Gene Flow Constrains and Facilitates Genetically Based Divergence in Quantitative Traits

Author(s): Sarah W. Fitzpatrick, Corey A. Handelsman, Julian Torres-Dowdall, Emily W. Ruell, E. Dale Broder, John A. Kronenberger, David N. Reznick, Cameron K. Ghalambor, Lisa M. Angeloni, and W. Chris Funk

Source: Copeia, 105(3):462-474.

Published By: The American Society of Ichthyologists and Herpetologists

https://doi.org/10.1643/CI-16-559

URL: http://www.bioone.org/doi/full/10.1643/CI-16-559

Copeia 105, No. 3, 2017, 462–474

Gene Flow Constrains and Facilitates Genetically Based Divergence in Quantitative Traits

Sarah W. Fitzpatrick[1,2,3], Corey A. Handelsman[4], Julian Torres-Dowdall[5], Emily W. Ruell[4], E. Dale Broder[6], John A. Kronenberger[4,7], David N. Reznick[8], Cameron K. Ghalambor[4,7], Lisa M. Angeloni[4,7], and W. Chris Funk[4,7]

Theory predicts that gene flow will decrease phenotypic differences among populations. Correlational studies have in some cases documented constraining effects of gene flow on phenotypic divergence and/or have also provided evidence for local differentiation despite high gene flow. However, correlative studies are unable to evaluate how gene flow affects genetically based phenotypic divergence or the extent to which gene flow constrains adaptive divergence. Translocation experiments using Trinidadian guppies provided an opportunity to test the effects of new gene flow on quantitative traits in native recipient populations. We measured a suite of traits in guppies reared in common garden environments before and multiple generations following gene flow from guppies that originated from a different environment. We interpreted our results in light of *a priori* predictions based on evolutionary theory and extensive background information about guppies and our focal populations. Although we could not include a spatiotemporal control that would allow us to be certain that the observed changes were directly caused by gene flow, we found that post-gene flow populations showed genetically based shifts in most traits. Whether traits shifted in predicted adaptive directions or whether they became more or less similar to the source population depended on the trait and initial conditions of the population. Our study provided a rare opportunity to test how recent gene flow affects genetically based changes in traits with known adaptive significance, and our results attest to the complex interactions between gene flow and selection.

GENETIC exchange among divergent populations (e.g., gene flow) can have wide-ranging effects on adaptive divergence, demography, and species' ranges, but is rarely the primary interest of studies on eco-evolutionary dynamics, which tend to focus on the role of selection (Hendry, 2017; Lowe et al., 2017). However, gene flow can interact with selection and cause eco-evolutionary outcomes that are demonstrably different from 'no gene flow' scenarios (Garant et al., 2007). For example, experimental addition of gene flow has shown changes in abundance (Kronenberger et al., 2016) and invasion success (Wagner et al., 2017) relative to 'no gene flow' controls, and experimental removal of gene flow has shown changes in phenotypic evolution (Riechert, 1993; Nosil, 2009). The challenge is understanding the specific role of gene flow on eco-evolutionary dynamics and phenotypic evolution given complex interactions among gene flow, drift, and selection (Garant et al., 2007; Guillaume and Whitlock, 2007; Hendry, 2017).

Evolutionary theory predicts that gene flow should reduce phenotypic divergence among populations by homogenizing allele frequencies at loci affecting traits (Haldane, 1948; Slatkin, 1978; Garcia-Ramos and Kirkpatrick, 1997). In this way, gene flow is often considered a constraining force that limits local adaptation within a species (Mayr, 1963; Lenormand, 2002). Evidence for this in nature is provided by the commonly documented positive relationship between levels of genetic and phenotypic divergence (King and Lawson, 1995; Calsbeek and Smith, 2003; Hendry and Taylor, 2004; Nosil and Crespi, 2004). In other words, populations that are more genetically isolated are often more phenotypically distinct. Indeed, two experimental reductions of gene flow showed predicted increases in phenotypic differentiation (Riechert, 1993; Nosil, 2009). However, there are also examples of substantial phenotypic divergence among populations that experience high gene flow, suggesting that gene flow does not play a purely constraining role (Saint-Laurent et al., 2003; Hoekstra et al., 2004; Fitzpatrick et al., 2015; Moody et al., 2015). In fact, gene flow can promote adaptive evolution by providing beneficial alleles and increasing additive genetic variation, thereby causing a faster response to selection (Swindell and Bouzat, 2006). In addition, gene flow into small, inbred populations can mask deleterious alleles and reduce the occurrence of detrimental traits (Keller and Waller, 2002).

There are several reasons why it has proven difficult to evaluate how gene flow affects adaptive divergence in natural populations (Garant et al., 2007; Hendry, 2017). First, the correlations between genetic and morphological divergence often used to evaluate the constraining role of gene flow are typically limited to traits measured on wild-caught individuals. Therefore, the genetic basis of these traits is unknown,

---

[1] W.K. Kellogg Biological Station, 3700 E. Gull Lake Drive, Michigan State University, Hickory Corners, Michigan 49083; Email: sfitz@msu.edu. Send reprint requests to this address.
[2] Department of Integrative Biology, Michigan State University, East Lansing, Michigan.
[3] Ecology, Evolutionary Biology, and Behavior Program, Michigan State University, East Lansing, Michigan.
[4] Department of Biology, 1878 Campus Delivery, Colorado State University, Fort Collins, Colorado 80523.
[5] Lehrstuhl für Zoologie und Evolutionsbiologie, Department of Biology, University of Konstanz, Konstanz, Germany.
[6] Interdisciplinary Research Incubator for the Study of (in)Equality, University of Denver, 2199 S. University Blvd., Denver, Colorado 80208.
[7] Graduate Degree Program in Ecology, Colorado State University, Fort Collins, Colorado 80523.
[8] Department of Biology, University of California, Riverside, California 92521.

Submitted: 30 November 2016. Accepted: 18 April 2017. Associate Editor: D. M. Green.
From "Eco-Evolutionary Dynamics in Cold Blood," an ASIH-sponsored symposium at the 2016 Joint Meeting of Ichthyologists and Herpetologists in New Orleans, Louisiana.

DOI: 10.1643/CI-16-559

due to a lack of either extensive pedigrees or common-garden experiments (Merilä and Hendry, 2014). Understanding the genetically based effects of gene flow on traits is important, as phenotypic plasticity can maintain phenotypic divergence between populations even when gene flow imposes a constraint on genetic divergence (Crispo, 2008). Second, determining cause and effect from these patterns is confounded because adaptive divergence may, in turn, cause reduced gene flow (Rasanen and Hendry, 2008). Finally, understanding how traits relate to fitness in a given environment is difficult, but necessary, because drift can cause populations to become phenotypically differentiated by chance (Keller and Taylor, 2008). Populations may become phenotypically divergent in the absence of local adaptation, in which case homogenizing gene flow would not constrain adaptation *per se*, only neutral divergence. Therefore, rigorously testing whether gene flow constrains adaptive divergence requires clear predictions about selection and adaptation in different environments, a manipulation of gene flow that allows pre- versus post-gene flow comparisons, and the ability to assess whether gene flow caused genetically based changes in traits.

Trinidadian guppies (*Poecilia reticulata*) provide a system in which the above criteria could be met. Namely, we could test the effects of experimentally induced gene flow on quantitative traits, measured in a common garden environment, with *a priori* predictions about the adaptive direction in which traits tend to vary with respect to the environment. Trinidadian guppies are a model system for studying rapid adaptation in the wild (Endler, 1980; Reznick et al., 1990, 1997; Magurran, 2005). Divergent selection pressure typically associated with level of predation has resulted in genetically based adaptive differences that have evolved mostly in parallel across independent drainages (Reznick and Bryga, 1996; Reznick et al., 1996). Multiple translocation experiments in which guppies from high predation localities were introduced into low predation environments have provided evidence for rapid, genetically based adaptation to the release of predation (Endler, 1980; Reznick and Bryga, 1987), though not in all cases (Gordon et al., 2009). Extensive gene flow from introduced populations has been documented throughout native populations at far downstream distances (Shaw and Carvalho, 1992; Fitzpatrick et al., 2015). Despite this high gene flow from originally divergent introduced populations, guppy phenotypes from downstream populations were generally well matched to their local predation regime (Fitzpatrick et al., 2015), providing further evidence for at least partly deterministic selection of similar traits in similar environments. Whether this adaptive phenotypic divergence was genetically based or maintained through phenotypic plasticity could not be determined from traits measured in the wild. Guppies within the introduced populations have shown initial plasticity in some traits, followed by genetically based phenotypic evolution (Reznick and Bryga, 1987; Karim et al., 2007; Handelsman et al., 2013, 2014), as predicted during colonization of a new environment (Ghalambor et al., 2007). However, the extent to which gene flow has caused genetically based changes in traits in the native populations that existed downstream from introduction sites was previously unknown.

We conducted a series of common garden assays to test for effects of gene flow on genetically based phenotypic evolution in two native populations that received gene flow from an adaptively divergent source population. We first measured a suite of traits from descendants of wild caught guppies captured in two focal low predation (LP) sites. Next, guppies from a high predation (HP) source site were introduced upstream from the two focal reaches (Fig. 1A) as part of a separate study by D. Reznick and colleagues (as outlined in Travis et al., 2014). We measured the same suite of traits in fish from this source population in the common garden environment. We then replicated the common garden assay using guppies captured at the same low predation focal sites approximately ten guppy generations after gene flow from the upstream introduction experiments. Migration was expected to be unidirectional and downstream from introduction sites into the native populations due to waterfall barriers that limit upstream dispersal. Indeed, subsequent to the introduction experiments, we documented extensive gene flow caused by an influx of migrants originating from the introduction sites (Fig. 1B; Fitzpatrick et al., 2016). Ideally, our design would include a temporal control, namely, a population that did not receive gene flow sampled at the same points to ensure temporal consistency aside from gene flow, but we were unable to include this control in our study. Despite this, we believe that we have a unique ability to assess how specific traits of ecological importance were impacted by a manipulation of gene flow.

We took advantage of *a priori* understanding of Trinidadian guppies, including detailed natural history knowledge of our specific focal guppy populations and their environment, to make predictions about the effects of gene flow. Fish (Gilliam et al., 1993), invertebrate communities (Zandonà et al., 2011), abiotic characteristics and resource levels (Kohler et al., 2012), and phenotypic variation of guppies (Torres Dowdall et al., 2012a; Bassar et al., 2013) have been previously characterized for the drainage in our study, providing an unusually fine-scale understanding of the selective environment. Guppy populations found in upland headwater tributaries consistently show low levels of genetic variation and are subject to strong genetic drift, likely caused by founder effects, as these populations are typically colonized by one or few individuals (Crispo et al., 2005; Barson et al., 2009; Baillie, 2012). Our focal sites represented the highest upstream extent of guppies prior to the introduction experiments and indeed showed extremely low levels of neutral genetic variation before gene flow (Table 1), even compared to other upland populations found throughout Trinidad (Fig. 1C). As expected when populations experience substantial drift, non-parallel phenotypes were previously documented despite the similarity of the environment with respect to predation (Torres Dowdall et al., 2012a; Fitzpatrick et al., 2014). However, selection could also explain phenotypic non-parallelisms if there were non-predator related environmental differences between these two streams. In general, there is an increasing appreciation of the highly context-dependent nature of phenotypic variation across native guppy populations (Gordon et al., 2009; Fitzpatrick et al., 2014; Pérez-Jvostov et al., this volume, 2017).

We developed *a priori* predictions about how traits would respond to gene flow based on evolutionary theory, previous background information about this model system (i.e., predicted adaptive direction of traits in low predation environments), and specific details about our focal populations (i.e., taking into account initial non-parallelism in native low predation populations). Our predictions fall under two primary hypotheses: the *'gene flow constrains divergence'* hypothesis, and the *'divergence in the face of gene flow'* hypothesis (Fig. 2). Under the first hypothesis, as generally

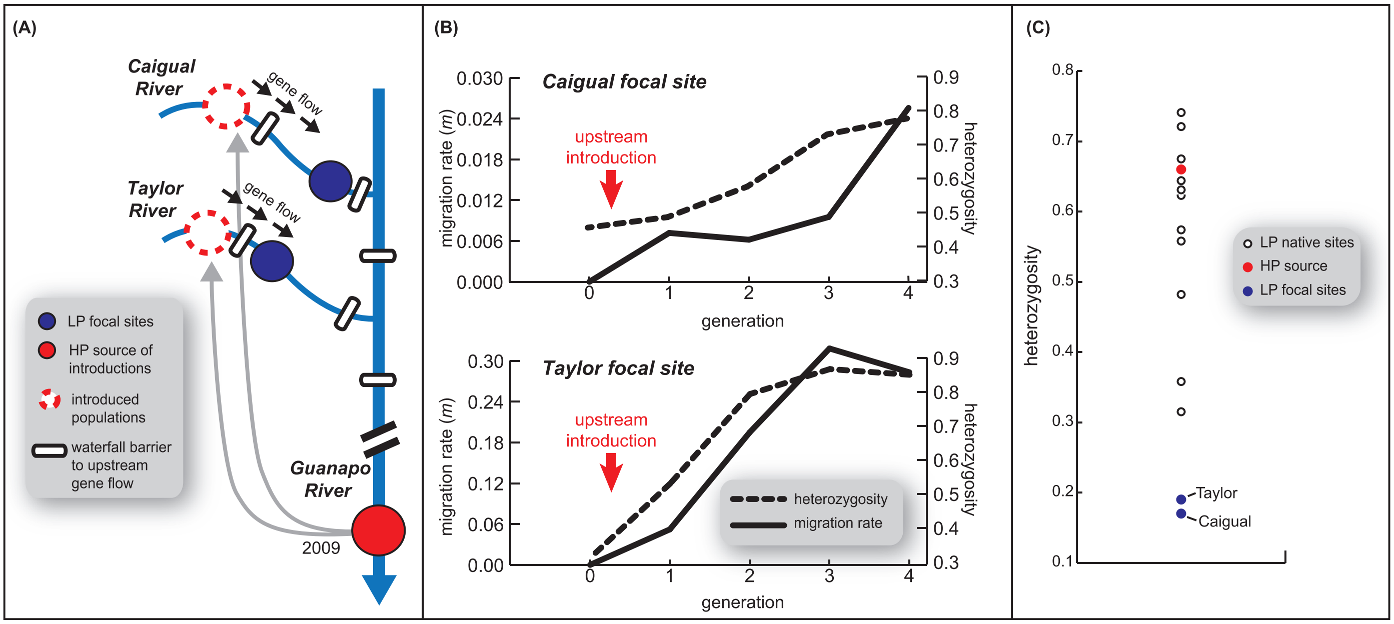

Fig. 1. (A) Schematic diagram of the introduction scenario that provided the ability to test the effects of gene flow from guppies originating from an adaptively divergent high predation (HP) source population (solid red) into two native low predation (LP) sites (solid purple). Introduction sites (dashed red) were located upstream from native focal sites, and gene flow was expected to be unidirectional and downstream. (B) Rates of migration (*m,* solid line) and heterozygosity (*H,* dashed line) estimated in the focal populations after the upstream introductions took place (noted by red arrow). Estimate of *m* is based on mark-recapture data, and *H* is based on 12 microsatellite loci detailed in Fitzpatrick et al. (2016). (C) Heterozygosity estimates from the high predation source population (solid red), the two native low predation (LP) focal sites before gene flow (solid purple), and 11 low predation guppy populations found in other headwater tributaries throughout the Northern Range mountains in Trinidad (open circles). All estimates are based on averages across the same ten microsatellite loci described in Fitzpatrick et al. (2015) and Baillie (2012).

predicted by theory, we expected gene flow to cause traits to become more similar to the source population, thereby eroding initial divergence (Fig. 2A). However, field measurements of traits suggest that locally adapted traits are maintained despite gene flow from an originally divergent source (Fitzpatrick et al., 2015). If this divergence has a genetic basis and natural selection is strong enough to overcome gene flow, it would provide evidence that divergence can be maintained despite gene flow (Fig. 2D). It is important to note that these hypotheses are not intended to be alternatives to one another and in fact they could both be true at the same time. That is, strong divergent selection could cause some divergence while gene flow constrains the optimal level of divergence. While keeping that in mind, we believe that our predictive framework is a useful way to evaluate the overall effects of gene flow on quantitative traits.

We incorporated our understanding of initial conditions (i.e., low genetic variation and some non-parallel phenotypes) of the native recipient populations into our predictions. In small populations, unpredictable allele frequency changes due to drift should lead to genetic and phenotypic heterogeneity among populations. Initial non-parallel divergence caused by low genetic variation could therefore be eroded by gene flow as both populations converge to become more similar to the source (Fig. 2B). However, if initial non-parallelisms were indeed caused by low genetic variation, and thus a lack of variation to respond to selection, we might expect the interaction between gene flow and selection to result in post-gene flow traits that match the expected direction of divergence across the predation regime (solid gray line in Fig. 2E). If initial non-parallelisms were instead due to differences in the selective environment between the neighboring low predation streams, we would expect the non-parallelism to be maintained (dashed line in Fig. 2E).

Finally, if traits did not show initial divergence across predation regime, under the *'gene flow constrains divergence'* hypothesis, we would expect no change in traits following gene flow (Fig. 2C). But if a lack of genetic variation prevented adaptive divergence in small native low predation populations before gene flow, we would expect the interaction of selection and gene flow to cause parallel divergence in

Table 1. Summary of guppy populations used in the quantitative trait analyses. Years correspond to timing of field collections. Samples from 2008 were collected prior to gene flow from upstream introduction experiments (pre), whereas 2011 samples were collected after gene flow (post). Sample sizes refer to number of G_2 reared individuals included in trait analyses. Population genetic parameters N_e, effective population size, A_r, allelic richness, and H, heterozygosity were estimated with ten microsatellite loci as described in Fitzpatrick et al. (2015).

Year	Population type	Predation level	Stream	# males	# females	N_e	A_r	H
2008	source	high	Guanapo	23	31	988 (208, inf)	12	0.80
	pre	low	Taylor	15	31	2 (0.5,74)	2	0.19
	pre	low	Caigual	21	28	3 (1,43)	2	0.17
2011	post	low	Caigual	24	19	921 (195, inf)	11	0.78
	post	low	Taylor	18	13	229 (99, inf)	9	0.71

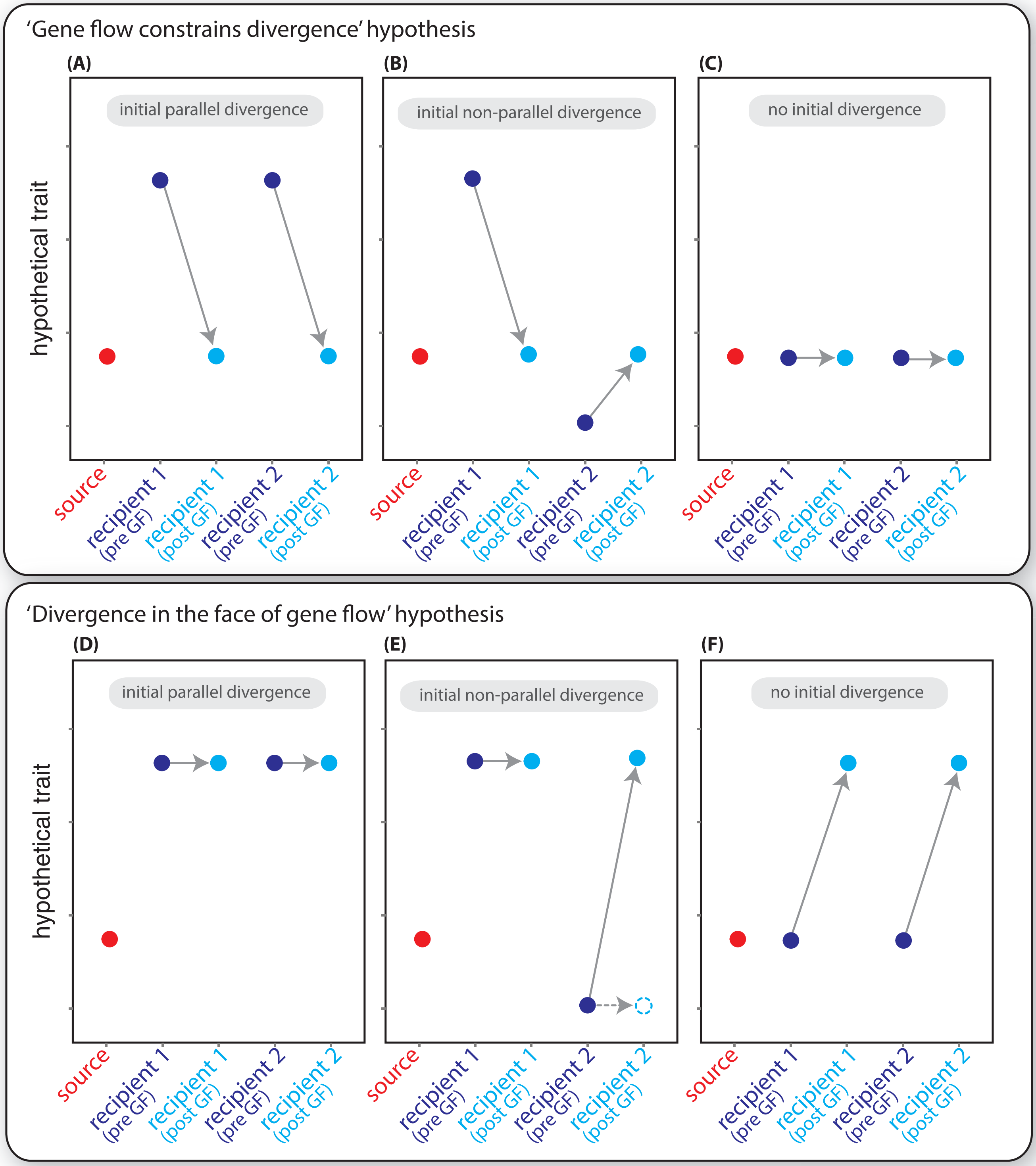

Fig. 2. Predicted trait responses depending on the role of gene flow and patterns of initial divergence between high predation source and native low predation populations. Under the *'gene flow constrains divergence'* hypothesis, traits will resemble the source population after gene flow (A–C). Under the *'divergence in the face of gene flow'* hypothesis, trait divergence from the source population will be maintained or increase after gene flow (D–F), unless selection favors a high predation-like phenotype (dashed line in E). Differences among the three predictions under each hypothesis are based on initial patterns of trait variation before gene flow.

post-gene flow populations (Fig. 2F). Testing these predictions in a system amenable to experimentation in both wild and laboratory environments provided a unique opportunity to interpret the effects of gene flow on phenotypic evolution of adaptive traits.

MATERIALS AND METHODS

Field sampling and rearing guppies in a common garden.— Populations included in our study were sampled from three sites within the Guanapo watershed in the Northern Range

Mountains of Trinidad. Two low predation (LP) headwater tributaries (Taylor River and Caigual River) and the high predation (HP) river (Guanapo) that served as the source for introduction experiments were sampled prior to introductions. Between January and April of 2008, 25–30 male and 25–30 female guppies were captured from each site with butterfly nets and transported to Colorado State University under an export permit granted by Trinidad's Fisheries Division of the Ministry of Food Production. In 2011, approximately ten guppy generations after high predation guppies from the Guanapo site were introduced into previously guppy-free sites upstream from native LP Taylor and LP Caigual populations (Travis et al., 2014), we re-sampled and transported 25–30 male and 25–30 female guppies from these same two low predation sites (Fig. 1A). Details about numbers sampled and population genetic parameters before and after gene flow are provided in Table 1.

We conducted two common garden assays using lab protocols designed to be as similar as possible to quantify genetic differentiation in traits before and after gene flow from an introduced, adaptively divergent source population. The pre-gene flow common garden assay consisted of native Taylor, Caigual, and Guanapo populations sampled in 2008. The post-gene flow assay consisted of Taylor and Caigual populations sampled in 2011. Genome-wide SNP data suggest all individuals in the 2011 sample are hybrids between Taylor or Caigual and Guanapo (Fitzpatrick, unpubl. data). We did not include the Guanapo population in 2011 due to concerns that high levels of gene flow from upstream introduction experiments confound this as a control site.

To minimize maternal and other environmental effects on traits, wild-caught guppies were reared at Colorado State University for two generations in custom-made recirculating systems under standardized lab conditions (described in Torres-Dowdall et al., 2012b; Handelsman et al., 2013; Ruell et al., 2013). Wild-caught females were randomly outcrossed with unique males to produce first generation (G_1) laboratory-born individuals, which were also randomly outcrossed to produce the second-generation (G_2) laboratory-born individuals used in this study. We observed low lab mortality and low crossing failure rates using this protocol, ensuring that selection to laboratory conditions should not be a major factor in our study.

Quantifying phenotypic traits.—We measured a series of life history, body shape, and coloration traits, which have previously been shown to exhibit adaptive divergence based on the local predation regime in guppies. We measured two life history traits (age and size at maturity) on both males and females following previously published methods (Reznick, 1982; Torres Dowdall et al., 2012a). Based on previous field and common garden studies, we expected guppies adapted to low predation environments to exhibit a slow life history with later maturation at larger body sizes than populations that experience high predation (Reznick, 1982; Reznick and Endler, 1982; Reznick and Bryga, 1996). A slowed life history is thought to be favored under low predation conditions where competition for resources in high density environments is a stronger fitness determinant than reproducing before being preyed upon, as in high predation environments (Bassar et al., 2013). Guppy body shape has also been shown to exhibit mostly parallel patterns of divergence corresponding to predation regime (Alexander et al., 2006; Hendry et al., 2006; Torres-Dowdall et al., 2012b). Specifically, fusiform bodies with dorsal orientation of the mouth are thought to improve escape ability in high predation localities (O'Steen et al., 2002), whereas water flow and resource acquisition in low predation habitats favor deeper bodies with a more terminal orientation of the mouth (Robinson and Wilson, 1995; Alexander et al., 2006). Increased male coloration tends to evolve in low predation environments in response to strong sexual selection, whereas inconspicuous males are favored by natural selection in high predation localities (Endler, 1980; Brooks and Endler, 2001).

To measure male and female life history traits (age and size at maturity), second-generation (G_2) juveniles were first anesthetized with tricaine methanesulfonate (MS-222) at 29 days after birth and sexed. At the age of four weeks, juvenile males can be differentiated from females based on the presence/absence of melanophores in a triangular patch that appears on the ventral abdomen, which is present only in females thereafter (Reznick, 1982). One male and one female per full-sibling family were housed individually, and all were reared under the same conditions until reaching sexual maturity. G_2 females were crossed with randomly chosen unrelated G_1 males on a weekly basis. Males were added to the female tank in the evening and removed the following morning so as not to interfere with food rations given to females. Tanks were checked daily for the presence of the first brood, and we considered female age at maturity as the number of days until first parturition. Males were considered to be sexually mature when the apical hood became even with the tip of the gonopodium (Reznick, 1990). Males were checked weekly for the first appearance of the apical hood and then checked daily until reaching maturity.

At maturity, both males and females were anesthetized, spread laterally on a white background alongside a metric ruler, and digitally photographed using a Canon EOS Rebel XSi SLR digital camera (Canon U.S.A., Inc., Melville, NY). We quantified variation in size and body shape at male and female maturity with landmark-based geometric morphometrics using the photographs taken on the day of maturity (James and Marcus, 1993). Body size and shape were characterized by eight homologous landmarks and six semi-landmarks digitized with TPSDig2 (Rohlf, 2010) from images of each specimen. We used centroid size (square root of sum of squared distances of each landmark to the location on the fish that minimizes that sum) as our estimate for overall body size (Bookstein, 1991). Male and female raw landmark coordinates were analyzed separately; first they were subjected to a Procrustes fit whereby variation from position, orientation, and isometric size is removed from the data (Rohlf and Slice, 1990).

Morphological analyses were performed in two steps within the 'geomorph' package in R v3.1.3 (Adams and Otárola-Castillo, 2013). First, we performed a Procrustes ANOVA (Goodall, 1991) to test for population differences after removing the variance in body shape accounted for by centroid size. For both sexes we found that shape differed by population, independent of size (males: $P = 0.001$; females: $P = 0.01$). However, we did not find a significant interaction between size and shape (males: $P = 0.317$; females: $P = 0.14$). Next, we performed a principal components analysis (PCA) using the covariance matrix of Procrustes coordinates. The first two PCA axes (PC1, PC2) explained 57.5% of the total differentiation in shape for males and 53.2% for females and were considered separate 'traits' for further analyses. We examined thin-plate spline deformation grids to facilitate biological interpretation of observed shape differences (Supplementary Fig. 1; see Data Accessibility). For both males and females, the first two PC axes generally corresponded to variation in body depth, length of caudal peduncle, and position of the mouth and eye.

We quantified male coloration at maturity using traditional outline methods following previously established protocols for measuring color in guppies (Ruell et al., 2013). We used photographs taken on the day of maturity (described above). Illumination of males in photographs was held constant by using a single camera without flash and lighting with two full-spectrum fluorescent lights that were permanently fixed on either side of the camera. All photographs were taken at a single location in a windowless room. Body area and color outlining was conducted using the freehand tool in ImageJ 1.46r. One person (J.A.K.) counted total number of distinct pigment-based color elements and assigned them to three categories of color (black, orange, and yellow-white). Total body area and area of each color element were measured and three metrics of color from these methods were extracted: i) total area of all color elements, ii) total number of distinct color elements, and iii) total area of orange. To correct for male body size, total area of all color elements and total area of orange were measured as proportions of total body area.

Statistical analyses.—We evaluated our predictions about how gene flow should affect genetically based changes in traits by fitting linear mixed effects models for each univariate trait. Population type (pre-gene flow, post-gene flow, or source) was included as a fixed effect with stream included as a random effect (following Table 1). Each trait was thus modeled individually using maximum likelihood, and significance of overall population differences was tested with likelihood ratio tests against the null model that included only the random effect. Residual plots were used to determine that model assumptions of normality and homoscedasticity were met. Female age at maturity was square-root transformed to normalize the data prior to analysis. Models were carried out with package 'lme4' in R (Bates and Maechler, 2009). We then performed *post hoc* Tukey's HSD tests with the 'multcomp' R package to determine significant pairwise differences and test our specific predictions about divergence among pre- and post-gene flow phenotypes within low predation sites compared to the source population (Hothorn et al., 2008).

In addition to testing our predictions based on means and variances of univariate traits, we examined the effect of gene flow on multivariate trait responses using discriminant analyses on principal components (DAPC; Jombart et al., 2010). This method determines the degree to which populations overlap in multivariate trait space, or how 'ecologically exchangeable' they are (Hendry et al., 2013). Under the *'gene flow constrains divergence'* hypothesis, we expect individuals from post-gene flow populations to be more similar and therefore show higher exchangeability with the source population. Whereas, under the *'divergence in the face of gene flow'* hypothesis, we expect little overlap with the source population and possibly increased exchangeability between low predation populations after gene flow. We conducted separate DAPCs for sets of male (life history, body shape, and coloration) and female (life history and body shape) traits. For each DAPC, we determined the assignment probability for a given individual in post-gene flow populations into each of the five candidate populations (CA-pre, CA-post, TY-pre, CA-post, source). We then determined the mean and 95% confidence intervals for the proportion of post-gene flow populations that were correctly classified into all other populations.

RESULTS

We compared the two LP populations before and after gene flow to the HP source population for 11 total traits: four life history traits (male age and size at maturity; female age and size at maturity), four morphometric traits (male PC1 and PC2; female PC1 and PC2), and three male color traits (total color area, total orange area, and number of distinct color elements).

Support for the 'gene flow constrains divergence' hypothesis.—Six out of the 11 traits we measured before and again ten generations after the onset of gene flow supported the hypothesis that gene flow homogenizes traits (Fig. 3A–F). That is, these traits became more similar to the source population after gene flow. Most of these traits (female body shape axis PC2, male body shape axis PC1, and all three color metrics) were initially divergent from the source in both LP populations and shifted in parallel towards the source population after gene flow. Initial patterns of divergence in the three metrics of coloration were opposite to the expected direction in guppies; the high predation source population was generally more colorful than native low predation populations (Fig. 3D–F). Given that we know female guppies typically prefer increased coloration, the apparent homogenization caused by gene flow in these traits could be due to the combined effects of selection in the LP environment as well as homogenizing gene flow. In contrast, the shifts in body shape axes were in the direction expected to be maladaptive. Higher values of female body shape axis PC2 correspond to an upturned mouth position relative to the eye and higher male body shape axis PC1 values correspond to an elongated caudal peduncle, both of which are more typical of the high predation ecotype. Male age at maturity was also consistent with the *'gene flow constrains divergence'* hypothesis (compare Fig. 3C to 2B). Initial non-parallel divergence between the two native low predation sites was diminished as post-gene flow Caigual males shifted to an earlier maturation comparable to the source and pre-gene flow Taylor, but post-gene flow Taylor males did not change (Fig. 3C).

Support for the 'divergence in the face of gene flow' hypothesis.—Female age at maturity (Fig. 4A) was the only trait for which adaptive divergence was maintained (Caigual) or facilitated (Taylor). Although age at maturity shifted slightly earlier (towards the source population) in Caigual, it was not found to be significantly different from the pre-gene flow population. Pre- and post-gene flow differences in this trait were also non-significant in Taylor, but age at maturity shifted later (in the predicted adaptive direction), enough to be considered significantly different from the source, and there was also an increase in variation for this trait.

Several traits became divergent from the source population following gene flow, but opposite to the expected adaptive direction. For example, both male and female size at maturity substantially decreased following gene flow, maturing at sizes even smaller than the high predation source site (Fig. 4B, C). Female body shape axis PC1 and male body shape axis PC2 also exhibited divergence away from the source population. Thin-plate spline deformations indicate that post-gene flow

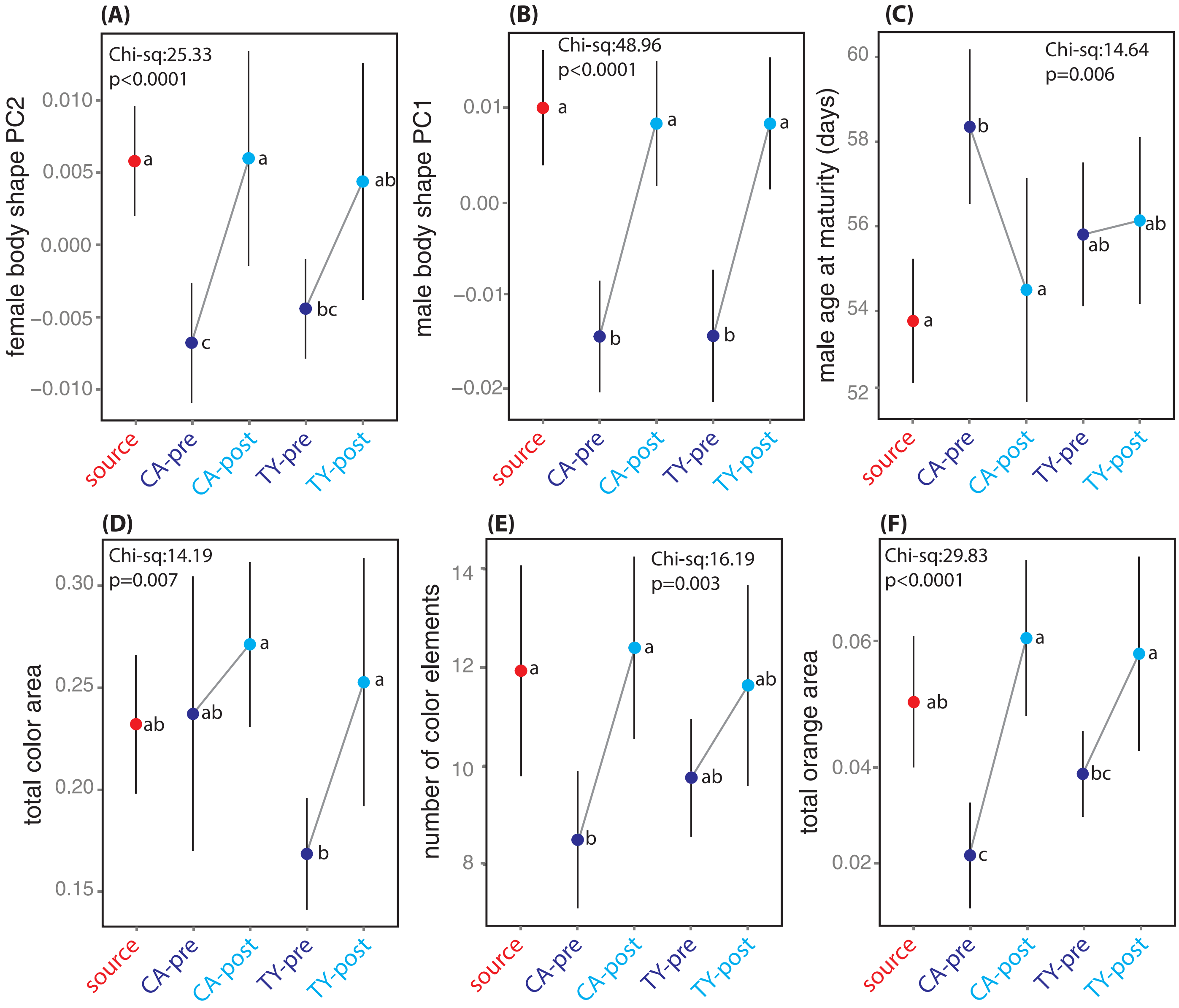

Fig. 3. Means and 95% confidence intervals for six traits that conformed to the *'gene flow constrains divergence'* hypothesis: (A) Female body shape PC2, (B) male body shape PC1, (C) male age at maturity, (D) total color area, (E) number of color elements, and (F) total orange area. Population IDs on x-axes correspond to population summaries in Table 1 with Caigual abbreviated as CA and Taylor abbreviated as TY. Chi-squared statistics correspond to the likelihood ratio test described in the text. Lowercase letters indicate significant differences among populations based on *post hoc* Tukey's HSD tests.

guppies evolved deeper bodies (Supplementary Fig. 1; see Data Accessibility).

Ordination plots from the DAPC exchangeability analyses also provide support for the *'divergence in the face of gene flow'* hypothesis. Namely, Caigual and Taylor post-gene flow populations are phenotypically distinct from the source population, and this result was consistent for both females and males (Fig. 5). Post-gene flow males and females from both streams had very low probabilities of being classified into the source population (CA [Caigual] females: 0.08±0.05; CA males: 0.02±0.01; TY [Taylor] females: 0.06±0.05; TY males: 0.06±0.07). In contrast, post-gene flow Caigual and Taylor populations were phenotypically similar to each other based on multivariate traits. The highest rates of 'misclassification' were individuals from post-gene flow Caigual that were assigned to post-gene flow Taylor (CA females: 0.25±0.06; CA males: 0.45±0.13), and vice versa (TY females: 0.40±0.13; TY males: 0.31±0.08).

DISCUSSION

In general, we found that gene flow induced genetically based shifts in quantitative traits. Most phenotypes measured on individuals from the same sites and reared for two generations in a common garden environment differed depending on whether they were sampled before or approximately ten generations after gene flow from a source population that was originally adapted to a different environment. While gene flow is recognized as one of the classical evolutionary forces, its role in shaping phenotypic evolution and eco-evolutionary dynamics remains controversial (Mayr, 1963; Ehrlich and Raven, 1969; Rasanen and Hendry, 2008; Ellstrand, 2014). Our study provided a rare opportunity to test how a recent onset of gene flow affected

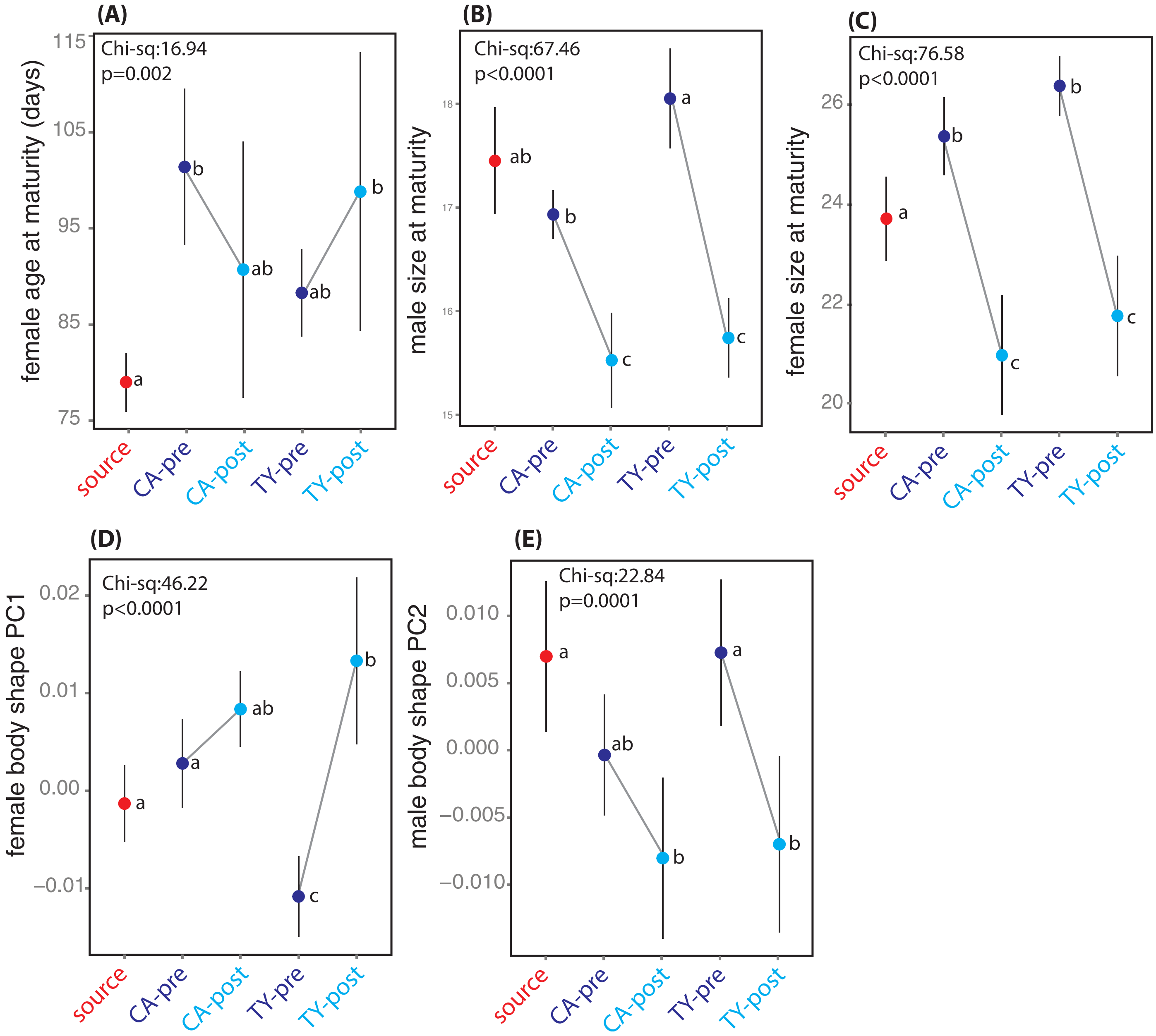

Fig. 4. Means and 95% confidence intervals for five traits that conformed to the *'divergence in the face of gene flow'* hypothesis: (A) age at female maturity, (B) male size at maturity, (C) female size at maturity, (D) female PC1, and (E) male PC2. Population IDs on x-axes correspond to population summaries in Table 1 with Caigual abbreviated as CA and Taylor abbreviated as TY. Chi-squared statistics correspond to the likelihood ratio test described in the text. Lowercase letters indicate significant differences among populations based on *post hoc* Tukey's HSD tests.

genetically based changes in traits with known adaptive significance, and our results indeed attest to gene flow's "multifarious" effects (Garant et al., 2007).

Does gene flow constrain divergence?—In the absence of other evolutionary forces, gene flow should homogenize allele frequencies between distinct populations, making them more phenotypically similar (Slatkin, 1978). Following this theory, under the *'gene flow constrains divergence'* hypothesis, we predicted that gene flow from a genetically distinct source population would cause traits in recipient populations to become similar to the source after gene flow. We found support for this hypothesis in just over half of traits we measured (Fig. 3A–F). We did not measure the fitness consequences of these changes, but the body shape and life history traits that fit the *'gene flow constrains divergence'* pattern (Fig. 3A–C) shifted in the maladaptive direction based on what we would predict for guppies in a low predation environment. That is, female guppies gained a more upturned mouth, male guppies evolved an elongated caudal peduncle, and Caigual males evolved an earlier age of maturation. These shifts towards the typical high predation ecotype suggest that gene flow constrained adaptive divergence for these traits. Interestingly, we observed no change in male age at maturity in the Taylor. Previous work suggested that a young age at maturity might be an adaptive anomaly for this stream due to selection from non-predator induced sources of mortality (Fitzpatrick et al., 2014). Thus, our result could be an example of the scenario represented by the dashed line in Figure 2E, namely, maintenance of non-parallel divergence in the face of gene flow.

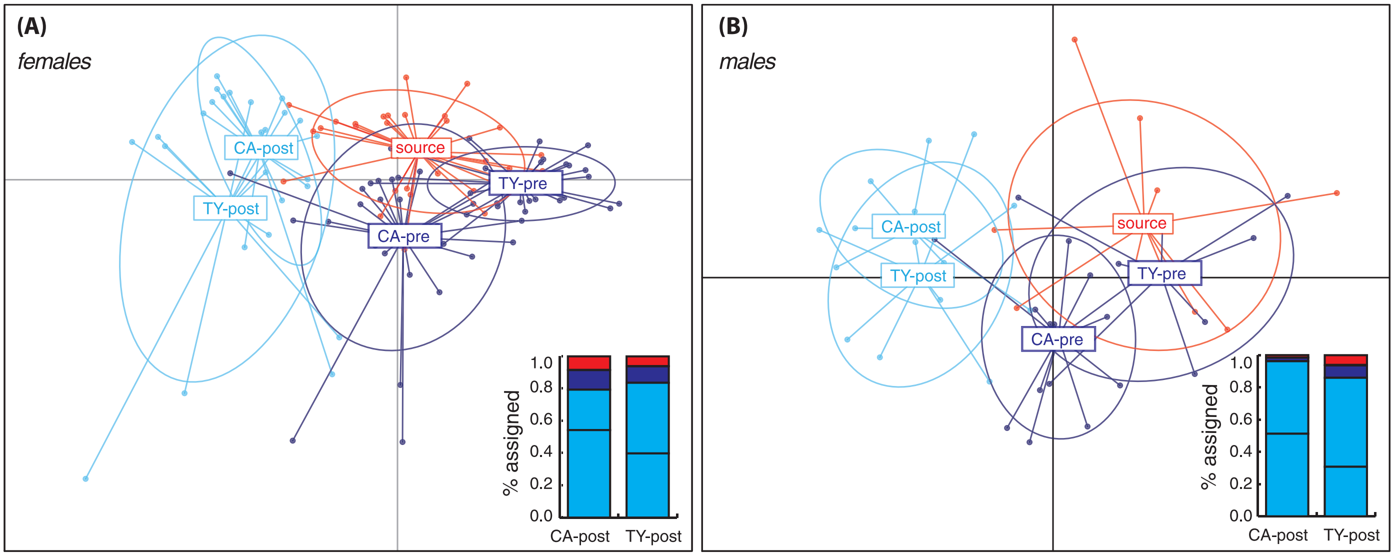

Fig. 5. Ordination plots and group classification based on discriminant analysis of principal components (DAPC) for multivariate sets of female (A) and male (B) phenotypic traits. Colors correspond to *a priori* groups based on population origin: pre-gene flow low predation sites in dark blue, post-gene flow low predation sites in light blue, and source of gene flow in red. Stacked bar graph insets show the mean proportion of individuals from each population assigned to each other population.

All metrics of male coloration also became more similar to the source population and therefore fit the *'gene flow constrains divergence'* hypothesis. However, the initial divergence pattern we observed in color metrics (i.e., greater coloration in high predation source) is opposite to what is commonly documented when comparing low versus high predation sites (Endler, 1980; Houde, 1997; Magurran, 2005), but see Weese et al. (2010) and Millar and Hendry (2012) for examples that highlight complexities to this paradigm. One possible explanation for the initially low coloration in native Caigual and Taylor is that pre-gene flow populations were limited by a lack of genetic variation to evolve high coloration. This is consistent with extremely low neutral genetic variation observed before gene flow (Table 1). Indeed, inbreeding is known to reduce coloration in guppies (Sheridan and Pomiankowski, 1997; van Oosterhout et al., 2003; Johnson et al., 2010). Although we were unable to measure inbreeding depression in our focal populations *per se*, the native populations exhibited extremely low levels of genetic diversity, even compared to other upland guppy populations throughout Trinidad (Fig. 1C). Thus, although the homogenizing role of gene flow is generally considered to reduce fitness and limit adaptation (Garcia-Ramos and Kirkpatrick, 1997; Lenormand, 2002), in this case it may have caused a shift in the direction that would presumably increase fitness. It is also possible that selection acting on the increased genetic variation caused by gene flow contributed to the increase in post-gene flow coloration, given that the trait means of post-gene flow populations tended to be higher than the high predation source population (Fig. 3D–F). Furthermore, increased coloration has been shown to be one of the fastest traits to evolve within introduced guppy populations (Endler, 1980), and the interaction between gene flow and selection for increased coloration in upstream populations could have caused a rapid evolutionary response in this trait. Previous work has shown that female guppies from low predation environments do not discriminate against male guppies from high predation sites in the same drainage (Schwartz et al., 2010), suggesting that female choice was not likely acting as a barrier to gene flow.

Does divergence occur in the face of gene flow?—Delayed age at female maturity was maintained in the Caigual, and evolved in the Taylor, despite gene flow from a population that matures at an early age (Fig. 4A). Age at female maturity therefore fits the prediction under our *'divergence in the face of gene flow'* hypothesis. Delayed female maturity, along with increased time between broods, is likely favored by selection in low predation environments because it increases development time for offspring to reach a larger size at birth (Reznick and Bryga, 1996). Larger offspring are thought to have higher fitness in this environment due to the gape-limited predator *Rivulus hartii* that selectively feeds on smaller size classes of guppies and increased competitive ability in a low resource environment (Bassar et al., 2013). Therefore, it is conceivable that divergence in this trait could be maintained by strong selection even under high gene flow that homogenizes other traits. One mechanism for this is differential introgression throughout the genome where gene flow homogenizes populations at neutral or nearly-neutral loci but locally adaptive loci are maintained through differential selection (Poelstra et al., 2014; Soria-Carrasco et al., 2014). Thus, even in the face of substantial gene flow from initially maladapted upstream populations, the alleles that underlie delayed female maturity may persist in the population and aid in the rapid recovery of local adaptation following gene flow.

We also observed divergence away from the source population in male and female body size and body shape axes (Fig. 4B–E), but these traits diverged in a presumably maladaptive direction. We attribute the decrease in body size of our laboratory populations at least in part to genotype by environment interactions, because large sizes at male maturity in post-gene flow Caigual and Taylor populations have been maintained in the wild (Fitzpatrick et al., 2015). Interactions among genetic divergence, plasticity, and gene flow are complex and poorly understood (Crispo, 2008; Thibert-Plante and Hendry, 2011), and we do not have clear expectations about how these interacting forces would affect observed patterns in body size. Growth rate, size at maturity, and offspring size have been shown to be highly plastic in guppies (Krause and Liesenjohann, 2012; Torres-Dowdall et al., 2012b; Handelsman et al., 2013) In fact, the source population in our study and other low predation populations have been shown to plastically respond to environmental cues in traits like size and morphology but not age at maturity (Handelsman et al., unpubl. data). One theory is that a chronic stress response, such as alteration in cortisol levels (Fischer et al., 2014), could reduce growth. It is possible that an unknown stressor in the post-gene flow lab environment induced this plastic response.

In addition to univariate trait analyses, we felt it was important to include the multivariate exchangeability analysis because phenotypic traits can relate to each other through genetic correlations and therefore respond to direct and indirect selection, gene flow, and drift (Lande and Arnold, 1983). Multivariate trait responses provided support for divergence in the face of gene flow from the source site. Interestingly, post-gene flow populations were not only distinct from the source but more similar and 'ecologically exchangeable' with each other. This could be due to directional selection in the low predation environment, but multivariate trait responses are more difficult to interpret biologically.

Putting the effects of gene flow in context.—The gene flow scenario we studied here differs from how gene flow is usually incorporated into standard population genetic models, and from other classic empirical systems that have addressed similar questions. First, levels of migration increased throughout our study (Fig. 1B) and were much higher than what is typically observed between adaptively divergent populations in nature (Slatkin, 1985). Second, before the onset of gene flow, recipient populations were small, isolated, had low genetic variation, and were potentially inbred (Table 1). Finally, although the original source population was adapted to a different environment, high predation guppies possess many universally beneficial characteristics such as high genetic variation (Barson et al., 2009) and high fecundity (Reznick, 1982). Later generation immigrants in our study may not only have retained those characteristics, but also likely started to evolve important low predation traits. Considering these characteristics of the immigrants and the depressed state of the recipient populations, the beneficial impacts of gene flow may be exaggerated in our study compared to more standard examples of maladaptive gene flow between divergent populations, such as between *Timema* stick insects adapted to different host plants (Nosil and Crespi, 2004), or lake versus stream stickleback (Hendry et al., 2002). But despite these differences, we also observed constraints on adaptive divergence in some traits, similar to previous studies. In fact, more univariate traits seemed to be constrained ($n = 6$) than to diverge in the adaptive direction ($n = 1$).

A novel contribution of our study was the ability to compare variation in phenotypes from the same populations before and after gene flow, thereby avoiding the confounding factors of geography and causality (i.e., does gene flow constrain adaptive divergence or vice versa?). Furthermore, gene flow caused by secondary contact between once-isolated populations is increasingly common under invasion scenarios and climate-induced range shifts and is also a potential management strategy to increase variation in small, genetically depauperate populations (Allendorf et al., 2001; Currat et al., 2008; Crispo et al., 2011). Our study thus directly addresses a growing need to gain a better understanding of how human-mediated gene flow affects evolution of fitness-related traits in order to effectively manage imperiled populations.

Most studies evaluating how gene flow affects phenotypes are limited to measuring traits in the wild (Merilä and Hendry, 2014). However, ambiguity about the relative influence of genetic change versus direct environmental effects on phenotypes restricts the scope of inference in these studies (Crispo, 2008). Previous work on guppies sampled downstream from multiple introduction experiments showed that locally adapted traits, when measured on wild-caught fish, are generally maintained in the face of high gene flow (Fitzpatrick et al., 2015). But the extent to which observed phenotypic divergence across the predation gradient was genetically based or maintained through plasticity could not be discerned by measuring traits in the wild. Thus, an advantage of this study was the ability to test the effect of gene flow on genetically based changes in traits measured in the common garden assays. Ideally, we would have included pre-gene flow populations measured in the common garden at the second time point (2011) to control for any temporal differences in the lab environment, but unfortunately these control populations no longer exist. We interpreted shifts in lab-measured traits from G_2 individuals as evidence for a genetic response to gene flow because maternal and other indirect environmental effects were reduced, and the common garden environment was highly controlled.

Conclusions.—We provided evidence that gene flow has caused genetically based changes in traits. Differences observed between populations and among traits confirm that gene flow does not have a single evolutionary role (Slatkin, 1987; Garant et al., 2007). As predicted by theory, we showed that most traits were homogenized by gene flow. However, our finding of an increase in male coloration after gene flow suggests that homogenization does not necessarily constrain adaptation, especially if recipient populations have experienced high genetic drift prior to gene flow. We also showed evidence in one trait for which the adaptive direction was maintained despite high levels of gene flow, suggesting that strong selection can counteract high levels of gene flow. Given that many of the traits we found to resemble the high predation ecotype in the common garden showed local adaptation in the wild (Fitzpatrick et al., 2015), our results point to the complex interactions between plasticity, genetic divergence, and gene flow that shape phenotypic diversity in the wild. Over contemporary time, gene flow has the potential to be a much larger source of genetic variation than mutation, but also has the potential to quickly erode local differentiation.

DATA ACCESSIBILITY

Supplemental material is available at http://www.copeiajournal.org/ci-16-559.

ACKNOWLEDGMENTS

We thank P. Bois, J. Gerberich, A. Perez-Umphrey, and J. Smith for assistance collecting data for this project. All experimental methods were approved by the Colorado State University Institutional Animal Care and Use Committee (protocol no. 12-3818A). Collection permits were graciously provided by the Fisheries division of Trinidad's Ministry of Food Production, Land and Marine Affairs. This manuscript benefited from comments from J. Conner and the Funk-Hoke

Lab at CSU. This project was funded by National Science Foundation grants to W.C.F. and L.M.A. (DEB-0846175), D.N.R. (EF-0623632), C.K.G. (DEB-0846175), NSF graduate research fellowships to E.D.B. and S.W.F., and a Society for the Study of Evolution Rosemary Grant Student Research Award to S.W.F. This is Kellogg Biological Station Contribution no. 1960.

LITERATURE CITED

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

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

Allendorf, F. W., R. F. Leary, P. Spruell, and J. K. Wenburg. 2001. The problems with hybrids: setting conservation guidelines. Trends in Ecology and Evolution 16:613–622.

Baillie, L. 2012. Genetic population structure of the Trinidadian guppy (*Poecilia reticulata*) across Trinidad and Tobago. Unpubl. Master's thesis, Dalhousie University, Nova Scotia, Canada.

Barson, N. J., J. Cable, and C. van Oosterhout. 2009. Population genetic analysis of microsatellite variation of guppies (*Poecilia reticulata*) in Trinidad and Tobago: evidence for a dynamic source-sink metapopulation structure, founder events and population bottlenecks. Journal of Evolutionary Biology 22:485–497.

Bassar, R. D., A. López-Sepulcre, D. N. Reznick, and J. Travis. 2013. Experimental evidence for density-dependent regulation and selection on Trinidadian guppy life histories. American Naturalist 181:25–38.

Bates, D., and M. Maechler. 2009. lme4: linear mixed-effects models using S4 classes. R package version 0.999375-32.

Bookstein, F. L. 1991. Morphological Tools for Landmark Data: Geometry and Biology. Cambridge University Press, Cambridge, U.K.

Brooks, R., and J. A. Endler. 2001. Direct and indirect sexual selection and quantitative genetics of male traits in guppies (*Poecilia reticulata*). Evolution 55:1002–1015.

Calsbeek, R., and T. B. Smith. 2003. Ocean currents mediate evolution in island lizards. Nature 426:552–555.

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

Crispo, E., P. Bentzen, D. N. Reznick, M. T. Kinnison, and A. P. Hendry. 2005. The relative influence of natural selection and geography on gene flow in guppies. Molecular Ecology 15:49–62.

Crispo, E., J.-S. Moore, J. A. Lee-Yaw, S. M. Gray, and B. C. Haller. 2011. Broken barriers: human-induced changes to gene flow and introgression in animals. BioEssays 33:508–518.

Currat, M., M. Ruedi, R. J. Petit, and L. Excoffier. 2008. The hidden side of invasions: massive introgression by local genes. Evolution 62:1908–1920.

Ehrlich, P. R., and P. H. Raven. 1969. Differentiation of populations. Science 165:1228–1232.

Ellstrand, N. C. 2014. Is gene flow the most important evolutionary force in plants? American Journal of Botany 101:737–753.

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

Fischer, E. K., R. M. Harris, H. A. Hofmann, and K. L. Hoke. 2014. Predator exposure alters stress physiology in guppies across timescales. Hormones and Behavior 65:165–172.

Fitzpatrick, S. W., J. C. Gerberich, L. M. Angeloni, L. L. Bailey, E. D. Broder, J. Torres-Dowdall, C. A. Handelsman, A. López-Sepulcre, D. N. Reznick, C. K. Ghalambor, and W. C. Funk. 2016. Gene flow from an adaptively divergent source causes rescue through genetic and demographic factors in two wild populations of Trinidadian guppies. Evolutionary Applications 9:879–891.

Fitzpatrick, S. W., J. C. Gerberich, J. A. Kronenberger, L. M. Angeloni, and W. C. Funk. 2015. Locally adapted traits maintained in the face of high gene flow. Ecology Letters 18:37–47.

Fitzpatrick, S. W., J. Torres-Dowdall, D. N. Reznick, C. K. Ghalambor, and W. C. Funk. 2014. Parallelism isn't perfect: could disease and flooding drive a life-history anomaly in Trinidadian guppies? American Naturalist 183: 290–300.

Garant, D., S. E. Forde, and A. P. Hendry. 2007. The multifarious effects of dispersal and gene flow on contemporary adaptation. Functional Ecology 21:434–443.

Garcia-Ramos, G., and M. Kirkpatrick. 1997. Genetic models of adaptation and gene flow in peripheral populations. Evolution 51:21–28.

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. Functional Ecology 21:394–407.

Gilliam, J. F., D. F. Fraser, and M. Alkins-Koo. 1993. Structure of a tropical stream fish community: a role for biotic interactions. Ecology 74:1856–1870.

Goodall, C. 1991. Procrustes methods in the statistical analysis of shape. Journal of the Royal Statistical Society Series B: Statistical Methodology 53:285–339.

Gordon, S. P., D. N. Reznick, M. T. Kinnison, M. J. Bryant, D. J. Weese, K. Rasanen, N. P. Millar, and A. P. Hendry. 2009. Adaptive changes in life history and survival following a new guppy introduction. American Naturalist 174:34–45.

Guillaume, F., and M. C. Whitlock. 2007. Effects of migration on the genetic covariance matrix. Evolution 61:2398–2409.

Haldane, J. 1948. The theory of a cline. Journal of Genetics 48:277–284.

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. Integrative and Comparative Biology 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*). Integrative and Comparative Biology 54:794–804.

Hendry, A. P. 2017. Eco-evolutionary Dynamics. Princeton University Press, Princeton, New Jersey.

Hendry, A. P., R. Kaeuffer, E. Crispo, C. L. Peichel, and D. I. Bolnick. 2013. Evolutionary inferences from the analysis of exchangeability. Evolution 67:3429–3441.

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

Hendry, A. P., and E. B. Taylor. 2004. How much of the variation in adaptive divergence can be explained by gene flow? An evaluation using lake-stream stickleback pairs. Evolution 58:2319–2331.

Hendry, A. P., E. B. Taylor, and J. D. McPhail. 2002. Adaptive divergence and the balance between selection and gene flow: lake and stream stickleback in the Misty system. Evolution 56:1199–1216.

Hoekstra, H. E., K. E. Drumm, and M. W. Nachman. 2004. Ecological genetics of adaptive color polymorphism in pocket mice: geographic variation in selected and neutral genes. Evolution 58:1329–1341.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. Biometrische Zeitschrift 50:346–363.

Houde, A. E. 1997. Sex, Color, and Mate Choice in Guppies. Princeton University Press, Princeton, New Jersey.

James, R. F., and L. F. Marcus. 1993. A revolution morphometrics. Trends in Ecology and Evolution 8:129–132.

Johnson, A. M., G. Chappell, A. C. Price, F. Helen Rodd, R. Olendorf, and K. A. Hughes. 2010. Inbreeding depression and inbreeding avoidance in a natural population of guppies (*Poecilia reticulata*). Ethology 116:448–457.

Jombart, T., S. Devillard, and F. Balloux. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genetics 11:94.

Karim, N., S. P. Gordon, A. K. Schwartz, and A. P. Hendry. 2007. This is not déjà vu all over again: male guppy colour in a new experimental introduction. Journal of Evolutionary Biology 20:1339–1350.

Keller, L. F., and D. M. Waller. 2002. Inbreeding effects in wild populations. Trends in Ecology and Evolution 17:230–241.

Keller, S. R., and D. R. Taylor. 2008. History, chance and adaptation during biological invasion: separating stochastic phenotypic evolution from response to selection. Ecology Letters 11:852–866.

King, R. B., and R. Lawson. 1995. Color-pattern variation in Lake Erie water snakes: the role of gene flow. Evolution 49: 885–896.

Kohler, T. J., T. N. Heatherly, R. W. El-Sabaawi, E. Zandonà, M. C. Marshall, A. S. Flecker, C. M. Pringle, D. N. Reznick, and S. A. Thomas. 2012. Flow, nutrients, and light availability influence Neotropical epilithon biomass and stoichiometry. Freshwater Science 31:1019–1034.

Krause, E. T., and T. Liesenjohann. 2012. Predation pressure and food abundance during early life alter risk-taking behaviour and growth of guppies (*Poecilia reticulata*). Behaviour 149:1–14.

Kronenberger, J. A., W. C. Funk, J. W. Smith, S. W. Fitzpatrick, L. M. Angeloni, E. D. Broder, and E. W. Ruell. 2016. Testing the demographic effects of divergent immigrants on small populations of Trinidadian guppies. Animal Conservation 20:3–11.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210–1226.

Lenormand, T. 2002. Gene flow and the limits to natural selection. Trends in Ecology and Evolution 17:183–189.

Lowe, W. H., R. P. Kovach, and F. W. Allendorf. 2017. Population genetics and demography unite ecology and evolution. Trends in Ecology and Evolution 32:141–152.

Magurran, A. E. 2005. Evolutionary Ecology: The Trinidadian Guppy. Oxford University Press, Oxford, U.K.

Mayr, E. 1963. Animal Species and Evolution. Harvard University Press, Cambridge, Massachusetts.

Merilä, J., and A. P. Hendry. 2014. Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. Evolutionary Applications 7:1–14.

Millar, N. P., and A. P. Hendry. 2012. Population divergence of private and non-private signals in wild guppies. Environmental Biology of Fishes 94:513–525.

Moody, K. N., S. N. Hunter, M. J. Childress, R. W. Blob, H. L. Schoenfuss, M. J. Blum, and M. B. Ptacek. 2015. Local adaptation despite high gene flow in the waterfall-climbing Hawaiian goby, *Sicyopterus stimpsoni*. Molecular Ecology 24:545–563.

Nosil, P. 2009. Adaptive population divergence in cryptic color-pattern following a reduction in gene flow. Evolution 63:1902–1912.

Nosil, P., and B. J. Crespi. 2004. Does gene flow constrain adaptive divergence or vice versa? A test using ecomorphology and sexual isolation in *Timema cristinae* walking-sticks. Evolution 58:102–112.

O'Steen, S., A. J. Cullum, and A. F. Bennett. 2002. Rapid evolution of escape ability in Trinidadian guppies (*Poecilia reticulata*). Evolution 56:776–784.

Pérez-Jvostov, F., A. P. Hendry, G. F. Fussmann, and M. E. Scott. 2017. Experimental assessment in nature of the ecological effects of a specialist parasite. Copeia 105.

Poelstra, J. W., N. Vijay, C. M. Bossu, H. Lantz, B. Ryll, I. Müller, V. Baglione, P. Unneberg, M. Wikelski, M. G. Grabherr, and J. B. W. Wolf. 2014. The genomic landscape underlying phenotypic integrity in the face of gene flow in crows. Science 344:1410–1414.

Rasanen, K., and A. P. Hendry. 2008. Disentangling interactions between adaptive divergence and gene flow when ecology drives diversification. Ecology Letters 11: 624–636.

Reznick, D. 1982. Genetic determination of offspring size in the guppy (*Poecilia reticulata*). American Naturalist 120: 181–188.

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

Reznick, D. N. 1990. Plasticity in age and size at maturity in male guppies (*Poecilia reticulata*): an experimental evaluation of alternative models of development. Journal of Evolutionary Biology 3:185–203.

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

Reznick, D. N., 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. N., F. H. Rodd, and M. Cardenas. 1996. Life-history evolution in guppies (*Poecilia reticulata*: Poeciliidae). IV. Parallelism in life-history phenotypes. American Naturalist 147:319–338.

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: 1934–1937.

Riechert, S. E. 1993. Investigation of potential gene flow limitation of behavioral adaptation in an aridlands spider. Behavioral Ecology and Sociobiology 32:355–363.

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

Rohlf, F. J. 2010. TPS Programs. Department of Ecology & Evolution. State University of New York, Stony Brook.

Rohlf, F. J., and D. Slice. 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. Systematic Biology 39:40–59.

Ruell, E. W., C. A. Handelsman, C. L. Hawkins, H. R. Sofaer, C. K. Ghalambor, and L. Angeloni. 2013. Fear, food and sexual ornamentation: plasticity of colour development in Trinidadian guppies. Proceedings of the Royal Society B: Biological Sciences 280:20122019.

Saint-Laurent, R., M. Legault, and L. Bernatchez. 2003. Divergent selection maintains adaptive differentiation despite high gene flow between sympatric rainbow smelt ecotypes (*Osmerus mordax* Mitchill). Molecular Ecology 12: 315–330.

Schwartz, A. K., D. J. Weese, P. Bentzen, M. T. Kinnison, and A. P. Hendry. 2010. Both geography and ecology contribute to mating isolation in guppies. PLoS ONE 5: e15659.

Shaw, P. W., and G. R. Carvalho. 1992. Genetic consequences of an artificial introduction of guppies (*Poecilia reticulata*) in N. Trinidad. Proceedings of the Royal Society B: Biological Sciences 248:111–116.

Sheridan, L., and A. Pomiankowski. 1997. Fluctuating asymmetry, spot asymmetry and inbreeding depression in the sexual coloration of male guppy fish. Heredity 79:515–523.

Slatkin, M. 1978. Spatial patterns in the distributions of polygenic characters. Journal of Theoretical Biology 70: 213–228.

Slatkin, M. 1985. Gene flow in natural populations. Annual Review of Ecology and Systematics 163:393–430.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science 236:787–792.

Soria-Carrasco, V., Z. Gompert, A. A. Comeault, T. E. Farkas, T. L. Parchman, J. S. Johnston, C. A. Buerkle, J. L. Feder, J. Bast, T. Schwander, S. P. Egan, B. J. Crespi, and P. Nosil. 2014. Stick insect genomes reveal natural selection's role in parallel speciation. Science 344:738–742.

Swindell, W. R., and J. L. Bouzat. 2006. Gene flow and adaptive potential in *Drosophila melanogaster*. Conservation Genetics 7:79–89.

Thibert-Plante, X., and A. P. Hendry. 2011. The consequences of phenotypic plasticity for ecological speciation. Journal of Evolutionary Biology 24:326–342.

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

Torres Dowdall, J., C. A. Handelsman, and E. W. Ruell. 2012a. Fine-scale local adaptation in life histories along a continuous environmental gradient in Trinidadian guppies. Functional Ecology 26:616–627.

Travis, J., D. N. Reznick, R. D. Bassar, A. Lopez-Sepulcre, R. Ferriere, and T. Coulson. 2014. Do eco-evo feedbacks help us understand nature? Answers from studies of the Trinidadian guppy. Advances in Ecological Research 50:2–36.

van Oosterhout, C., R. E. Trigg, G. R. Carvalho, A. E. Magurran, L. Hauser, and P. W. Shaw. 2003. Inbreeding depression and genetic load of sexually selected traits: how the guppy lost its spots. Journal of Evolutionary Biology 16:273–281.

Wagner, N. K., B. M. Ochocki, K. M. Crawford, A. Compagnoni, and T. E. X. Miller. 2017. Genetic mixture of multiple source populations accelerates invasive range expansion. Journal of Animal Ecology 86:21–34.

Weese, D. J., S. P. Gordon, A. P. Hendry, and M. T. Kinnison. 2010. Spatiotemporal variation in linear natural selection on body color in wild guppies (*Poecilia reticulata*). Evolution 64:1802–1815.

Zandonà, E., S. K. Auer, S. S. Kilham, J. L. Howard, A. López-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. Functional Ecology 25:964–973.