life histories (e.g., Reznick 1982; Reznick et al. 1996), morphology (e.g., Alexander and Breden 2004), behavior (e.g., Magurran and Seghers 1991, 1994; Huizinga et al. 2009), and swimming performance (e.g., Ghalambor et al. 2003). Many of these traits have a genetic basis and have been shown to evolve on contemporary time scales (reviewed in Reznick et al. 1997). Additionally, environmental cues can induce adaptive plastic responses in several of these same traits. For example, in response to visual or chemical cues from predators, guppies alter habitat use (Botham et al. 2006), age at maturity (Rodd et al. 1997; Gosline and Rodd 2008), shoaling behavior (Huizinga et al. 2009), and other life-history traits (Dzikowski et al. 2004). Similarly, in response to changes in food quantity or type, guppies exhibit adaptive plasticity in head morphology (Robinson and Wilson 1995), and age and size at maturity (Reznick 1990). Thus, although we expect guppy populations to show local adaptation in behavioral, morphological, and life-history traits, we also predict these same traits to exhibit some degree of plasticity in response to environmental conditions during development. What remains unclear, however, is the degree to which predator-induced plasticity explains the magnitude of population differentiation, the role of plasticity during the process of local adaptation, and the degree to which populations differ in the magnitude of plasticity they exhibit.

Here, we experimentally reared family lines of guppies derived from two high- and low-predation population pairs under conditions mimicking the ancestral, high- and derived, low-predation environments by exposing them to chemical cues from a natural predator of guppies. Specifically, we assessed whether exposure to these predator cues induces plastic changes in habitat use (measured as position in the water column), head morphology, and size at maturity to test if the magnitude and direction of plasticity has diverged between high- and low-predation populations. This suite of traits comprises phenotypes that have been shown to be correlated with fitness in high- and low-predation guppy populations through their effects on food acquisition, predator avoidance, and mate acquisition (Seghers 1973; Reznick 1982; Reznick et al. 2001; Magurran 2005; Robinson and Wilson 1995; Langerhans 2010).

Material and Methods

EXPERIMENTAL SUBJECTS

Gravid *P. reticulata* females were collected from high- and low-predation localities from the Yarra and Guanapo rivers located in

the Northern Range Mountains on the island of Trinidad, West Indies. The Yarra River is one of the major drainages on the Northern Slope region, and the Guanapo River is part of the larger Caroni drainage on the southern slope of the Northern Range Mountains. Previous work has identified these regions as containing two distinct genetic lineages of guppies that have independently evolved in response to the presence and absence of predators (e.g., Willing et al. 2010). Within drainages, we collected fish from two localities: (1) a high-predation locality where guppies coexist with a diversity of large piscivorous fish, and (2) a low-predation locality that lacks large piscivorous fish. Within the Yarra drainage, individuals from the high-predation population were collected approximately 2-km upstream from the confluence with the ocean, whereas individuals from the low-predation population were collected from a small upstream tributary of the Yarra River (see Reznick et al. 1996 for details). In the Guanapo drainage, individuals from the high-predation population were collected approximately 7-km upstream from the confluence of the Guanapo River with the higher order Caroni River. Individuals from the low-predation population were collected from the Taylor stream, a small tributary to the Guanapo River (see Gilliam et al. 1993 for details).

Wild-caught females from each population were kept individually either in 10-L glass tanks without recirculating water (Yarra fish) or in 1.5-L tanks in recirculating systems (Guanapo fish) (12L:12D, temperature $25 \pm 1^\circ\text{C}$; Reznick 1982) and fed twice daily (AM: TertraminTM tropical fish flake paste, PM: hatched *Artemia* cysts). We propagated two lab-born generations from these wild collected females to generate distinct F2 family lines. To maximize genetic variation within populations and to minimize any confounding maternal effects, F2 family lines were generated by randomly crossing lab-born fish within each generation and population. Not all collected fish were successfully propagated to the second generation, thus our sample size varies among populations. Of the original families per population in the Yarra drainage, 11 of the low-predation and 13 of the high-predation families persisted through two lab-reared generations, whereas 15 low-predation and 23 high-predation families persisted from the Guanapo drainage.

EXPERIMENTAL DESIGN

We conducted a common garden experiment where F2 families from all four populations were reared in the presence or absence of predator cues. Chemical cues are thought to play an important role in predator detection in guppies (Nordell 1998; Brown and Godin 1999; Dzikowski et al. 2004; Huizinga et al. 2009). Previous work has identified specialized epidermal cells, called club cells, which release an alarm pheromone when ruptured during a predation event (Pfeiffer 1974). We fed guppies to pike cichlids (*Crenicichla* spp.)—a common predator of guppies in the southern slope of

the Northern Range of Trinidad—to mimic the chemical cues in nature and ensure that both the alarm pheromone and any predator kairomones (chemicals excreted by predators) were present in the water (see below).

Within 24 h postparturition, families of F2 litters were evenly split between two tanks ($n = 2\text{--}10$ fish per tank) corresponding to one of two treatments: with or without predator cues. To simulate predator cues, fish from the Yarra populations were reared in nonrecirculating 10-L tanks, where we exchanged water from tanks housing pike cichlids maintained on a diet of two guppies daily. Every three days, 1 L of dechlorinated tap water (without predator cue treatment) and 1 L of predator-conditioned water (with predator cue treatment) were exchanged in each tank and all observations occurred within 24 h following water exchanges. Fish from the Guanapo drainage were reared in 1.5-L tanks that were part of a recirculating system. A pike cichlid (fed a diet of two guppies daily) was maintained in the sump of the system to generate the predator cue treatment, and an identical system lacking a pike cichlid was used for the without predator cue treatment. Thus, in both types of tanks, the water in the predator cue treatment contained both predator kairomones and guppy alarm cues that were expected to act in concert to induce plastic responses to perceived predation risk (Nordell 1998; Dzikowski et al. 2004; Schoepfner and Relyea 2005).

DATA COLLECTION

We measured three dependent variables: vertical position in the water column (a proxy for habitat use), male size at maturity, and head morphology. The vertical position of fish in the water column was measured prior to morning feedings. Each trial consisted of recording the number of fish at the water surface (i.e., within 1 cm of the surface), and the number of fish throughout the remainder of the water column (i.e., between the bottom of the tank and 1 cm of the surface) once per minute for 5 min. The average of these five observations was used to quantify the weekly proportion of fish using the surface versus the remainder of the water column. In all cases, fish were not fed between 12 and 15 h prior to observations in effort to standardize any effect of hunger on behavior. To test if there was an acclimation or habituation effect to the chemical cues, we conducted a pilot study on three-, four-, and five-week-old fish, in which we measured the vertical position of fish from the Yarra drainage. We found no effect of age on the proportion of fish at the surface ($F_{2,102.4} = 0.560$, $P = 0.572$; P -values for all interactions were >0.5), suggesting no habituation to the treatment over time. Therefore, water column use was only measured at maturity in subsequent observations.

Once male guppies reached maturity, as determined by the development of the anal fin following Reznick (1982), they were anesthetized with tricainemethanesulfonate (MS-222; 100 mg/L) and photographed. Photographs were used to calculate size at

maturity by measuring the standard length to the nearest 0.01 mm (ImageJ Software; Abramoff et al. 2004) and to measure head shape. Geometric morphometrics was used to quantify variation in head shape between individuals (Rohlf and Marcus 1993). To capture the variation in head shape, four homologous landmarks and one sliding semilandmark (Bookstein 1997) were digitized from digital photographs with tpsDig2 (Rohlf 2003; Fig. 3). A Generalized Procrustes Analysis (Tpsrelw software; Rohlf 2003) was performed to align the sliding semilandmark among all specimens and to remove isometric size, rotational, and position effects on Cartesian coordinates (Bookstein 1991).

STATISTICAL ANALYSIS

A mixed model analysis of variance (ANOVA) was used to test for differences in the proportion of fish that were found at the surface of the tank and differences in standard length at maturity. We used drainage, treatment, natural predation regime (hereafter predation regime), and their interactions as fixed factors, and family as a random effect. We square root-arcsine transformed the proportion of fish at the surface to closer approximate normality in the data.

The analysis of head morphology was performed in two steps. First, a multivariate analysis of covariance (MANCOVA, performed in R version 2.13; <http://www.r-project.org>) was used to test for differences in head shape due to the effects of drainage, treatment, and predation regime with centroid size (Bookstein 1991) included as a covariate. The MANCOVA model evaluates how each factor in the model explains variation in head shape (matrix of aligned landmarks coordinates). Further, we estimated the partial variance (η^2), the effect size of each model parameter. We found drainage of origin to be the dominant effect in the MANCOVA (Table 1), and therefore, we subsequently analyzed each drainage separately to look for qualitatively similar responses to the experimental treatments.

To visualize how head shape in each high- and low-predation population pair responded to the two rearing environments, we performed a Canonical Variate Analysis (CVA). In MorphoJ, CVA was performed on the aligned specimen coordinates (Klingenberg 2011). Predation regime and rearing treatment were used as classifiers in the CVA to identify axes of shape that maximize the difference in head shape among populations in each treatment. Discriminant function analysis was used to show how rearing treatment corresponds to variation in head shape (MorphoJ; Klingenberg 2011). The discriminant function analysis finds the linear combination of the shape variables that best separates the groups reared with and without predator cues. The variation in head shape along the discriminant axis was depicted with wire frame outlines that capture the nature of shape change (MorphoJ; Klingenberg 2011).

Given that fish from the two drainages were reared in different systems (nonrecirculating 10-L tanks vs. 1.5-L tanks in the

recirculating system), drainage effect and rearing conditions are confounded factors in our experimental design, thus we explicitly considered how rearing system might influence the interpretation of any effects of drainage, or its interaction with other factors, on variance observed in our study. All analyses were performed in JMP (JMP, version 9. SAS Institute Inc., Cary, NC, 1989–2011) except where otherwise noted.

Results

The presence of predator cues during development caused guppies to significantly shift their vertical position to within 1 cm of the water column surface, whereas in the absence of the predator cues guppies were rarely observed at the water column surface ($F_{1,56.91} = 278.45$, $P < 0.0001$; Fig. 1). However, no differences between drainages or predation regime within drainages were observed (all P values, including those for interaction terms >0.05 ; Fig. 1). In the presence of predator cues, 64.9% of the fish were observed at the surface of the water, whereas only 3.1% of the fish were observed at the surface in the treatment without predator cues (Fig. 1). Families did not significantly differ in their use of

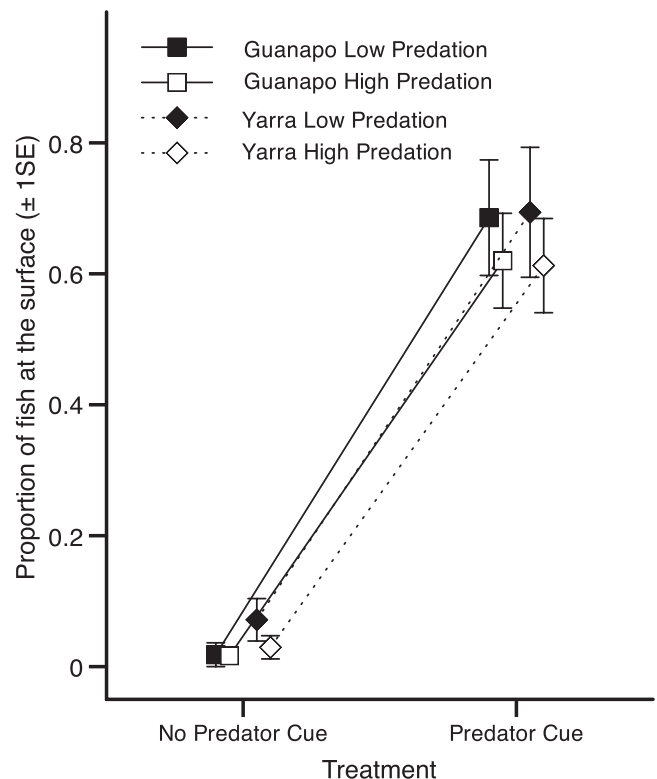


Figure 1. Mean and SEs of proportion of fish at surface from low- (filled symbols) and high- (open symbols) predation regimes from the Guanapo (square symbols) and the Yarra (diamond symbol) drainages reared in the presence or absence of predator cues. Symbols are slightly displaced to facilitate visualization of the error structure.

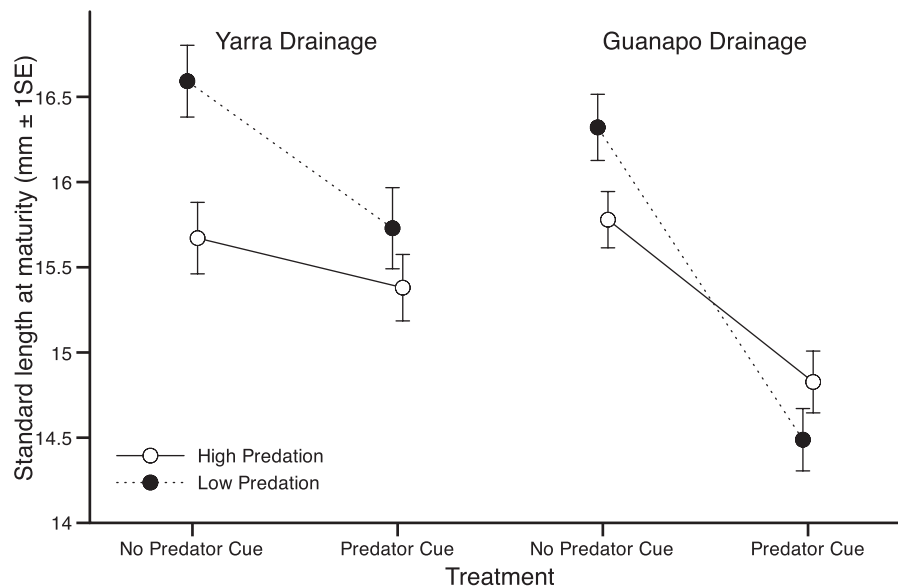


Figure 2. Mean and SEs of standard length at maturity from low- (filled symbols) and high- (open symbols) predation regime guppies reared in the presence or absence of predator cues. The right panel depicts fish from the Guanapo drainage, and the left panel depicts fish from the Yarra drainage. Symbols are slightly displaced to facilitate visualization of the error structure.

the water column, or in how they reacted to the presence or absence of predator cues (95% confidence interval for the variance component: -0.01 , 0.02 and -0.02 , 0.06 , respectively).

We found that in both drainages, the size at maturity was significantly larger in fish from low-predation regimes than their counterparts from high-predation regimes when reared in the absence of predator cues (Fig. 2). However, no differences in size were observed between populations when reared in the presence of predator cues, resulting in a significant interaction between predation regime and treatment (Fig. 2, treatment \times predation regime interaction $F_{1,48.19} = 8.046$, $P = 0.007$). Furthermore, we found a higher degree of plasticity (i.e., a steeper slope) for size at maturity in fish from the Guanapo drainage than in fish from the Yarra drainage across treatments, as differences in size between fish reared with and without predator cues were greater for Guanapo fish than it was for Yarra fish (Fig. 2, treatment \times drainage interaction $F_{1,43.83} = 11.690$, $P = 0.001$). This higher degree of plasticity in Guanapo guppies appears to be independent of the natural predation regime (i.e., high- vs. low-predation), as we did not find a significant interaction between drainage, treatment, and predation regime (P -value > 0.05). All main effects were significant (drainage: $F_{1,45.35} = 6.066$, $P = 0.018$; predation regime: $F_{1,50.40} = 4.397$, $P = 0.041$; treatment: $F_{1,44.60} = 53.923$, $P < 0.0001$). Variation among families explained 24% of the observed variation in size at maturity (95% confidence interval for the variance component estimate: 0.01 , 0.36). However, families did not significantly differ in their response to the presence or absence of predator cues (95%

confidence interval for the variance component estimate: -0.19 , 0.19).

Fish reared with predator cues also developed longer and shallower heads than fish reared without predator cues (Fig. 3A). A multivariate analysis of covariance (with centroid size as a covariate) revealed that head morphology differed between high- and low-predation guppies, between fish from the Yarra and Guanapo drainages, and in response to the presence/absence of predator cues in the rearing environment (Table 1). However, independent of the rearing environment, the magnitude of the difference in head shape between fish from high- and low-predation regimes is greater in fish from the Yarra Drainage than in fish from the Guanapo Drainage (Table 1; all other interactions were non-significant).

We found drainage of origin to be the dominant effect in our linear model, explaining more than 80% of the partial variance (Table 1). Thus, we conducted a post-hoc analysis on each drainage separately to look for qualitatively similar responses to the experimental treatments. For this, we used CVA to visualize trends in head shape variation as a function of treatment and predation regime. The first canonical variate axis (CV1; Fig. 3B) explained between 77% and 80% of the total variation, and shows a trend for fish from different predator regimes to differ in the degree to which they exhibit plasticity when reared in the absence of predator cues than when reared in the presences of predator cues (Fig. 3B). The second canonical variate axis (CV2; Fig. 3B) explained an additional 15–20% of the variation, and further separates high- and low-predation populations when reared in the

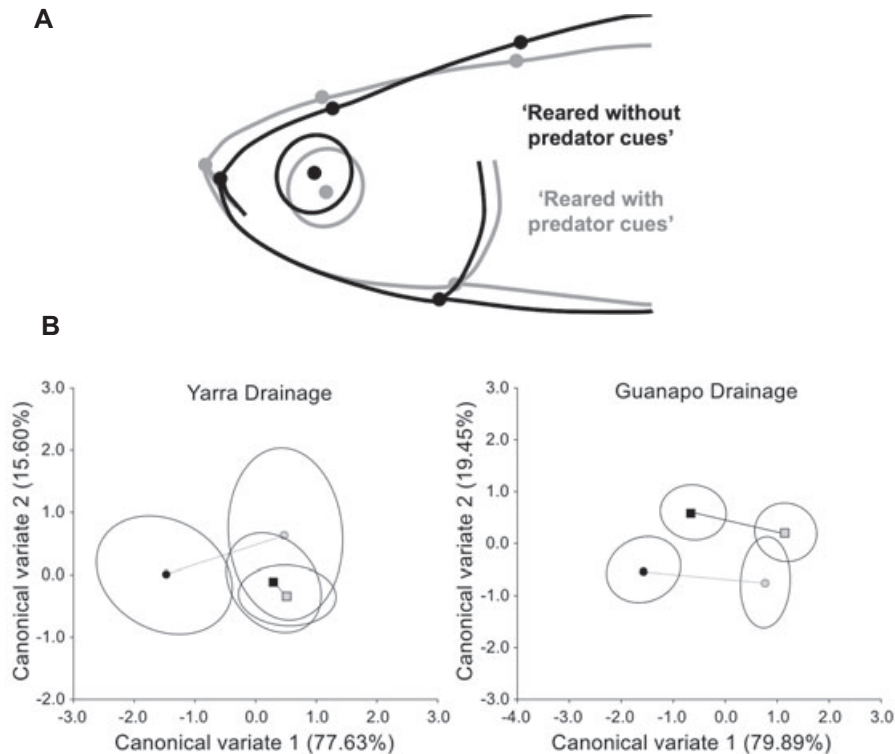


Figure 3. (A) Changes in head shape due to the effect of rearing environment identified by discriminant function analysis (Klingenberg 2011). When reared in the presence of predator cues, guppies develop a more fusiform head shape (gray lines). In the absence of predator cues, the angle from the rostrum to the posterior terminus of the head increased (black line). Solid circles represent landmarks used for morphological analysis. (B) Mean and 95% confidence ellipses for CV1 and CV2 scores from low- (circles) and high- (squares) predation regime guppies reared in the presence (gray symbols) or absence (black symbols) of predator cues. Analyses were performed for each drainage separately. Note that low-predation genotypes responded more strongly to the treatment than the high-predation genotypes, and that the 95% confidence ellipses overlap for the high- and low-predation populations in the presence of the predator cue, but not in its absence.

absence of predator cues (Fig. 3B). However, the high-predation populations between drainages differ in their plastic response because head shape in the Yarra high predation is invariant to the treatment (Fig. 3B).

Discussion

Adaptive phenotypic divergence in response to different environmental conditions can be due to local adaptation, phenotypic plasticity, or both (Reznick and Travis 1996; Schlichting and Pigliucci 1998). Common garden studies using treatments designed to simulate natural environmental variation can experimentally test whether phenotypic divergence represents evolved genetic differences or a plastic response to the environment (Gotthard and Nylin 1995; Kawecki and Ebert 2004; Williams et al. 2008; Hereford 2009). Here, in a comparison of guppies from high- and low-predation environments from two different drainages, we found evidence for evolution in some, but not all, traits thought to be under divergent selection from predators.

Yet, the evidence for evolutionary divergence was dependent on the rearing environment. When second-generation lab-born fish were reared in the presence of chemical cues produced by pike cichlids consuming guppies, no differences were found between guppies from high- and low-predation environments in any of the examined traits, regardless of their drainage of origin (Figs. 1, 2, 3). However, when fish were reared in the absence of these predator cues, guppies from high- and low-predation environments did exhibit significant differences in size at maturity and a similar pattern was found in head shape (Figs. 2, 3).

The observed differences between guppies from high- and low-predation regimes suggest a genetic basis to what are thought to be adaptive differences between guppy populations occurring in high- and low-predation environments, but also indicate that plasticity in two of the three analyzed traits has evolved. The divergence in the slope of the reaction norms between high- and low-predation populations within drainages occurs because guppies from low-predation regimes express a low-predation phenotype in the common garden environment that mimics the derived

Table 1. Statistical results of MANCOVA model testing treatment effects of head shape in male Trinidadian guppies. Drainage tested for fixed differences among the Yarra and Guanapo drainages and their respective rearing design; Predation tested for differences between fish from high- and low-predation regime origin within drainages; and Treatment tested for the plastic response to being reared in the presence or absence of predator chemical cues. Centroid size was used as a covariate. The percentage of the partial variance (η^2) explained by each factor or interaction is in the last column.

	df	df _{den}	Wilk's test	P	η^2
Drainage	6	110	82.795	<0.0001	0.802
Predation	6	110	4.225	0.004	0.153
Treatment	6	110	12.957	<0.001	0.215
D×P	6	110	2.651	0.021	0.125
D×T	6	110	1.094	NS	0.061
P×T	6	110	1.052	NS	0.055
D×P×T	6	110	1.023	NS	0.053
Centroid size	6	110	6.136	0.002	0.168

native environment (predator cues absent) and a high-predation phenotype in the environment that mimics their high-predation ancestral environment (predator cues present). These results are consistent with models that view the evolution of reaction norms as a by-product or indirect consequence of selection acting on mean trait values in different environments (e.g., Via and Lande 1985; Gotthard and Nylin 1995; Via et al. 1995; Czesak et al. 2006). Such models predict that when the cross-environment genetic correlation for a trait is low, selection in one environment can change the trait value independently of changes in the mean value of the same trait in a different environment. Thus, any changes in the slope of the reaction norm occur as a by-product or indirect effect of the response to selection in the respective environments (Falconer 1990; Czesak et al. 2006). Here, our results suggest that adaptation to the low-predation environment results in divergence from the ancestral high-predation populations and a change in the slope of the reaction norm for size at maturity and head morphology (Figs. 2, 3). These results qualitatively differ from other studies of plasticity in guppies, where adaptive divergence between high- and low-predation populations has not resulted in changes to the slope of the reaction norm (e.g., Huizinga et al. 2009). However, the dichotomous predation environments examined in this study preclude the ability to evaluate other models arguing that selection acts directly on plasticity through changes in the coefficients that describe the shape of the reaction norm (e.g., Gavrillets and Scheiner 1993a,b). Nevertheless, our results are similar to those found by Hairston and De Meester (2008), where the evolution of predator-induced plasticity in *Daphnia* populations was driven by selection in one environment, rather than changes in slope across all environments. Similarly, selection

experiments in the seed beetle *Stator limbatus* found that the evolution of host plant induced plasticity in egg size was associated with the selection on trait values, but also depended on the environment where selection occurred (Czesak et al. 2006). Below, we first discuss why the phenotypic differences between guppies from the different predation regimes are likely to be adaptive in support of the argument that plasticity evolved as a by-product of adaptation to the low-predation environment. We then discuss these results in the context of the role plasticity is likely to play in the process of colonization and adaptation to novel environments.

PLASTICITY AND DIVERGENCE IN BEHAVIOR, MORPHOLOGY, AND SIZE AT MATURITY

We found that the magnitude of plasticity and divergence between the ancestral, high- and derived, low-predation populations varied depending on the trait being examined. In all populations, individuals exhibited a significant behavioral shift toward the surface of the water column when reared in the presence of the predator cue, whereas when reared in the absence of the cue, few, if any, individuals occupied the surface of the water column (Fig. 1). The similarity of these behavioral responses across populations is notable for several reasons. First, the populations from the two drainages were evaluated in different rearing tanks (nonrecirculating 10-L tanks vs. 1.5-L tanks in a recirculating system), but still exhibited indistinguishable responses. Second, the degree of plasticity (i.e., the slope of the reaction norm) has been conserved across drainages and populations. Finally, the lack of differentiation between populations within drainages suggests no evolutionary divergence has occurred in response to different predation regimes. Such predator-induced behavioral changes are presumably adaptive (Seghers 1973; Reznick et al. 2001; Botham et al. 2006), because a shift in habitat use toward the surface reduces susceptibility to a predator strike by reducing the dimensionality of the environment, which in turn may allow for earlier detection of predators and longer strike distances (Walker et al. 2005). However, it should be noted that no study to date has tested whether prey species at the water surface are more likely to survive a predator encounter compared to those lower in the water column.

In contrast to the behavioral response, when comparing populations within drainages, we found evidence for evolutionary divergence between predation regimes for size at maturity; males from the low-predation populations were significantly larger at maturity than males from the high-predation populations (Fig. 2). However, this result was largely driven by a significantly greater increase in the size of low-predation males when reared in the absence of the predator cue (Fig. 2). Past studies have emphasized the important fitness consequences of maturing at a smaller size under high-predation conditions, and at a large size under low-predation conditions (e.g., Reznick 1982; Magurran 2005).

In low-predation localities, sexual selection via female mate preferences favors a larger male body size (Reynolds and Gross 1992; Magellan et al. 2005). In contrast, males in high-predation localities are under selection to mature at a smaller age and size because they experience high mortality rates (Reznick 1982) and coexist with a suite of larger predators that selectively prey on larger guppies (Liley and Seghers 1975). Indeed, similar to our results, past common-garden studies carried out in the absence of any predator cues have also demonstrated a genetic basis to the smaller size at which high-predation guppies mature (Reznick 1982; Reznick et al. 1990; Reznick and Bryga 1996; Magurran 2005). Thus, the ability to mature at a larger size would be predicted to be both an adaptive plastic response in the absence of predators, and an evolutionary response driven by sexual selection and relaxed selection from predation. Yet, the ability to exhibit plasticity in size at maturity when reared in the absence of the cue appears to be a derived response, as both high-predation populations examined in this study had shallower reaction norms compared to their low-predation counterparts (Fig. 2). There were also differences between drainages, as both high- and low-predation regime guppies from the Guanapo drainage matured at a significantly smaller size than the Yarra populations (Fig. 2). A plausible explanation for this pattern is that pike cichlids, which were used to simulate predation risk in this study, are common guppy predators in the Guanapo drainage and throughout the southern slope of the Northern Range Mountains, whereas they are absent from most of the northern slope rivers. Thus, Guanapo populations may be more sensitive to the chemical cues of the pike cichlid owing to their longer history of coexistence, although evidence for this greater sensitivity was not found in the behavioral or morphological traits we measured (Figs. 1, 3B). Alternatively, the concentration of the predator cues may have been higher in the recirculating systems the Guanapo fish were reared in, compared to the noncirculating tanks of the Yarra fish. This could explain the observed variation between drainages as there is evidence that prey responses can be sensitive to the abundance of predator kairomones (Van Buskirk et al. 2011).

Lastly, we found that head morphology differed between drainages, predation regimes, and the rearing environment (Table 1). Similar to our findings for size at maturity, our post-hoc canonical variate analyses suggest that within drainages, high- and low-predation populations only diverged when fish were reared in the absence of predator cues (Fig. 3B). In the absence of the pike cichlid cue, the low-predation populations from both drainages developed a greater angle from the tip of the rostrum to the ventral and dorsal posterior terminus of the head, yielding a more stout head shape with the mouth in a more anterior position and aligned with the eye (Fig. 3B). However, in the presence of the pike cichlid cue, fish from all populations developed a more fusiform head with a dorsal shift in the position of the

mouth, and were indistinguishable from each other (Fig. 3B). These results suggest a trend for an evolutionary transition from a less plastic, ancestral morphology in the high-predation environment to a more plastic morphology with respect to the shape of the head and associated trophic structures (Fig. 3B). Past work has shown that the position of food resources in the water column can induce plastic changes in guppy trophic morphology (Robinson and Wilson 1995), suggesting that a behavioral shift away from the surface of the water column could play a role in developing a stout head with a more terminal mouth position. Moreover, recent work on the trophic ecology of guppies found low-predation populations to have a more generalized diet compared to high-predation populations (Bassar et al. 2010; Zandonà et al. 2011). A highly specialized diet could favor a more canalized head morphology as observed in the high-predation Yarra population, but additional work is needed to relate foraging behavior and diet to trophic morphology. An alternative explanation is that a fusiform head shape may influence escape performance, as a more fusiform head shape correlates with the presence of predators across several fish taxa (Langerhans 2010). A more streamlined head could increase escape performance from predators if it reduces drag (Langerhans 2010). Collectively, as with size at maturity, the difference between high- and low-predation populations in the absence of the predator cue suggests evolutionary divergence following colonization and adaptation to the low-predation environment.

THE ROLE OF PLASTICITY IN NEW ENVIRONMENTS

There has been renewed interest in understanding the possible role phenotypic plasticity plays in the process of adaptation to new environments (e.g., Price et al. 2003; West-Eberhard 2003; Crispo 2007; Ghilambor et al. 2007; Lande 2009), and experimental studies that compare reaction norms between ancestral and derived populations and environments can empirically test whether evolutionary divergence follows the direction of plasticity. Theory predicts that when populations experience strong directional selection, due either to changes in habitat conditions or colonization of new habitats, adaptive plasticity can increase the probability of population persistence, and in turn favor the evolution of increased plasticity and peak shifts toward the new local optimum (e.g., Baldwin 1896; Price et al. 2003; Lande 2009; Chevin and Lande 2010). However, empirical studies of natural populations supporting such theory are generally absent because of the challenges of capturing the role of plasticity during the initial stages of colonization. Instead, an increasing number of studies have compared reaction norms across environments in ancestral and derived populations (e.g., Yeh and Price 2004; Wund et al. 2008) or the plastic versus evolved responses to native and novel environments (e.g., Chapman et al. 2000; Losos et al. 2000), to infer the role plasticity might play when individuals

are exposed to new environments and subsequently experience evolutionary divergence. For example, the African cichlid, *Pseudocrenilabrus multicolor*, shows adaptive plasticity by modifying its gill size in response to hypoxic conditions, likely facilitating initial colonization and subsequent local adaptation to hypoxic swamps (Chapman et al. 2000). Similarly, the marine ecotype of the threespine stickleback, *Gasterosteus aculeatus*, exhibits patterns of phenotypic plasticity in trophic morphology consistent with derived ecotypes that successfully colonized and adapted to freshwater benthic and limnetic (Day et al. 1994; Wund et al. 2008). Below, we discuss how the patterns of plasticity observed in the current study contribute to our understanding of how plasticity and selection jointly facilitate local adaptation.

We observed patterns of adaptive plasticity in behavior, morphology, and life-history traits that likely contribute to population persistence among high- and low-predation habitats in nature. Additionally, we found evidence for evolutionary divergence between high- and low-predation populations consistent with directional selection and local adaptation occurring in the low-predation environment following colonization. Specifically, it appears that an initial behavioral response of high-predation guppies invading a low-predation environment would be to shift habitat use away from the water column surface in effort to better exploit a limited forage base. Because this behavioral response is “complete” or identical to the response of the locally adapted, low-predation populations (Fig. 1), it suggests the plastic response shields the population from directional selection (e.g., Price et al. 2003; Ghalambor et al. 2007). Such adaptive plasticity may explain why we found no evidence of divergence between populations. Alternatively, there might not be sufficient genetic variation in this trait for selection to act upon, which is suggested by the nonsignificant family effect in our model. In contrast, when reared in the absence of the predator cues, the high-predation populations exhibit a plastic response in head shape and size at maturity that is “incomplete” compared to the low-predation populations (Figs. 2, 3). By incomplete, we mean the plastic responses are in the direction favored by selection, but do not result in trait values produced by the locally adapted low-predation populations. Thus, although these plastic responses may be adaptive relative to a nonplastic genotype, there is still an opportunity for directional selection to act (e.g., Price et al. 2003). In addition, there appears to be sufficient standing genetic variation in the size at which individuals mature, as almost 25% of the observed variation was due to variance among families. Indeed, the evolutionary divergence from the ancestral, high- to derived, low-predation populations likely reflects the role of selection and local adaptation following colonization of the low-predation environment (Via and Lande 1985; Lande 2009). As a consequence, the derived low-predation populations exhibit steeper slopes in their reaction norms (Figs. 2, 3). The evolution of increased plasticity following

colonization of a new environment has been interpreted as evidence for the “Baldwin Effect” (compare Figs. 2, 3 with Fig. 1B in Crispo 2007).

Our results are also in contrast with other theoretical models that predict that plasticity should be lost following adaptation to a new environment through genetic assimilation (e.g., Lande 2009), because it is costly to maintain (e.g., van Tienbergen 1991; Moran 1992; DeWitt et al. 1998; but also see Van Buskirk and Steiner 2009), or due to random processes in the absence of selection to retain it (e.g., Masel et al. 2007). Instead, we found that the low-predation genotypes were still able to express a high-predation phenotype when reared in the presence of a predator cue (Figs. 1, 2, 3). One hypothesis for the observed retention of predator-induced plasticity is that it is maintained by selection because juvenile and smaller adult guppies do suffer some predation from *Rivulus hartii*. However, if this were a size-specific response, we would have expected predator-induced changes in behavior, and perhaps other traits, to be reduced once guppies reached a size class sufficient to preclude predation from *R. hartii*. We found no age effect on the proportion of fish at the surface in fish from the Yarra drainage (see Material and Methods for details), suggesting that the observed behavior was not size- or age specific. Thus, if low-predation genotypes invade high-predation environments, we would predict that the traits examined herein would not evolve because low-predation fish show a “complete” adaptive response to predator cues so there would be insufficient phenotypic variation for selection to act on. This asymmetry in plasticity between high- and low-predation ecotypes could have important implications for the interactions between gene flow and selection among localities (Crispo 2008). For instance, introgression via migrants from upstream, low-predation populations into downstream, high-predation populations should not have negative fitness implications based on the three traits examined here. However, immigrants from high-predation locales moving into low-predation populations should have lower relative fitness and be selected against because of the incomplete plastic responses in head shape and size at maturity.

In conclusion, this study provides empirical evidence for the evolution of adaptive plasticity between ancestral and derived populations of guppies as a by-product of local adaptation to environments with reduced mortality selection via predation. The colonization and establishment of populations in the derived environment could have been facilitated by plasticity in habitat use, size at maturity, and head shape that existed in the ancestral high-predation population. Heritable phenotypic divergence between high- and low-predation population in size at maturity, and, to a lesser extent head shape, could be attributed to the subsequent effects of directional selection in the new environment. Thus, these results support the view that adaptive evolution can proceed by increasing adaptive plasticity (e.g., Crispo 2007; Lande 2009).

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