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Phenotypic Plasticity Changes Correlations of Traits Following Experimental Introductions of Trinidadian Guppies (*Poecilia reticulata*)

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Synopsis Colonization of novel environments can alter selective pressures and act as a catalyst for rapid evolution in nature. Theory and empirical studies suggest that the ability of a population to exhibit an adaptive evolutionary response to novel selection pressures should reflect the presence of sufficient additive genetic variance and covariance for individual and correlated traits. As correlated traits should not respond to selection independently, the structure of correlations of traits can bias or constrain adaptive evolution. Models of how multiple correlated traits respond to selection often assume spatial and temporal stability of trait-correlations within populations. Yet, trait-correlations can also be plastic in response to environmental variation. Phenotypic plasticity, the ability of a single genotype to produce different phenotypes across environments, is of particular interest because it can induce population-wide changes in the combination of traits exposed to selection and change the trajectory of evolutionary divergence. We tested the ability of phenotypic plasticity to modify trait-correlations by comparing phenotypic variance and covariance in the body-shapes of four experimental populations of Trinidadian guppies (*Poecilia reticulata*) to their ancestral population. We found that phenotypic plasticity produced both adaptive and novel aspects of body-shape, which was repeated in all four experimental populations. Further, phenotypic plasticity changed patterns of covariance among morphological characters. These findings suggest our ability to make inferences about patterns of divergence based on correlations of traits in extant populations may be limited if novel environments not only induce plasticity in multiple traits, but also change the correlations among the traits.

Introduction

Colonization of novel environments can alter selective pressures and act as a catalyst for rapid evolution in nature (Thompson 1998; Hendry and Kinnison 1999; Reznick and Ghalambor 2001; Hairston et al. 2005; Carroll et al. 2007). Indeed, most empirical examples of rapid adaptive evolution follow colonization events and are associated with shifts in the selective landscape (Reznick and Ghalambor 2001). Theory and empirical studies suggest that the ability

of a population to exhibit an adaptive evolutionary response to these novel selection pressures should reflect the presence of sufficient additive genetic variance and covariance for individual and correlated traits (Lande 1979; Lande and Arnold 1983; Roff 1997).

At the genetic level, genetic covariance among correlated traits arises from the pleiotropic effects of genes and linkage, and is characterized as the genetic variance–covariance matrix (**G**) in multivariate

selection models (Lande 1979). At the phenotypic level, **G** is manifested as the degree to which phenotypic traits are correlated with each other and can be similarly described by the phenotypic variance-covariance matrix (**P**) (Olson and Miller 1958; Cheverud 1982, 1988; Revell et al. 2007, 2010). The structure of **G** and **P** jointly determine how a set of correlated traits should respond to selection (Lande 1979; Lande and Arnold 1983; Roff 1997). When there is a high degree of genetic and phenotypic integration, traits are not free to evolve independently, thus biasing the path of evolutionary divergence in multiple traits away from the directional selection gradient (Lande 1979; Lande and Arnold 1983; Björklund 1996; Schluter 1996; Walker 2007; Kirkpatrick 2009; Chenoweth et al. 2010; Revell et al. 2010). Conversely, when directional selection gradients parallel the major axis of genetic variance, evolution should be rapid and the path of phenotypic divergence should be toward the new fitness-optimum (Schluter 1996; Merilä and Björklund 2004). Yet, the ability of **G** and **P** to provide predictive insight into patterns of evolutionary divergence and constraint depends on the stability of genetic correlations across space and time.

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

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

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

Phenotypic plasticity and evolution in Trinidadian guppies

Natural populations of Trinidadian guppies are found throughout the tropical streams of Trinidad's Northern Range Mountains and provide a model system for studying rapid evolutionary change in nature (Reznick et al. 1997). Guppies that occupy larger rivers and streams experience intense predation from a suite of piscivorous fishes (Reznick 1982; Reznick and Endler 1982; Reznick et al. 1996). In contrast, guppies in smaller headwater and tributary streams experience lower extrinsic mortality due to predation (Reznick 1982; Reznick and Endler 1982; Reznick et al. 1996). Differences in predation and other environmental covariates, such as food-resources or stream-velocity, are correlated with rapid adaptive divergence in life histories (Reznick and Endler 1982), behavior (Seghers 1974; Endler 1995; Godin and Briggs 1996; Templeton and Shriner 2004), and body-morphology (Layman et al. 2003; Langerhans and Dewitt 2004; Alexander et al. 2006; Hendry et al. 2006; Burns et al. 2009). Previous studies have experimentally translocated guppies from streams where they experience high predation into streams with low predation and found rapid evolution in the aforementioned traits (Reznick and Bryga 1987, 1996; Reznick et al. 1990, 1997). However, these studies have not captured the initial phenotypic changes that arise immediately following colonization.

Body-shape in fishes shows consistent patterns of integration and divergence due to the functional constraints imposed by aquatic environments (Langerhans and Dewitt 2004; Walker 2010). Moreover, guppies show parallel patterns of divergence in body-shape that correspond to predation regimes in the wild (Alexander et al. 2006; Hendry et al. 2006), making morphology of the body a candidate phenotype to investigate the stability of trait-correlations during divergence. Specifically, guppies from high-predation locales have more fusiform bodies and a dorsal orientation of the mouth (Alexander et al. 2006) that show genetic and plastic responses

to stream-velocity, predation-risk, and foraging behavior (Robinson and Wilson 1995; Ghalambor et al. submitted for publication). In contrast, patterns of water-flow and the acquisition of resources in low-predation habitats leads to a phenotype characterized by deeper bodies with a more terminal and anterior orientation of the mouth (Alexander et al. 2006).

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

Methods

Sampling of guppy populations

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

Paired introductions were conducted across two consecutive years (Handelsman et al. 2013; Arendt et al. 2014). In March 2008, descendants from the ancestral population were introduced into the Lower Lalaja and Upper Lalaja tributaries of the Guanapo River (hereafter Intro-1 and Intro-2, respectively). Each stream was stocked with 38 gravid females and 38 mature males. To minimize the potential for founder effects and standardize genetic diversity

in each stream, males and females were randomly crossed and introduced into alternate streams with the consequence that the introduced females carried sperm stores from the 38 males that they were mated with. Then, the females were paired in the introduction site with 38 new males. Paired random crosses were employed to prevent biased mating that may arise from females' mate-choice. Additionally, previous laboratory experiments (Reznick 1982; Reznick and Bryga 1987; Torres-Dowdall et al. 2012; Handelsman et al. 2013) regularly produce viable progeny from paired crosses and have observed low failure rates in such crosses. In March 2009, this protocol was replicated in the upper reaches of the Caigual and Taylor tributaries of the Guanapo River (hereafter Intro-3 and Intro-4, respectively), but 45 males and females were introduced into each site.

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

Mature males ($n=67$) from the ancestral population were captured, anesthetized in MS-222 (0.85 mg ml^{-1} ; ethyl 3-aminobenzoate methane sulfonic acid salt) (Sigma-Aldrich, St Louis, MO) buffered with sodium bicarbonate, and photographed (see below) in January of 2008 prior to the introductions. The pairs of experimental populations were sampled 3 months after they were established (May of 2008 for Intro-1 and Intro-2 and May of 2009 for Intro-3 and Intro-4) to assess first-generation recruits. Under laboratory conditions, the ancestral population had an inter-brood interval of 25 days and males matured within 54 days (Handelsman, unpublished data). Therefore, first-generation

recruits were expected to be mature, but there had not been sufficient time yet for a second brood to mature. Thus, our sampling design is intended to capture the initial plastic changes associated with developing in a low-predation stream. We collected and photographed all mature males (i.e., first generation recruits) from each population (Intro-1: $n=208$, Intro-2: $n=302$, Intro-3: $n=194$, Intro-4: $n=286$). Females were excluded because, as live-bearers, their body-shape changes throughout gestation and can complicate interpretations of shape.

Analysis of body-shape

We analyzed variation in lateral body-shape with geometric morphometrics (Rohlf and Marcus 1993; Zelditch et al. 2004). We used eight homologous landmarks and six semi-landmarks (Bookstein 1997) acquired from digital images to characterize the lateral body-shape of adult male guppies. Lateral photographs of the left side of each fish were taken with Nikon D60 digital SLR cameras equipped with Nikkor 50-mm macro lenses (Nikon Inc., Melville, NY) mounted on tripods. The height of the tripod was adjusted to yield an 8-cm field of view that was determined sufficient to eliminate any parallax within the lens area occupied by a guppy. To standardize the position of fish and to expose homologous landmarks, a fine-tipped artist's paintbrush was wetted and used to straighten the specimen and spread the median fins. A ruler was placed in each picture to show scale. Landmarks were digitized with TPSDig2 (Rohlf 2013). We isolated geometric shape by removing variation due to size, position, and orientation, with a Generalized Procrustes Superimposition (Rohlf and Slice 1990; Goodall 1991; Dryden and Mardia 1998) using the geomorph package in R (Adams and Otárola-Castillo 2013). Procrustes distance was used to optimize the position of semi-landmarks with the geomorph package in R (Adams and Otárola-Castillo 2013). Specifically, semi-landmarks were slid along tangent lines and optimized by minimizing the Procrustes distance between adjacent landmarks (Bookstein 1997; Rohlf 2010). The superimposed coordinates (Procrustes coordinates) were used in all further analysis of shape.

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

The **P**-matrix was calculated from all 24 principal components.

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

Phenotypic plasticity

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

P-matrix comparisons

Following Roff et al. (2012), we employed several complementary statistical methods to compare **P** matrices among the ancestral population and the experimental populations. Specifically, we used the jump-up approach to the Flury method (Phillips and Arnold 1999; Roff and Mousseau 2005), modified Mantel test (Goodnight and Schwartz 1997), Bartlett's test (Goodnight and Schwartz 1997), Jackknife-MANOVA test (Roff 2002), and the jackknife-eigenvalue test (Kirkpatrick 2009; Roff et al. 2012). The principal components generated from rotating the Procrustes coordinates in MorphoJ (see above) (Klingenberg 2011) were used as traits to produce and compare **P** matrices. However, the Jackknife-MANOVA requires a full-rank model.

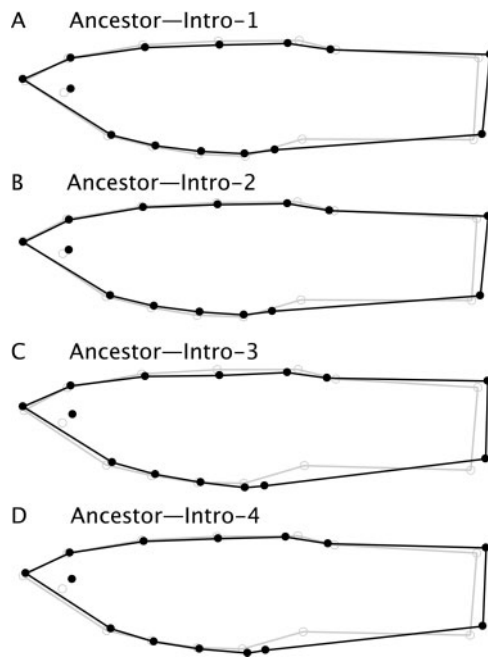


Fig. 1 Deformation grids depicting the discriminant function that describes changes in shape between the ancestral population from a high-predation stream (gray outline) and A) Intro-1, B) Intro-2, C) Intro-3, and D) Intro-4 (black outlines). Separate discriminant-function analyses were run for each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

Given our sample sizes, the models became rank deficient if more than 22 principal components were included. Thus, we ran all models with the first 22 principal components. These 22 Principal components captured 99.97% of the sample variance.

Results

Phenotypic plasticity

The first-generation recruits in all four experimental populations showed divergence in body-shape in response to being moved from high-predation to low-predation streams (Fig. 1). Phenotypic divergence in the introduced populations is likely due to phenotypic plasticity, given that the mean values of traits changed while the phenotypic variance increased (Fig. 2; Tables 1 and 2), and because sampling bias, founder effects, or selection favoring certain phenotypes should reduce phenotypic variance in conjunction with shifting the mean values. Moreover, the plastic response produced parallel changes in all four populations (Figs. 1 and 2). Specifically, the eye underwent a dorsal and posterior shift, the caudal peduncle was elongated, and the

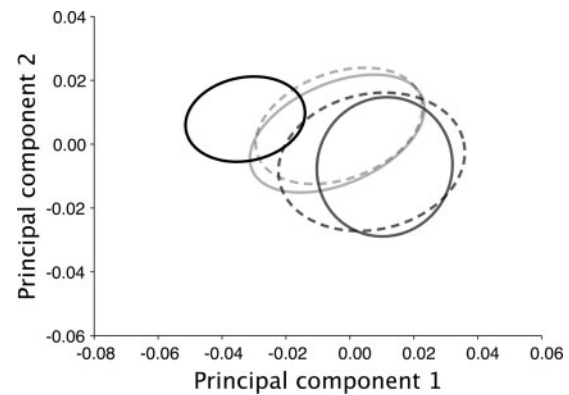


Fig. 2 Bivariate plot of correlations between principal component 1 (40.8% of the sample variance) and principal component 2 (21.0% of the sample variance). Moving descendants of the ancestral population (black ellipse) into four low-predation streams (Intro-1, light gray ellipse; Intro-2, light gray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

insertion of the anal fin underwent an anterior and ventral shift that resulted in a deeper body.

Comparisons of P-matrices

We used five methods to contrast the structure of the **P** matrices between the ancestral populations and the four experimental populations. All methods produced congruent results and suggest that the **P** matrix in the experimental populations diverged from the ancestral population (Table 3). Specifically, these comparisons tested the null hypotheses that the **P** matrices are proportional (Table 3; Flury method and modified Mantel test), contain equal elements (Table 3; Flury method and Jackknife-MANOVA), share common principal components (Table 3; Flury method), are of equal size (Table 3; Bartlett's test), and have equal eigenvalues (e.g., total variance (Table 3; Jackknife-eigenvalue). In all tests, we rejected the null hypotheses of matrix equality and found support for repeated divergence between the four experimental populations and their source population (Table 3).

Discussion

Adaptive evolution is a function of the strength of natural selection and the genetic architecture of the underlying traits targeted by selection (Lande 1979; Lande and Arnold 1983; Roff 1997). As genetic effects (e.g., pleiotropy and linkage) can place constraints on whether phenotypic divergence parallels multivariate selection gradients, the structure of **G**

Table 1 Population means and standard deviations for each principal component

	N	PC1	SD	PC2	SD	PC3	SD	PC4	SD
Ancestor	67	−0.0327	0.0123	0.0078	0.0088	−0.0087	0.0070	−0.0137	0.0077
Intro-1	208	−0.0040	0.0180	0.0033	0.0122	0.0031	0.0091	−0.0023	0.0072
Intro-2	302	−0.0034	0.0172	0.0057	0.0120	0.0013	0.0089	−0.0009	0.0065
Intro-3	194	0.0109	0.0140	−0.0071	0.0144	0.0013	0.0094	0.0012	0.0079
Intro-4	286	0.0068	0.0192	−0.0055	0.0143	−0.0025	0.0098	0.0050	0.0073
	N	PC5	SD	PC6	SD	PC7	SD	PC8	SD
Ancestor	67	−0.0043	0.0069	−0.0059	0.0082	0.0023	0.0053	0.0005	0.0059
Intro-1	208	−0.0027	0.0064	0.0011	0.0053	−0.0002	0.0050	−0.0016	0.0044
Intro-2	302	−0.0007	0.0066	0.0007	0.0059	−0.0002	0.0054	0.0006	0.0045
Intro-3	194	0.0022	0.0063	0.0016	0.0055	−0.0016	0.0065	−0.0008	0.0046
Intro-4	286	0.0023	0.0066	−0.0013	0.0055	0.0009	0.0056	0.0009	0.0045
	N	PC9	SD	PC10	SD	PC11	SD	PC12	SD
Ancestor	67	−0.0013	0.0044	0.0013	0.0057	0.0023	0.0042	−0.0010	0.0033
Intro-1	208	0.0007	0.0034	−0.0014	0.0039	−0.0008	0.0034	0.0004	0.0031
Intro-2	302	0.0002	0.0034	0.0006	0.0036	−0.0004	0.0031	−0.0002	0.0030
Intro-3	194	−0.0022	0.0041	−0.0011	0.0035	0.0013	0.0031	0.0001	0.0034
Intro-4	286	0.0010	0.0040	0.0008	0.0035	−0.0004	0.0032	0.0002	0.0030
	N	PC13	SD	PC14	SD	PC15	SD	PC16	SD
Ancestor	67	0.0014	0.0031	0.0012	0.0028	−0.0001	0.0024	0.0000	0.0021
Intro-1	208	−0.0006	0.0025	−0.0007	0.0024	0.0000	0.0020	0.0001	0.0018
Intro-2	302	−0.0008	0.0024	0.0002	0.0022	0.0001	0.0022	0.0002	0.0018
Intro-3	194	0.0008	0.0024	−0.0002	0.0024	0.0005	0.0023	−0.0002	0.0020
Intro-4	286	0.0004	0.0025	0.0002	0.0024	−0.0004	0.0021	−0.0002	0.0019
	N	PC17	SD	PC18	SD	PC19	SD	PC20	SD
Ancestor	67	0.0001	0.0016	−0.0008	0.0020	0.0000	0.0013	0.0003	0.0013
Intro-1	208	0.0000	0.0014	0.0003	0.0012	−0.0002	0.0010	0.0000	0.0007
Intro-2	302	−0.0001	0.0014	0.0001	0.0011	0.0001	0.0011	−0.0002	0.0008
Intro-3	194	0.0000	0.0015	−0.0001	0.0015	0.0000	0.0011	0.0001	0.0010
Intro-4	286	0.0001	0.0014	0.0000	0.0014	0.0000	0.0012	0.0001	0.0008
	N	PC21	SD	PC22	SD	PC23	SD	PC24	SD
Ancestor	67	0.0001	0.0002	−0.0001	0.0003	0.0000	0.0001	0.0000	0.0001
Intro-1	208	0.0000	0.0002	0.0000	0.0002	0.0000	0.0001	0.0000	0.0001
Intro-2	302	0.0000	0.0002	0.0000	0.0002	0.0000	0.0001	0.0000	0.0001
Intro-3	194	0.0000	0.0002	0.0000	0.0002	0.0000	0.0001	0.0000	0.0001
Intro-4	286	0.0000	0.0004	0.0000	0.0002	0.0000	0.0002	0.0000	0.0001

and **P** are important determinants of a population's response to selection. Indeed, the importance of genetic correlations is perhaps exemplified in agricultural breeding programs aiming to maximize yield when the environment is constant, and selection is strong (Moose et al. 2004; Powell and Norman 2006). In natural populations, however, changes in the environment act both as a source of selection and as a trigger for developmental plasticity that can alter genetic and phenotypic correlations. We found that

the translocation of guppies that had evolved under conditions of high predation to four replicate low-predation habitats resulted in parallel plastic changes in body-shape and in the underlying pattern of trait-correlations (Fig. 1; Tables 2, 3). Thus, the same genetic background develops a predictable change in body-shape (Fig. 1), and pattern of trait-correlations (Table 3) simply by developing in a new environment. Such results challenge the frequent assumption of stability of the structure of phenotypic covariance

and have important implications for the ability of **G** and **P** to forecast patterns of phenotypic divergence. We discuss these implications in more detail below.

Correlations among quantitative traits and the traits themselves can be sensitive to environmental variation during ontogeny (Sgrò and Hoffmann 2004). The observation that patterns of genetic correlation are dependent on the environmental context in which they are measured suggests that the ability to infer genetic constraints on evolutionary responses may be difficult to generalize when only taking measurements in a single environment (Sgrò and Hoffmann 2004). For example, comparisons of the same genetic background in different environments reveal that the direction of genetic correlations can change in response to temporal stability of the habitat (Newman 1988a, 1988b, 1989) and of the abundance of resources (Service and Rose 1985; Gebhardt and Stearns 1988). Complex phenotypes also are plastic in response to changing environmental conditions, and the body-morphology of fishes can be particularly sensitive to environmental conditions. For example, Parsons and Robinson (2006) compared body-shape of ancestral and derived ecomorphs of the pumpkinseed sunfish (*Lepomis gibbosus*) and found that correlated patterns of phenotypic plasticity had evolved in the novel environment. Similarly, in common garden experiments, Ghalambor et al. (submitted for publication) found

parallel patterns of plasticity in body-shape of Trinidadian guppies from high-predation and low-predation locales that were contingent upon combinations of water velocity and perceived risk of predation that mimicked natural habitats of guppies. Although these examples of correlated plastic responses provide compelling evidence that phenotypic plasticity can produce parallel shifts in multiple aspects of the phenotype, it remains unclear whether plasticity played a role in altering the trajectory of phenotypic divergence that would have been predicted given the structure of trait-correlations in the ancestral population. Thus, more monitoring of populations that have recently colonized new environments are needed, if we are to evaluate the role of plasticity during divergence of correlated traits.

Trinidadian guppy populations have repeatedly diverged in a suite of life-history, behavioral, and morphological characters in what is regarded as a classic example of rapid adaptive evolution (Reznick and Bryga 1987; Endler 1995; Magurran 2005), but the role of plasticity for single or multiple traits in the evolutionary process remains unclear. In previous translocation experiments, experimental populations of guppies have been shown to exhibit rapid patterns of parallel phenotypic divergence (Reznick et al. 1990, 1997), and while phenotypic plasticity may play a role in rapid evolution (Torres-Dowdall et al. 2012; Handelsman et al. 2013), no studies to date have quantified plastic changes in natural populations. Here, we show that body-shape of field-collected individuals that founded the populations and their first-generation recruits exhibited a deepening of the body and a dorsal shift in position of the eye relative to the mouth (Fig. 1; Table 2) that is consistent with patterns of divergence between native high-predation and low-predation populations of guppies (Alexander et al. 2006). These plastic responses are assumed to be adaptive given that they are in the same direction as those observed in native populations subject to low predation (Ghalambor et al. submitted for publication). However, we also

Table 2 Discriminant function analysis testing for plastic changes in body shape between the ancestral population and each experimental population

	Procrustes distance	Cross-validated classification (%)	P-values ^a
Ancestor—Intro-1	0.035	99.7	<0.001*
Ancestor—Intro-2	0.035	99.5	<0.001*
Ancestor—Intro-3	0.051	100	<0.001*
Ancestor—Intro-4	0.047	99.7	<0.001*

^aP-values for permutation tests with 10,000 permutation runs.
*Significant after Holm's sequential Bonferroni correction.

Table 3 Summary of pairwise comparisons of **P** matrices

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

^aCommon principal component.

found an elongation of the caudal peduncle that contrasts with the expected direction of divergence (Alexander et al. 2006), suggesting that the initial patterns of plasticity following colonization of a new environment may also include non-adaptive responses (Ghalambor et al. 2007). An initially non-adaptive plastic response to a new environment should impose strong selection on a trait (Ghalambor et al. 2007; Handelsman et al. 2013); thus, subsequent work can test the prediction that the caudal peduncle should evolve more quickly than do other traits.

In addition to overall plastic changes in body-morphology, we found the covariance structure differed between the ancestral genotype and all four experimental populations. Specifically, we found that when compared with the ancestral genotype, elements of **P** in the experimental populations were not equal (Table 3; Flury Hierarchy, Jackknife-MANOVA), had unequal eigenvalues (i.e., total variance) (Table 3; Jackknife-eigenvalue), were unequal in size (Table 3; Bartlett's test), were not proportional (Table 3; Flury Hierarchy, Modified Mantel test), and did not share common principal components (Table 3; Flury Hierarchy). Thus, translocation of the ancestral genotype (high predation) into low-predation streams changes the correlations between traits and may therefore influence the evolutionary trajectory of these populations to the new selection pressures they experience. Had we examined the pattern of correlations among traits in the ancestral population to infer the evolutionary response to selection, we would have drawn different conclusions on how integration biases phenotypic divergence.

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

occurring low- or high-predation populations of guppies. The developmental mechanism responsible for this novel phenotype is not known, but likely involves changes in gene expression in response to the low-predation environment (Gunter et al. 2013). The critical question is whether this new combination of traits is a long-term constraint imposed by plasticity. Monitoring these introduced populations through time will shed light on whether these attributes of shape will become decoupled, as is observed in naturally occurring low-predation populations, or whether they will impose a lasting constraint on the direction of the evolution of body-shape.

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

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