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LETTER

Locally adapted traits maintained in the face of high gene flow

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Abstract

Gene flow between phenotypically divergent populations can disrupt local adaptation or, alternatively, may stimulate adaptive evolution by increasing genetic variation. We capitalised on historical Trinidadian guppy transplant experiments to test the phenotypic effects of increased gene flow caused by replicated introductions of adaptively divergent guppies, which were translocated from high- to low-predation environments. We sampled two native populations prior to the onset of gene flow, six historic introduction sites, introduction sources and multiple downstream points in each basin. Extensive gene flow from introductions occurred in all streams, yet adaptive phenotypic divergence across a gradient in predation level was maintained. Descendants of guppies from a high-predation source site showed high phenotypic similarity with native low-predation guppies in as few as ~12 generations after gene flow, likely through a combination of adaptive evolution and phenotypic plasticity. Our results demonstrate that locally adapted phenotypes can be maintained despite extensive gene flow from divergent populations.

Keywords

Adaptive divergence, eco-evolutionary dynamics, gene flow, natural selection, *Poecilia reticulata*.

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INTRODUCTION

Gene flow plays a complex evolutionary role as it can either promote or constrain adaptation (Garant *et al*. 2007). Theory predicts that the level of adaptive divergence should reflect a balance between homogenising gene flow and diversifying selection, and that surprisingly low levels of genetic exchange between populations can be sufficient to counteract the diversifying forces of drift, mutation and directional selection (Haldane 1930). Such homogenisation can limit divergence among populations that occupy different selective environments, potentially pulling populations away from their adaptive peaks and reducing fitness (Garcia-Ramos & Kirkpatrick 1997). However, gene flow can also increase fitness by reducing inbreeding depression and infusing adaptive genetic variation (Tallmon *et al*. 2004). Understanding the effects of gene flow between adaptively differentiated populations represents a major eco-evolutionary and conservation puzzle. A fundamental question that remains is how much does gene flow actually constrain local adaptation within a species?

The complex role of gene flow is illustrated by a wide array of empirical findings. Evidence for its homogenising effect is provided by the inverse relationship often documented between levels of gene flow and phenotypic divergence (Hendry & Taylor 2004), and by studies that have experimentally reduced gene flow and documented subsequent divergence (Nosil 2009). The positive effects of gene flow are generally less appreciated, although several studies document adaptive divergence despite naturally high gene flow (Hoekstra *et al*. 2004) or an increase in hybrid fitness when divergent parents are crossed (Bijlsma *et al*. 2010). Conservation scenarios exemplify opposing effects of gene flow, where some species, such as native cutthroat trout, are threatened by the introgression of invasive alleles (Muhlfeld *et al*. 2009), while others, like the iconic Florida panther, have been rescued from the brink of extinction by assisted migration and hybridisation with immigrants (Johnson *et al*. 2010). Such opposing effects challenge the traditional view of gene flow's primarily constraining role, leading to uncertainty about the outcome of gene flow for locally adapted populations. Most studies examining recent gene flow in the wild are limited to case studies because replicated experiments under natural conditions typically are not feasible.

Repeated transplant experiments using Trinidadian guppies (*Poecilia reticulata*) – among the most compelling examples of natural selection driving phenotypic evolution in the wild – provided a novel opportunity to study gene flow and adaptive divergence in a replicated scenario in nature. Guppies show adaptive phenotypic divergence largely based on complexity of the piscivorous fish community at a given site. Life history (Reznick *et al*. 1996), morphological (Hendry *et al*. 2006), colour (Endler 1980) and behavioural (Seghers 1974) traits are known to be fitness-related, have an underlying genetic basis, and typically vary predictably across high- and low-predation environments. Between 1957 and 2009, Caryl Haskins, John Endler, David Reznick and colleagues introduced guppies originating from high-predation localities to guppy-free low-predation sites upstream of native guppy populations in six separate streams (Haskins, unpublished data, Endler 1980, Reznick & Byrga 1987, Travis *et al*. 2014). While the primary

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goal of the introduction experiments was to test for rapid adaptive evolution (Reznick *et al.* 1990, Reznick *et al.* 1997), and interactions between ecology and evolution, in the case of Travis *et al.* 2014, our goal was to assess the impact of elevated gene flow on neutral genetic and adaptive divergence from these experimentally introduced populations into downstream, native guppy populations. [Correction added on 13 November 2014 after first online publication: Additional references have been added to the above two sentences and in Table 1.]

Gene flow in drainages without introduction experiments is restricted by geographic features that limit upstream dispersal (distance and waterfall barriers), high mortality of downstream migrants caused by predation (Weese *et al.* 2011) and the small populations and slow life history typical of low-predation, upstream populations. As such, guppy populations are highly genetically differentiated within these natural drainages across Trinidad (Barson *et al.* 2009; Suk & Neff 2009; Baillie 2012). In contrast, the experimental introductions set up scenarios where high downstream gene flow is expected to occur because introduced guppies originating from high-predation environments are more fecund and initially have traits enabling them to persist at any point along the predation gradient (Fig. 1). Mating between divergent populations is expected because females often prefer novel males (Hughes *et al.* 1999). Indeed, extensive spread of immigrant alleles has been documented downstream from the oldest translocation site, suggesting downstream gene flow and hybridisation between the introduced and native population (Shaw *et al.* 1992; Becher & Magurran 2000).

In our study, we first confirmed elevated levels of gene flow by documenting the spread of introduced genotypes throughout multiple sites downstream from historical introductions and second, characterised the predator community and a suite of known fitness-related traits of guppies at each site. We tested the hypothesis that increased downstream gene flow

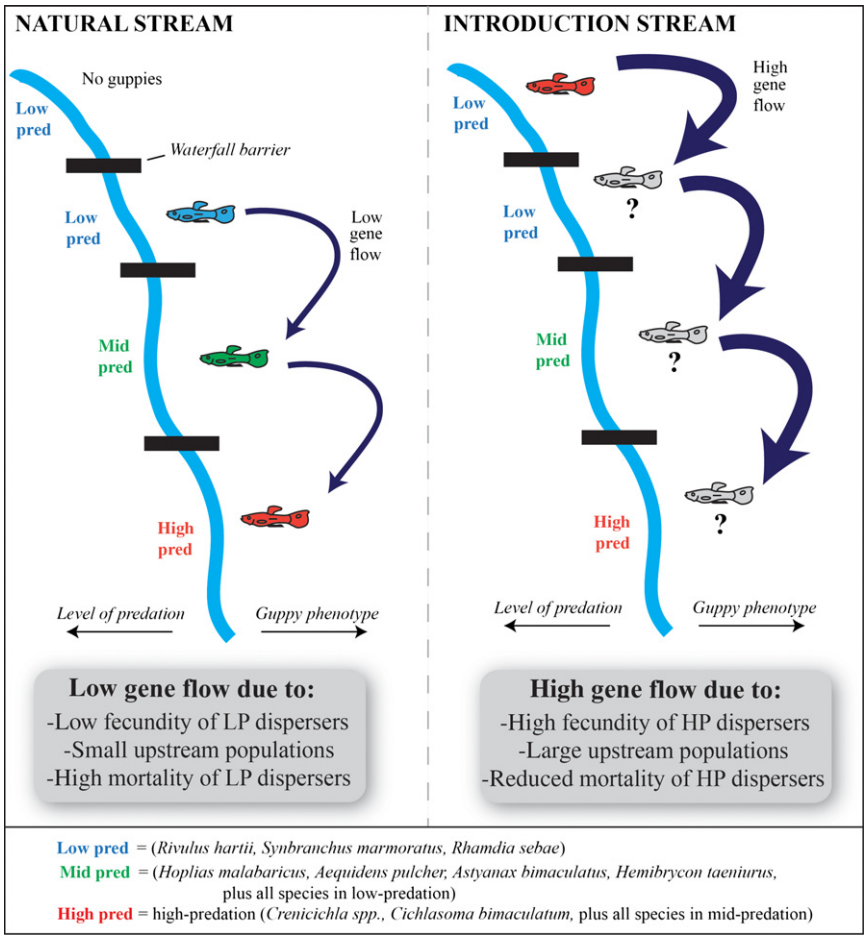

Figure 1 Conceptual diagram illustrating the expected differences in amount of gene flow between natural streams and streams with introduced populations. In both hypothetical streams, predation level is colour coded based on the species listed in the bottom key and increases in the downstream direction. Black rectangles indicate waterfall barriers that limit upstream fish dispersal. The colour of fish indicates traits matched to a certain level of predation (e.g., the blue fish has traits that are adaptive in a the low-predation environment). In the hypothetical natural stream, fish are perfectly matched to their level of predation and gene flow among populations is low based on biological factors listed in the grey box. In the hypothetical introduction stream, guppies from high-predation (HP) environments were translocated upstream of naturally occurring low-predation (LP) populations. Gene flow is expected to increase relative to natural levels for the reasons listed in the grey box, and the effect of elevated gene flow on locally adapted traits remains unknown (indicated by grey fish and question marks).

from an originally maladaptive source population will cause the loss of adaptive phenotypes. In addition, we tested the extent to which gene flow constrains locally adapted traits using guppies sampled from two native populations before introductions took place. These native populations provided a powerful comparison of neutral genetic and phenotypic divergence before and after gene flow.

METHODS

Field sampling

In January 2013, we sampled six streams where adaptively divergent, high-predation guppies were previously introduced upstream of naturally existing populations (Fig. 2). We sampled introduction and source sites from all introduction experiments and, where possible, up to four incremental sites downstream from the introduction (0, 500, 1000, 5000 m; Fig. 2; Table 1) to include the furthest downstream site that introduced guppies could reach within each drainage. The 0 m site was determined by prior surveys that noted the upstream extent of native guppies prior to the introduction (typically below a barrier waterfall). Thus, the 0 m site was not the site of introduction, but the first site of contact and potential gene flow from introduced populations into downstream native recipient populations. We refer to streams as the collection of sites sampled for each historic introduction experiment, and sites as sampling localities within streams. We sampled from six streams corresponding to the six introductions (Aripo, Caigual, El Cedro, L. Lalaja, Taylor and Turure). One stream (El Cedro) only had introduction and source sites because high-predation guppies were simply transplanted above a waterfall into a previously guppy-free, low-predation environment (Table 1). The predator community at each site was classified as high, mid or low based on fish species diversity, determined using snorkel surveys, personal communication with other researchers, and a published survey of quantitative abundance estimates of the icthyofauna within the Guanapo drainage (Gilliam *et al.* 1993; Fig. 1). Previous work on the guppy system indicates that the presence or absence of particular predators is indicative of the level of predation pressure that drives adaptive divergence of fitness-related traits (e.g., Reznick *et al.* 1996; Torres-Dowdall *et al.*, 2012a).

During the 2013 sampling, we collected 20 adult females and 20 adult males from each of 24 sites across six streams (n = 953 individuals; Table 1). In addition, we sampled 29 individuals from a native low-predation site in the Aripo drainage (native-Aripo) and 40 males that were sampled in 2009 from two streams at the 0 m site prior to upstream introductions (native-Caigual, native-Taylor). These purely native individuals allowed us to assess genetic and phenotypic divergence before and after gene flow. All fish were collected using butterfly nets. Because females have indeterminate growth, individuals were chosen to represent the range of adult sizes (> 14 mm) found at a site. All individuals were anesthetised with MS-222, had three scales sampled for genetic analyses and were photographed on their left side for phenotypic measurements (Fig. S1). See Appendix S1 for standardised photography procedures. Females were euthanised

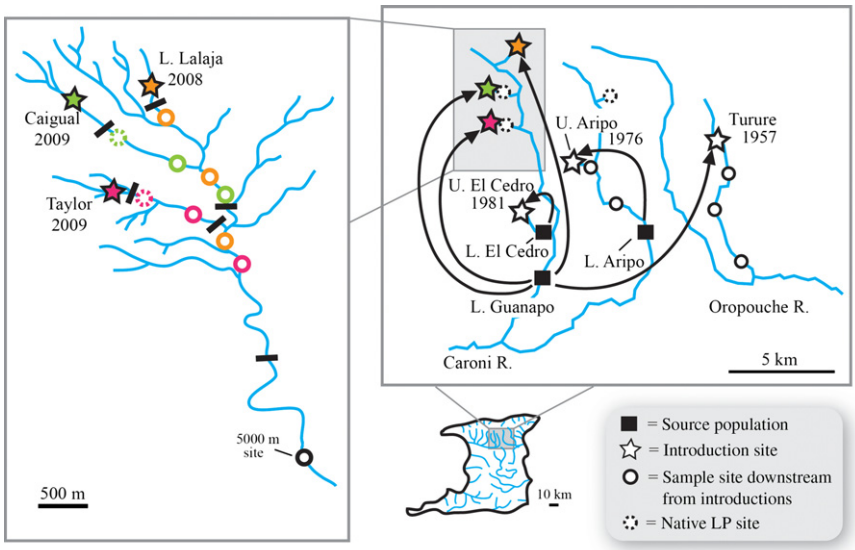

Figure 2 Map illustrating sampling scheme for our study. The island of Trinidad is shown at the largest scale, with a grey box indicating where all introductions took place. At the next spatial scale, six introduction scenarios are indicated by black arrows with the names of rivers and year of translocation. Black squares represent source sites and stars represent introduction sites. Coloured stars correspond to the introduction sites on the next inset with the smallest spatial scale. Circles indicate sites that were sampled downstream or in addition to introduction and source sites. Dashed circles indicate natural low-predation populations that were sampled before the introductions (in the case of the Guanapo drainage) or upstream from the Aripo introduction. All introduction sites are low-predation environments and all source sites are high-predation. Sites sampled downstream from introduction sites were characterised as low-, mid-, or high-predation based on complexity of fish community (Table 1).

Table 1 Site summary

Stream	Age of introduction	No. males/females introduced	Site	Coordinates	Predation level	No. males sampled	No. females sampled
Turure	1957 'old' (Haskins, *unpublished data*)	~100/~100	Introduction	N10°41.169′ W61°10.312′	Low	20	20
			0–500 m	N10°40.507′ W61°09.910′	Mid	20	20
			1000 m	N10°40.274′ W 61°09.869′	Mid	18	20
			5000 m	N10°39.413′ W61°10.081′	High	20	20
Aripo	1976 'old' Endler 1980	~100/~100	Native LP	N10°44.700′. W61°15.406′	Low	15	14
			Introduction	N10°40.241′ W61°13.865′	Low	20	20
			0 m	N10°40.179′ W61°13.737′	Mid	19	20
			500 m	N 10°40.030′ W61°13.672′	High	20	20
			1000 m/Source	N10°39.796′ W61°13.561′	High	20	20
El Cedro	1981 'old' Reznick & Bryga 1987	~50/~50	Introduction	N10°39.864′ W61°15.898′	Low	20	20
			Source	N10°39.735′ W61°15.910′	High	20	20
Lower Lalaja	2008 'recent' Travis *et al*. 2014	38/38	Introduction	N10°42.969′ W61°16.000′	Low	19	20
			0 m	N10°42.904′ W61°16.040′	Low	18	20
			500 m	N10°42.698′ W61°16.014′	Low	20	20
			1000 m	N10°42.422′ W61°15.892′	Mid	19	20
Caigual	2009 'recent' Travis *et al*. 2014	38/38	Introduction	N10°42.863′ W61°16.459′	Low	20	20
			0 m – Pre Intro	N10°42.768′ W61°16.289′	Low	19	0
			0 m	N10°42.768′ W61°16.289′	Low	20	20
			500 m	N10°42.741′ W61°16.104′	Low	20	20
			1000 m	N10°42.579′ W61°15.968′	Low	20	20
Taylor	2009 'recent' Travis *et al*. 2014	38/38	Introduction	N10°42.499′ W61°16.295′	Low	20	20
			0 m – Pre Intro	N10°42.472′ W61°16.277′	Low	18	0
			0 m	N10°42.472′ W61°16.277′	Low	20	20
			500 m	N10°42.418′ W61°16.096′	Low	20	20
			1000 m	N10°42.272′ W61°15.938′	Mid	20	20
Guanapo			5000 m*	N10°41.658′ W61°15.836′	High	20	20
Mainstem			Source†	N10°38.402′ W61°14.896′	High	20	20

*5000 m site for L. Lalaja, Caigual and Taylor.
†Source site for Turure, L. Lalaja, Caigual and Taylor.

with a lethal concentration of MS-222 and preserved individually in 7% formalin for later quantification of life-history traits (see below). Males were returned alive to their site of capture.

Characterising genetic divergence

To confirm high downstream gene flow from introduction sites, we characterised genetic variation, connectivity and population genetic structure within introduction streams at 10 neutral microsatellite loci (Table S1). Loci were selected to maximise overlap with previous studies that describe population genetic patterns in natural guppy populations (Crispo *et al*. 2006; Suk & Neff 2009; Baillie 2012). We genotyped all individuals, including native low-predation guppies sampled in three sites. DNA extraction, PCR conditions, estimates of genetic diversity and quality checking procedures are outlined in Appendix S1 and Table S1.

Natural guppy populations within a single drainage are typically genetically structured such that upstream headwater populations are more isolated, distinct, and have reduced genetic variation compared to downstream populations (Crispo *et al*. 2006; Weese *et al*. 2011; Baillie 2012). We assessed genetic differentiation among all sites within each stream from pairwise-F_{ST} values calculated in FSTAT 2.9.4 (Goudet 1995). F_{ST} is a population-level index ranging from 0 to 1, where low values indicate panmixia and higher values indicate increased differentiation among sites. We investigated spatial population structure along introduction streams using the Bayesian clustering algorithm STRUCTURE 2.2 (Pritchard *et al*. 2000). STRUCTURE analyses were performed separately for each introduction stream except all sites downstream from recent introductions within the Guanapo drainage were included in the same analysis because they share 5000 m and source sites. Admixture was assumed and the number of groups (k) ranged from one to the maximum number of sites within each stream, including source sites (Appendix S1). STRUCTURE analyses for Guanapo and Aripo introductions included the native guppies sampled in those streams either prior to introductions (Guanapo), or without upstream introductions (Aripo) to examine whether native fish were genetically distinct and whether the native genetic signature persists post-introduction.

Quantifying phenotypic traits

To assess adaptive divergence downstream from introductions, we quantified a suite of known fitness-related traits (colour, body shape and life history) from photographs and field-collected specimens. Polymorphic colouration of male guppies generally represents a local balance between sexual selection (females typically prefer more colourful males; Houde 1997) and predation intensity (more conspicuous males have higher mortality; Weese *et al*. 2010). Male colour was assessed with

an observer rank approach following Ruell *et al*. (2013), whereby individuals were visually ranked according to relative colouration. This method excels at producing a single comprehensive metric characterising qualitative differences in overall colouration resulting from the spatial interaction among diverse colour elements (i.e., specific colour/pattern combinations) and has been used to quantify colour in guppies (Ruell *et al*. 2013) and other taxa (e.g., Armenta *et al*. 2008). In this study, photographs of male guppies were randomly selected from each site and arranged on PowerPoint slides, such that each slide contained one photograph from each site within a stream (n = 20 slides per stream). Stream, site and fish identification were hidden from observers. Slideshows were presented in a dark room over the course of 1 day. Eight observers, ignorant of experimental design, but familiar with Trinidadian guppies, ranked fish for relative colouration based on four criteria: (1) number of different colours, (2) number of colour elements, (3) relative intricacy of colour elements and (4) relative size and brightness of colour elements. Observers assigned each fish a single ranking from 1 (least colourful) to 6 (most colourful). High repeatability of this method was confirmed by examining variation across observers and by duplicating the entire Taylor slideshow, unbeknownst to observers. We also obtained similar results using traditional colour outline analyses.

Guppy body shape varies somewhat predictably across environments (Hendry *et al*. 2006), influencing foraging ecology and swimming performance (Langerhans & Reznick 2010). We used geometric morphometrics to quantify variation in body shape among sites (Rohlf & Marcus 1993). Females were excluded from this analysis due to shape changes during pregnancy. Body shape of adult males was characterised by eight homologous landmarks and six semi-landmarks digitised with TPSDig2 (Rohlf 2010) from images of each specimen (Fig. S1). Raw coordinates were subjected to a Procrustes fit in MorphoJ whereby variation from position, orientation and isometric size is removed from the data (Klingenberg 2011). We performed between-group PCA with the Procrustes coordinates in R v3.1-108 (Mitteroecker & Bookstein 2011). Altogether, the first three PCA axes (PC1, PC2 and PC3) explained 93% of the total shape variation and were considered separate 'traits' for further analyses.

We measured a suite of life-history traits using photographs of males and field-preserved females following previously published methods (Reznick *et al*. 1996). Because male guppies have determinate growth, we estimated their size at maturity from photographs of adult fish. We extracted centroid size (square root of sum of squared distances of landmarks from their centroid) from the same landmarks used in morphometric analyses (Bookstein 1991). As female guppies bear live young, we measured three life-history traits from formalin-preserved females: number of offspring, offspring mass and reproductive allocation. Females were dissected under a microscope and embryos were counted and classified by developmental stage following Haynes (1995). After 1 week in a drying oven at 80°C, embryos and all non-reproductive tissue were weighed separately. To predict fecundity while controlling for female size, we used the common within-group slope but allowed intercepts to vary across sites. To estimate mean offspring mass, we divided total dry weight of the brood by the number of embryos. Reproductive allocation (proportion of the female's body mass dedicated to reproduction) was determined by dividing the dry weight of embryos by the sum of dry weight of embryos and non-reproductive tissue. Total embryo mass decreases as embryos consume yolk during development, and thus stage of embryo development was included as a covariate for calculating reproductive allocation and embryo mass.

Analysis of phenotypic divergence

If traits diverged according to predation regime, we would reject our hypothesis that gene flow completely constrains adaptive divergence. We tested this hypothesis with linear mixed effects models, where predation level (low, mid or high) was used as the fixed factor and stream and site were included as hierarchically nested random effects. We attempted to fit the maximal random effects structure (random intercepts and slopes; Barr *et al*. 2013) but were forced to simplify to the random-intercepts-only model to obtain convergence. Each trait was modelled individually using maximum likelihood, and significance of the predation effect was tested using likelihood ratio tests against the null model that included only random effects. Traits for which predation improved model fit were then re-fit with restricted maximum likelihood to obtain fitted values. Residual plots were used to determine whether model assumptions of normality and homoscedasticity were met. Embryo mass was log transformed and fecundity was square-root transformed to normalise the data prior to analysis. All models were carried out with package 'lme4' in R (Bates *et al*. 2009).

We next implemented a recently developed approach for classifying individuals with respect to a particular property (e.g., phenotypic traits, neutral genetic loci) to inform the degree to which populations overlap at these variables (Hendry *et al*. 2013). We evaluated exchangeability at neutral loci and phenotypic traits among native low-predation individuals from 0 m sites in Taylor and Caigual, individuals sampled from exactly the same sites post-introduction, and high-predation source individuals using discriminant analysis on principal components (DAPC) in R package 'adegenet' (Jombart *et al*. 2010). This method uses the full distribution of genotypes and phenotypes to evaluate the probability of classification of each individual into each sampled population and then uses the distribution of these classification probabilities to assess the level of exchangeability based on traits, genetic similarity, etc.

We used the exchangeability analysis to evaluate the extent that gene flow constrains adaptive divergence. If gene flow constrains adaptive divergence (i.e., if high-predation immigrants cause phenotypes in native low-predation populations to become more like the high-predation ecotype), we would expect low exchangeability, or 'misclassification', based on genetic markers between native and post-introduction populations (because high-predation immigrant genotypes will replace native genotypes) and low exchangeability among these populations based on traits (because high-predation phenotypes will replace native low-predation phenotypes). In con-

trast, we would expect post-introduction individuals that have experienced gene flow from the introduction site to overlap more with source individuals than with pre-introduction individuals at neutral genetic loci and possibly phenotypic traits, depending on the level of adaptive divergence.

We conducted one DAPC on genetic data using the 10 microsatellite loci and a second DAPC on four male phenotypic traits (male size, body shape – PC1, body shape – PC2 and body shape – PC3) that were measurable for both native and post-introduction individuals based on photographs. Ordination plots for genetic and phenotypic DAPCs were examined, and for each population, we calculated mean and 95% confidence intervals for the proportion of classifications into all other populations.

RESULTS

Genetic divergence

Multilocus genotype data from 1019 individuals (67 native and 953 from post-introduction sites) revealed extensive downstream gene flow from introduction sources in all streams. Assumptions of neutrality were met, loci were polymorphic (Table S2) and genotyping error rate was low (< 0.05%). Allelic richness and heterozygosity were universally high throughout recent introductions and increased in downstream sites sites of old introduction streams (Table S2). However, compared to native populations (average heterozygosity: 0.25), introduced populations and all those downstream from introductions had much higher levels of genetic variation (0.67). Genetic differentiation among sites from introduction streams was low: average pairwise-F_{ST} was 0.03, ranging from 0.01 to 0.12 (Fig. 3a; Table S3). In contrast, average level of genetic differentiation between natural sites before or without an upstream introduction was 0.21 and ranged from 0.07 to 0.27 (Fig. 3a).

STRUCTURE analyses revealed varying degree of fine-scale population structure associated with age of introduction. Although all introduction streams show universally high genetic connectivity based on low F_{ST} values, sites from older introductions exhibited more genetic partitioning than sites from recent introductions (Fig. 3b). Native populations sampled before or without upstream introductions clustered in genetic groups distinct from post-introduction sites, regardless of age of introduction.

Phenotypic divergence

Including predation level as a predictor usually improved the fit of our mixed models of phenotypic variation (Fig. 4, Table S4). Most traits were significantly affected by predation level, and variation in male colour, male size at maturity, and embryo mass matched the predicted adaptive direction (Fig. 4). Specifically, our results matched expectations that guppies from low-predation environments will be more colourful, reach a larger size at maturity and produce heavier embryos than their high-predation counterparts. Reproductive allocation and fecundity also showed significant variation with respect to predation, but did not match the expected direction across the predation gradient. Instead, we found that guppies

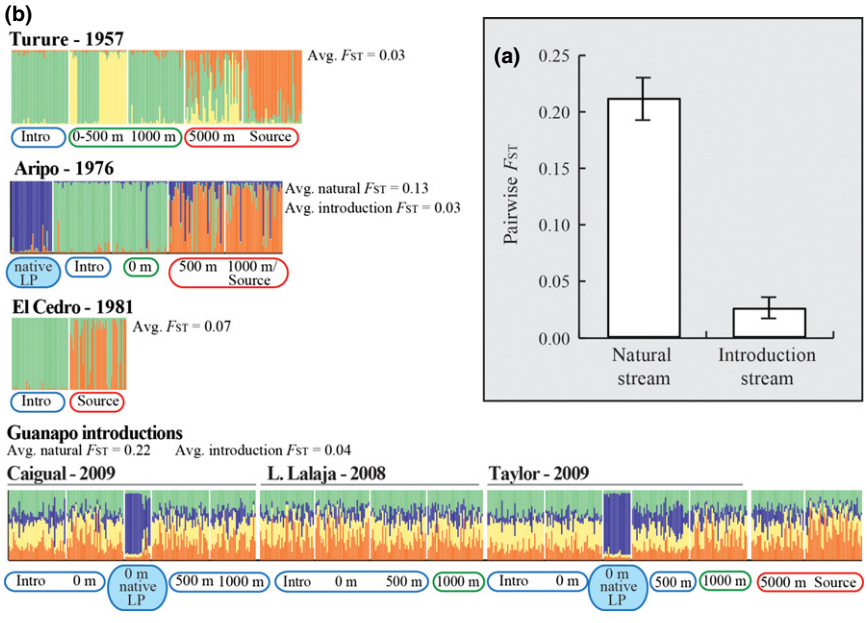

Figure 3 (a) Comparison of genetic differentiation (pairwise-F_{ST}) among all sites in natural streams vs. among all sites in streams after introductions took place. (b) Within stream STRUCTURE plots and average pairwise-F_{ST} values for all six streams that experienced an upstream introduction. Each line in the plots corresponds to an individual with colours representing the proportion of an individual's genotype assigned to a given genetic cluster. Old introductions show fine-scale genetic structure despite low genetic divergence (low F_{ST}). All sites from the three recent introductions conducted in the Guanapo drainage were included in the same analysis because they share the 5000 m and source sites. These recent introductions are more genetically homogeneous, with the exception of pre-introduction 0 m sites in Taylor and Caigual (shaded in blue) that are very distinct and genetically divergent (high F_{ST}) from the rest of the sites. Colored circles on the x-axes indicate the predation level at each site: blue = low, green = mid, red = high as defined in Fig. 1. All plots represent the (k) number of genetic clusters with the highest support (see Appendix S1).

sampled in mid-predation sites generally had higher female reproductive allocation. In addition, fecundity in low-predation environments was higher than high-predation populations, contrary to expectations of fewer, larger offspring in low-predation sites. The first two PC axes of male body shape did not show a significant predation effect (Table S4). However, the third PC axis was significantly affected by predation in the adaptive direction, with a ventral shift in mouth orientation (higher PC3 score) favoured in low-predation environments (Fig. 4).

Ordination plots from the DAPC exchangeability analyses showed differing levels of genetic and phenotypic similarity among individuals from the native low-predation population, the same site sampled several generations post-introduction, and the introduction source (Fig. 5). The DAPC on genetic data confirmed greater genetic similarity between individuals from the source site and those from the 0 m sites post-introduction, whereas native individuals sampled prior to the introduction were genetically distinct (Fig. 5a). Individual misclassification was generally low using genetic data; however, post-introduction and source populations were more exchangeable with each other than with the native populations. Conversely, the same analysis using phenotypic data reveals clustering by predation regime, regardless of population origin, and individuals from low-predation sites showed a high proportion of misclassification. Thus, native and post-introduction populations were highly exchangeable using phenotypic data (Fig. 5b).

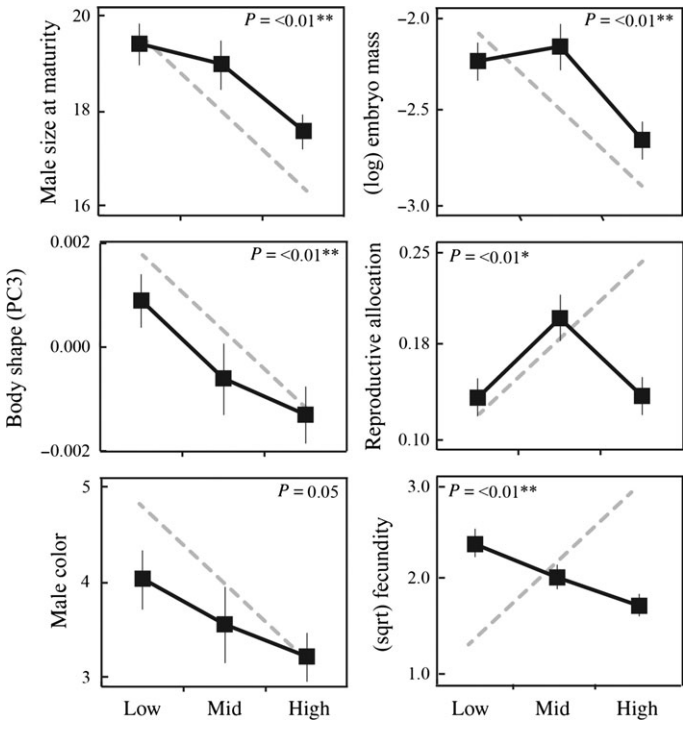

Figure 4 Mean values (± SE) of phenotypic traits that vary by predation level based on linear mixed effects models. Dashed grey lines indicate the expected adaptive direction of the trait across the predation gradient based on prior studies, but not the slope or actual trait values. Site was a nested random effect within stream in all models. *$P < 0.01$; **$P < 0.001$

DISCUSSION

Gene flow between adaptively divergent populations potentially threatens local genetic signature and may breakdown local adaptation. Alternatively, if natural selection is strong, and sufficient genetic variation exists, gene flow from adaptively divergent immigrants may do little to constrain local adaptation and could even rescue small populations or speed up adaptive evolution by increasing the 'working surface' of natural selection. Predicting the outcome of the interaction between gene flow and adaptive divergence remains difficult despite its importance for understanding the evolution of populations and, in some cases, how to best conserve them. Our study demonstrates two novel results in this respect. First, as predicted based on previous studies of gene flow in guppies, we documented repeated and extensive genetic homogenisation from introduced populations over a remarkably short time frame. Second, contrary to the hypothesis that gene flow substantially constrains adaptation, phenotypic divergence along a steep ecological gradient was maintained for multiple traits, despite high gene flow from introduced populations. These findings were consistent in all introduction replicates, providing strong evidence that gene flow did not overwhelm adaptation. Indeed, the additional genetic diversity may have even bolstered fitness within recipient populations.

Elevated gene flow downstream from introductions

Our genetic results provide evidence that higher than natural levels of gene flow has occurred from each of the introduced populations throughout all downstream distances. Consistent with an infusion of immigrant alleles, we found high levels of genetic variation in all sites downstream from introduced populations compared to native populations (Table S2). Second, we observed low genetic differentiation throughout all streams, and high similarity to source populations, indicating that these sites have experienced genetic connectivity in the recent past. For example, pairwise-F_{ST} between the site furthest downstream from the Turure introduction and its source population (Guanapo), sites that are located in geographically distinct east- and west-flowing basins, is an order of magnitude lower than typical levels of divergence between populations from these highly divergent basins (Baillie 2012). Due to non-equilibrium conditions of recent gene flow into isolated populations, F_{ST} cannot be used to infer the rate of gene flow *per se*. However, F_{ST} is an appropriate index of genetic differentiation among populations (Whitlock & McCauley 1999), which we can use to compare to population pairs of equivalent distance in streams without introductions. Indeed, the level of genetic divergence among sites was dramatically lower within introduction streams than natural levels of within-stream divergence, suggesting high connectivity throughout all introduction streams (Fig. 3a). Third, although STRUCTURE analyses (which are more sensitive than F_{ST} for identifying fine-scale genetic differences) uncovered subtle fine-scale population structure in old introduction streams, they show genetic homogeneity throughout the recent introductions within the Guanapo drainage (Fig. 3b). The genetic uniformity of individuals from introduction sites, the Guanap-

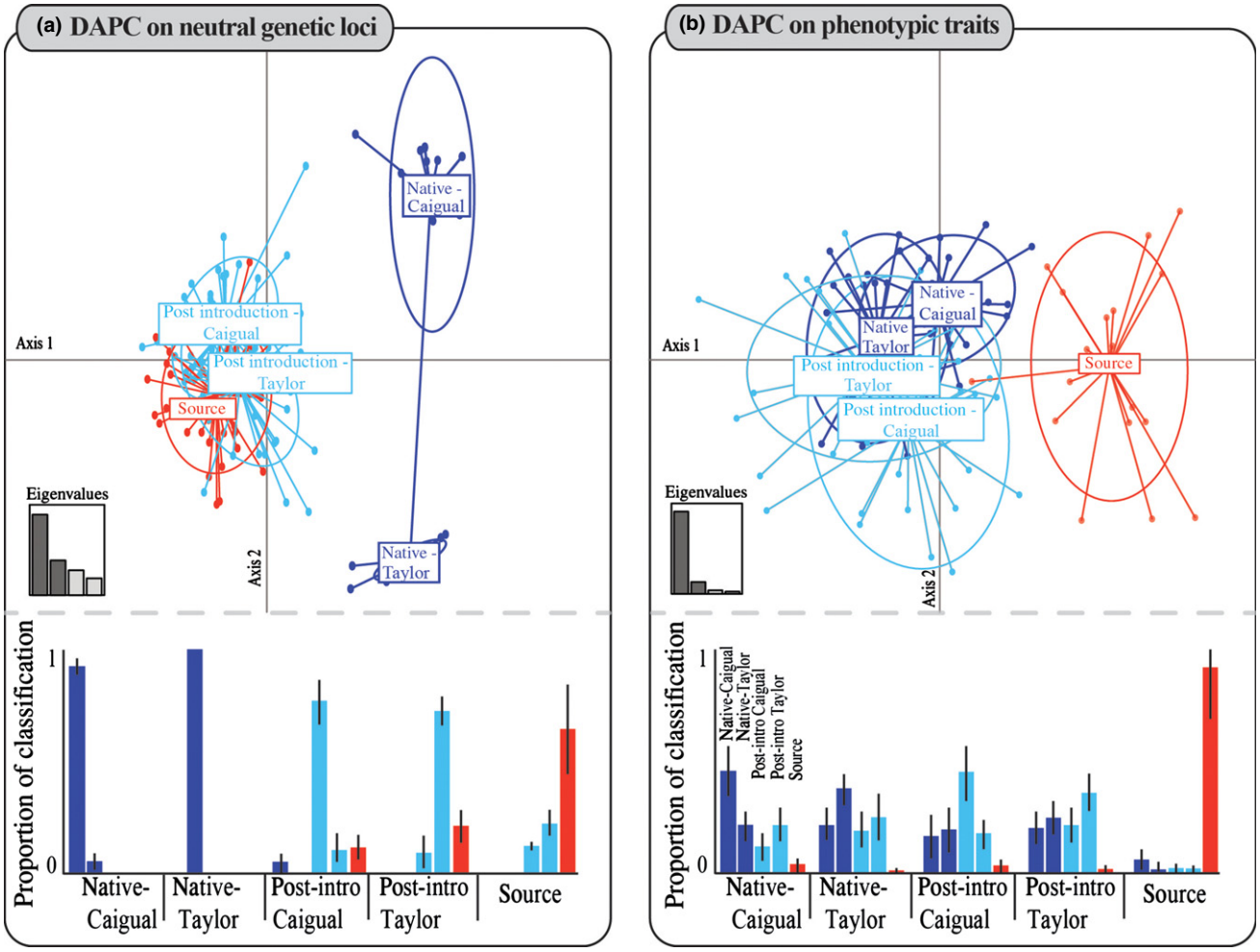

Figure 5 Ordination plots and group classification based on discriminant analysis of principal components (DAPC) for neutral genetic loci (a) and phenotypic traits (b). Colours correspond to *a priori* groups based on population origin: native low-predation in purple, the same sites post-introduction in blue and introduction source in red. Bar graphs below the dashed line show the mean (and 95% CIs) proportion of individuals from each population classified into each population. Each bar represents the classification of the population on the *x*-axis, as labelled for one set of bars in (b). The bottom-left insets show eigenvalues of the four principal components in relative magnitude.

o source population and all sites downstream is in stark contrast to the high genetic structure found between upstream native populations sampled before the introductions took place, and suggests high gene flow downstream from introduction sites on a rapid timeframe.

Differences in genetic structure between old and recent introduction streams attest to processes that naturally structure guppy populations, despite initially high gene flow from introduction sites. Total genetic differentiation based on F_{ST} remains low between all introduction sites and their source populations (Table S3), yet STRUCTURE analyses split all old introduction and source sites into distinct genetic clusters (Fig. 3b). We also discovered a downstream trend of increasing within-population genetic variation in old introduction streams (Table S2), which mirrors typical patterns of guppy gene flow in un-tampered streams (Crispo *et al.* 2006). Previous work shows that downstream rather than upstream gene flow is more common due to waterfall barriers and the direction of flow limiting upstream dispersal (Crispo *et al.* 2006), but also that male guppies moving from low-predation to high-predation sites have greater predator-induced mortality (Weese *et al.* 2011), which could decrease overall levels of downstream gene flow and contribute to the isolation of upstream populations. Over 100 guppy generations have elapsed since the old introductions occurred, a timeframe in which it is reasonable to expect the natural processes of genetic drift and restricted gene flow to cause genetic structure at neutral loci (Allendorf & Phelps 1980), likely explaining observed differences in genetic variation and structure.

Phenotypic divergence maintained despite extensive gene flow

If high downstream gene flow had swamped local adaptation, we expected a lack of phenotypic divergence across the predation gradient. Rather, we documented significant trait variation across the predation gradient, generally in adaptive directions predicted by extensive prior work on this system (Fig. 4, Fig. S2). Despite rapid and extensive gene flow from initially maladapted populations, males in low-predation environments tended to be more colourful, mature larger, have

ventrally shifted mouths, and gravid females had larger embryos, compared with those in high-predation environments. The two traits that did not completely parallel the expected adaptive direction (fecundity and reproductive allocation) are exactly those known to be most affected by seasonality (Reznick 1989). Female guppies tend to devote less energy to reproduction during the wet season (May–December) when resources are low (Reznick 1989). Our samples were collected at the start of the dry season, when females were likely still recovering from wet season conditions. Another possibility is that certain traits of high-predation guppies genuinely dominate and persist in post-introduction populations. Native guppies in low-predation environments likely show decreased fecundity due to physiological costs of producing larger offspring, not because selection favours fewer offspring. If, through higher levels of genetic variation, heterosis or transgressive segregation, immigrants or hybrids are physiologically able to produce larger embryos (as favoured in low-predation environments) but still retain high fecundity, this 'super' phenotype could be selectively favoured and contribute to the spread of introduced alleles.

Native individuals from two low-predation sites sampled prior to introductions provided direct comparisons of natural and post-introduction populations in terms of genetic and phenotypic divergence. Our analyses of genetic and phenotypic exchangeability revealed that ~12 generations after transplantation and gene flow within a low-predation environment, descendants of guppies from a high-predation site clustered with the native population in multidimensional trait space, showing high phenotypic exchangeability despite neutral genetic divergence (Fig. 5). Although traits in this analysis were limited to male size and shape axes, both size and morphological features that affect swimming performance are known to vary based on the environment, affect guppy fitness, and thus are likely under selection.

Adaptive evolution or phenotypic plasticity?

Phenotypic divergence across the predation gradient may have evolved in direct response to the environment if there is a genetic basis to the observed variation, or may represent a plastic response to environmental differences. We are unable to directly parse the relative contribution of phenotypic plasticity and adaptive evolution to observed trait divergence, but both processes are likely at play. Phenotypic plasticity is known to occur in guppies (Reznick & Bryga 1987; Torres-Dowdall *et al.* 2012b; Ruell *et al.* 2013), and to contribute to the establishment and persistence of populations in new environments (Ghalambor *et al.* 2007). However, previous common garden experiments have also documented a genetic basis for the same traits we measured (Table S5), and results from pre- and post-gene flow common garden studies suggest that gene flow causes genetically based changes in traits in two of our sites (Handelsman and Fitzpatrick, *unpublished data*). Thus, although plasticity likely plays a role, prior evidence of the genetic basis and rapid evolution of these traits, facilitated by strong selection and short generation times, suggests that adaptive evolution is also a process maintaining phenotypic divergence in the face of gene flow.

Adaptive trait divergence can also persist, despite homogenisation at neutral markers, through differential introgression across the genome (Soria-Carrasco *et al.* 2014). Selection will most strongly impact genomic regions that affect or are tightly linked to ecologically important traits. Simultaneously, homogenising effects of gene flow may continue throughout the rest of the genome at neutral or nearly neutral loci (Via 2009). Thus, what appears as near displacement of the native genotype based on neutral microsatellite loci may not be representative of the entire genome if locally adapted native loci or genomic regions are maintained by strong selection. Indeed, theoretical models of the introduction scenario studied here found that selection reduced gene flow at selected markers but not at unlinked neutral markers (Labonne & Hendry 2010).

Conservation implications

Predicting immigrant success and assessing their impact on native populations is a core goal of conservation biology as fragmentation leaves some populations isolated and in need of assisted gene flow, while incidental invasions and climate-induced range shifts result in other, distinct taxa coming into contact (Allendorf *et al.* 2001). In our system, the repeated success of translocated guppies appears to be a combination of 'invasive traits', mating system, genetic factors and the environment. Life-history traits such as high fecundity and a promiscuous mating system in which females prefer novel males likely contributed to the aggressive spread of introduced guppies. Furthermore, although introduced populations experienced initial founder effects (shown by loss of genetic diversity in introduction sites compared to the source population), standing genetic variation in source populations greatly exceeded that of native low-predation populations (Table S2). This characteristic of small, potentially inbred populations could render them vulnerable to invasion and predisposed to benefiting from gene flow. Finally, fitness of translocated individuals obviously depends on selective factors faced in their new environment. Previous reciprocal introductions (i.e., moving low-predation guppies into high-predation environments) revealed high mortality of low-predation guppies (Weese *et al.* 2011), so immigrant success in this system is one-way: populations that experience release from predation are able to persist and spread, even if initially maladapted to the new environment.

Summary

Our study demonstrates a replicated scenario where genetic homogenisation has not necessarily diminished adaptive divergence, as locally adapted phenotypes were maintained despite extensive immigrant gene flow. We caution that this scenario is likely most applicable to conspecific populations where selection for a local ecotype is strong, recipient populations are inbred, and possibly where phenotypic plasticity exists for rapid response. In addition, organisms with mating systems that prevent or slow accumulation of reproductive barriers between divergent populations may be less prone to outbreeding depression. We note that the spread of immi-

grant alleles was rapid and extensive, likely resulting in extinction of pure local genotypes. Whether such losses of native genetic signature represent a true detriment must be regarded as case specific; the costs may be outweighed by infusion of new genetic variation as with Florida panthers and the guppy case examined here. Predicting fitness effects of gene flow is imperative, as maintaining and restoring healthy ecosystems will rely on our ability to manage micro-evolution in the face of climate change and altered patterns of connectivity.

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AUTHORSHIP

WCF, LMA and SWF designed the study and collected the field data. JCG, JAK and SWF collected and analysed phenotypic trait data. SWF performed genetic analyses and wrote the first draft of the manuscript. All authors contributed substantially towards revisions.

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