DISSERTATION

TESTING THE EFFECTS OF GENE FLOW ON ADAPTATION, FITNESS, AND DEMOGRAPHY IN WILD POPULATIONS

Submitted by

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ABSTRACT

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Gene flow should reduce differences among populations, potentially limiting adaptation and population growth. But small populations stand to benefit from gene flow through genetic and demographic factors such as heterosis, added genetic variation, and the contribution of immigrants. Understanding the consequences of gene flow is a longstanding and unresolved challenge in evolutionary biology with important implications for conservation of biodiversity. My dissertation research addresses the importance of gene flow from evolutionary and conservation perspectives.

In the first study of my dissertation I characterized natural patterns of gene flow and genetic diversity among remaining populations of Arkansas darters (*Etheostoma cragini*) in Colorado, an endemic to drying streams of the Great Plains, and a candidate for listing under the US Endangered Species Act. I found low diversity and high isolation, especially among sites with low water availability, highlighting this as a species that might eventually benefit from a well-managed manipulation of gene flow.

I then turned to the Trinidadian guppy system to test the effects of gene flow using a model species for studying evolution in natural populations. My work capitalized on a series of introduction experiments that led to gene flow from an originally divergent population into native recipient populations. I was able to characterize neutral genetic variation, phenotypic variation, and population size in two native populations before the onset of gene flow. The goal

of my first study using this system was to evaluate the level of gene flow and phenotypic divergence at multiple sites downstream from six introduction sites. I found that traits generally matched expectations for local adaptation despite extensive homogenization by gene flow at neutral loci, suggesting that high gene flow does not necessarily overwhelm selection. I followed up on this study by measuring many of the same traits in a common garden environment before and after gene flow to test whether gene flow caused genetically based changes in traits, and to evaluate the commonly held *'gene flow constrains divergence'* hypothesis versus the *'divergence in the face of gene flow'* hypothesis. I found that gene flow caused most traits to evolve, but whether those changes constrained adaptation depended on initial conditions of the recipient population.

Finally, to link gene flow to changes in fitness and demography I conducted a large-scale capture-mark-recapture survey of two native populations beginning three months prior and following 26 months after upstream introductions took place. I genotyped all individuals from the first 17 months of this study to compare the relative fitness (survival and population growth rate) of native, immigrant, and hybrid guppies. In total this survey spanned 8-10 guppy generations and documented substantial increases in genetic variation and population size that could be attributed to gene flow from the introduction site. As a whole, the results from my research suggest that gene flow, even from a divergent population, can provide major demographic benefits to small populations, without necessarily diminishing locally important traits.

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1. INTRODUCTION

The nature of connectivity among wild populations can determine the ecological role and evolutionary trajectory of species. It is also a powerful reflection of the ways in which organisms use and are limited by their environment. Humans have altered many natural connectivity patterns by fragmenting landscapes, or causing biological invasions (Barrett, 2014), and the changing climate is expected to further impact connectivity (Crispo et al., 2011; Le Galliard et al., 2012). Understanding the extent to which populations are connected, and the ecological and evolutionary consequences of altering these patterns are crucial challenges for the conservation of biodiversity. These challenges provided the motivation for my dissertation research.

Dispersal, defined broadly as any movement of individuals or propagules across space (Ronce, 2007), is the ecological process through which populations are connected, and is the cause of gene flow when individuals reproduce and alter allele frequencies at a new location. Gene flow (i.e., the transfer of genetic material between populations) is one of the four classical mechanisms of evolutionary change, along with mutation, drift, and selection (Wright, 1931). Increasingly, the roles of gene flow, drift, and selection are appreciated as being relevant on an ecological timeframe, as they can determine the contemporary dynamics and fate of wild populations (Pelletier et al., 2007; Saccheri and Hanski, 2006). Although an understanding of abundances and distributions of organisms was intertwined with early development of evolutionary theory (Darwin, 1859; Fisher, 1930; Malthus, 1798; Wallace, 1858), empirically linking evolution and demography is a relatively recent endeavor with many remaining unanswered questions (Kokko and López-Sepulcre, 2007). For example, predicting demographic consequences of gene flow is a major challenge (Tallmon et al., 2004; Whiteley et al., 2015),

especially when interacting effects of drift and selection are also involved (Kinnison and Hairston, 2007).

In the absence of gene flow, small populations are subject to increased probability of mating among relatives, which can result in accumulation and fixation of deleterious alleles, and lead to reduced fitness (Keller and Waller, 2002). Negative fitness consequences of inbreeding have been consistently documented across taxa (Crnokrak and Roff, 1999), and directly linked to higher extinction risk in butterflies (Saccheri et al., 1998), *Drosophila* (Bijlsma et al., 2000), and plants (Newman and Pilson, 1997). Thus, demographic and fitness consequences caused by a *lack* of gene flow are widely appreciated. But, those same fitness consequences in response to the *occurrence* of gene flow are heavily debated.

Gene flow: the enigmatic evolutionary force

Traditionally, gene flow was considered as the "evolutionary glue" that held species together (Mayr, 1963). High natural rates of gene flow were assumed, and it was reasoned that gene flow should be the primary source of genetic variation for natural populations. But, Ehrlich and Raven (1969) argued that gene flow in nature was more restricted than commonly thought and might not be the cohesive force holding species together that Mayr (1963) advocated. In fact, they predicted that gene flow would eventually be discovered to play an insignificant role in evolution. After four decades of subsequent research, the contemporary view generally regards gene flow as indeed playing a significant, yet idiosyncratic role in the evolution of natural populations (Ellstrand, 2014; Garant et al., 2007).

Much theoretical attention has focused on the role of gene flow in constraining adaptive divergence (e.g., Garcia-Ramos and Kirkpatrick, 1997; Haldane, 1948; Hendry et al., 2001; Lenormand, 2002). Indeed, in the absence of drift and selection, gene flow will homogenize

allele frequencies and cause populations to become genetically and phenotypically similar (Slatkin, 1987). In nature, support for this homogenizing role of gene flow is inferred through the commonly documented inverse relationship between amount of gene flow and phenotypic divergence (e.g., Calsbeek and Smith, 2003; Hendry and Taylor, 2004), but studies that experimentally isolate gene flow as a constraint for adaptive divergence are rare (Nosil, 2009; Riechert, 1993). More recently, surprising levels of phenotypic divergence have been documented in the face high gene flow (Hendry et al., 2000; Hoekstra et al., 2004; Moody et al., 2015), suggesting that gene flow does not play a *purely* constraining role. Added genetic variation may actually enhance adaptation through increasing the efficacy of natural selection (Carlson et al., 2014; Swindell and Bouzat, 2006). The fitness benefits of gene flow to small populations could be additionally enhanced through heterosis, or a recovery from genetic load of inbreeding; a finding that has been experimentally shown in several natural and laboratory populations (Ebert et al., 2002; Pickup et al., 2013; Richards, 2000). At the population level, these fitness benefits from gene flow can cause "genetic rescue", an increase in population growth owing to the infusion of new alleles (Tallmon et al., 2004), through adaptive evolution, heterosis, or both.

Problems and promises of gene flow in conservation

Genetic factors associated with connectivity are at the heart of many issues in conservation biology. Many species with historically continuous distributions are now restricted to small and isolated patches (Fahrig, 2003). In other cases, isolated populations or species are brought into contact through biological invasions and climate-induced range shifts (Crispo et al., 2011). Hybridization between different species is a global concern for biodiversity loss (Allendorf et al., 2001; Muhlfeld et al., 2014). For example, in the Pacific Northwest, expansion of the Barred

Owl range and hybridization with the Northern Spotted Owl has led to concerns about the persistence of one of the most iconic species in conservation policy-making (Haig et al., 2004).

At the intraspecific level, issues of gene flow and conservation are muddled. Certainly, maintaining native genotypes is important for preserving unique evolutionary lineages, but at what cost? Without an adequate demographic buffer to withstand stochastic environmental disturbances, or enough genetic variation to adapt to a changing climate, small populations may increasingly face high extinction risk. Artificially induced gene flow resulting in genetic rescue could provide a powerful solution for buffering imperiled populations in the short term (Aitken and Whitlock, 2013; Edmands, 2007; Frankham, 2015; Whiteley et al., 2015). Already, genetic rescue has successfully caused the rebound of high profile species such as the Florida panther (Johnson et al., 2010) and Rocky Mountain bighorn sheep (Hogg et al., 2006). However, use of this management strategy remains controversial and perhaps under-utilized due to concerns that outbreeding depression will cause reduced fitness of offspring between genetically divergent parents (Frankham et al., 2011).

Isolated in the headwaters: gene flow quandaries of fishes in headwater streams

Species that occupy tributaries of headwater streams highlight the complex challenges with regards to population connectivity (Campbell Grant et al., 2007). Dispersal through dendritic stream networks tends to be hierarchical and unidirectional, which can isolate headwater populations (Fagan, 2002). These isolated populations often harbor unique alleles that increase overall genetic diversity of the species (Lowe and Likens, 2005), and in theory, without the homogenizing effects of gene flow they have the potential to become strongly locally adapted (Lenormand, 2002). But, limited migration could also leave these populations stranded at high risk for experiencing negative fitness consequences of inbreeding. Often, headwater stream

species exist in metapopulations, where connectivity is imperative for replenishing sink populations and colonizing new habitats (Hanski, 1998). Thus, altered patterns of connectivity and habitat fragmentation in dendritic networks have arguably more severe consequences than other systems, and may render stream-restricted species more vulnerable to extinction (Perkin and Gido, 2012).

Dissertation objectives: understanding gene flow in applied and model systems

My dissertation research aims to fill gaps in our understanding of the complex role of gene flow in nature. I focused on two species of freshwater fish that occupy headwater streams to test a variety of questions about gene flow. The first species represents an applied system; Arkansas darters (*Etheostoma cragini*) are native to the Great Plains, and a candidate species for listing under the US Endangered Species Act. The second species, Trinidadian guppies (*Poecilia reticulata*), is a model system for studying evolution in the wild.

My first study (**Ch.2**) characterized natural patterns of genetic diversity and connectivity among populations of Arkansas darters (*Etheostoma cragini*) in Colorado. I found overall low levels of genetic diversity and connectivity, but the variation that did exist was associated with habitat features related to water availability. These were the same habitat variables found to best predict darter occupancy in a previous study (Groce et al., 2012), suggesting that the drying expected to worsen in southeastern Colorado could threaten both genetic and demographic factors necessary for long term persistence. My results also showed little evidence of hatchery genotypes persisting in the wild, despite heavy augmentation efforts to a few natural darter populations. Stepping back to consider the situation facing Arkansas darters in Colorado (i.e., low diversity, low connectivity, reliance on water in a drought-stricken region, and poor

augmentation success), how could smart management of evolutionary processes on a contemporary timeframe contribute to maintaining healthy populations in Colorado?

To inform this hypothetical question, whether it applies to a species of conservation concern, or basic evolutionary ecology, I turned to the Trinidadian guppy system. The advantage of working with a model system is the opportunity to build from a wealth of knowledge previously compiled for the species. For guppies, we have a good understanding of the distribution of phenotypic traits and how they relate to fitness in a given environment (i.e., Endler, 1980; Reznick et al., 1996), geographic patterns of genetic diversity (Baillie, 2012; Barson et al., 2009; Crispo et al., 2006), mating system (Houde, 1997; Houde and Endler, 1990), and many other features of their biology and environment (Magurran, 2005). Much of our understanding about rapid adaptation in the guppies is due to a series of translocation experiments where guppies adapted to localities with many predators were introduced to headwater stream habitats above waterfall barriers that were previously lacking guppies and most predators (Endler, 1980; Reznick and Endler, 1982; Travis et al., 2014).

I took advantage of the opportunity offered by these introduction experiments to test the effects of gene flow on locally adapted traits, fitness, and population dynamics. First, I determined the overall extent of gene flow downstream from all introduction sites, and evaluated whether gene flow constrained local adaptation by measuring a suite of known fitness related traits in multiple downstream populations (**Ch. 3**). I then focused on two native populations that occurred in low predation headwater tributaries downstream from the most recent introduction experiments (conducted by David Reznick and colleagues in 2009), quantifying traits, genetic variation, and population sizes in these native populations just prior to upstream introductions. I transported guppies from these sites to Colorado State University in 2009 and again in 2011 to

measure traits in a common garden environment before and approximately 10 generations after the onset of gene flow (**Ch.4)**. This experiment allowed me to test the extent to which gene flow caused genetically based changes in quantitative traits. Finally, to test the effects of gene flow from the originally phenotypically and genetically divergent source on fitness and demography, I conducted a large-scale capture-mark-recapture survey in which I monitored changes to genetic composition, vital rates, and population dynamics of the two focal populations for multiple generations after gene flow (**Ch.5**).

Conclusions and significance

The overall goal of my dissertation was to empirically test how gene flow shapes genetic and phenotypic evolution, and to link those changes to fitness and demography. The results from my darter study revealed how the landscape can impact patterns of connectivity, and provided a case study for the genetic and demographic challenges of an isolated headwater species. My work on guppies illuminated mechanisms for how gene flow affects evolution and fitness. The finding that locally adapted traits were generally maintained despite high levels of neutral gene flow corroborated the guppy paradigm that similar phenotypes are strongly selected for across the predation gradient. What was surprising was the extent and pace that gene flow from introductions had washed out neutral genetic structure downstream from recent introductions, yet phenotypic divergence was maintained even in this drainage. My common garden experiment provided insight to whether this divergence was maintained by plasticity or evolution, showing that genetically based shifts in traits were generally in the direction of the divergent source population. Thus, interactions between gene flow, plasticity, and selection are likely causing the observed trait patterns in the wild. Finally, my long-term individual-based monitoring study provided a window to the genetic and demographic mechanisms of major demographic change

caused by gene flow. I documented genetic rescue in both streams, given that substantial increases in genetic diversity and population size were due in part to hybrid success. However, high rates of gene flow in one population led to a potentially worrisome outcome, namely, the near extinction of the "pure" native genotype.

This body of work sheds light on the role of gene flow as an important force determining the evolution and dynamics of (especially small) populations. In general, the benefits of gene flow seemed to outweigh the negative consequences. Although neutral genetic differentiation was greatly diminished, and gene flow caused genetically based constraint to some traits in a common environment, locally adaptive phenotypic differentiation was maintained in the wild. From a conservation standpoint, the combination of demographic and genetic rescue would be considered a success, given the dramatic boost in population sizes. Although many caveats and questions remain, my work highlights how gene flow is an important evolutionary force that can greatly influence the ecology of populations.

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2. WATER AVAILABILITY STRONGLY IMPACTS POPULATION GENETIC PATTERNS OF AN IMPERILED GREAT PLAINS ENDEMIC FISH[1]

Summary

Genetic, demographic, and environmental processes affect natural populations synergistically, and understanding their interplay is crucial for the conservation of biodiversity. Stream fishes in metapopulations are particularly sensitive to habitat fragmentation because persistence depends on dispersal and colonization of new habitat but dispersal is constrained to stream networks. Great Plains streams are increasingly fragmented by water diversion and climate change, threatening connectivity of fish populations in this ecosystem. We used seven microsatellite loci to describe population and landscape genetic patterns across 614 individuals from 12 remaining populations of Arkansas darter (*Etheostoma cragini*) in Colorado, a candidate species for listing under the U.S. Endangered Species Act. We found small effective population sizes, low levels of genetic diversity within populations, and high levels of genetic structure, especially among basins. Both at- and between-site landscape features were associated with genetic diversity and connectivity, respectively. Available stream habitat and amount of continuous wetted area were positively associated with genetic diversity within a site, while stream distance and intermittency were the best predictors of genetic divergence among sites. We found little genetic contribution from historic supplementation efforts, and we provide a set of management recommendations for this species that incorporate a conservation genetics perspective.

[1] Fitzpatrick, S.W., Crockett, H., and W.C. Funk. (2014) Water availability strongly impacts population genetic patterns of an imperiled Great Plains endemic fish. *Conservation Genetics* 15:771-788

Introduction

Solving complex conservation problems calls for integration across scales of time and space. Wildlife and fisheries management has traditionally focused on the demographic properties of populations, however determining which type of action is best-suited for the target species requires understanding the interplay between demography, genetics, and the environment (Lande 1988; Frankham 2005; Fagan and Holmes 2006). Recent studies have shown that ecological features that drive metapopulation dynamics, such as habitat area and connectivity, can also affect spatial genetic structure (Cosentino et al. 2011). Genetic diversity is often a useful predictor of abundance (McCusker and Bentzen 2010), but census size can be a poor predictor of effective population size (Luikart et al. 2010), indicating that processes underlying patterns of demography and genetics are not always similar. Ultimately, an understanding of the feedbacks between demography and genetics suggests that a loss of genetic variation through inbreeding and drift can reduce fitness, exacerbate population decline, and increase vulnerability to environmental stochasticity (Mills and Smouse 1994; Saccheri et al. 1998). Thus calling for conservation strategies that include a joint understanding of how demographic and genetic processes interact to affect overall population and metapopulation dynamics (Vander Wal et al. 2013).

Fishes that inhabit tributaries of fragmented stream networks are particularly prone to negative fitness consequences owing to the interaction between demographic, genetic, and environmental factors (Gaggiotti and Hanski 2004; Campbell-Grant et al. 2007; Labonne et al. 2008). Populations constrained to dendritic networks face hierarchical variation in climate, habitat quality, and ecological processes (e.g., dispersal, population growth, and community interactions), resulting in more severe consequences from disturbance and fragmentation (Fagan

2002; Benda et al. 2004). Studies of fishes inhabiting stream networks show that connectivity is influenced by both natural and anthropogenic landscape features (Meeuwig et al. 2010; Kanno et al. 2011), and that even a little fragmentation in dendritic networks can substantially increase local extinction risk by isolating upstream populations and reducing the potential for recolonization (Unmack 2001; Fagan et al. 2002; Letcher et al. 2007).

The North American Great Plains biome is one of the most imperiled on the continent (Samson et al. 2004). Loss of terrestrial prairie habitat and biodiversity has received much attention, but prairie streams and rivers are also highly impacted by anthropogenic modifications (Dodds et al. 2004). Although Great Plains streams are naturally dynamic, subject to intermittent flooding and climatic variation, anthropogenic impacts have severely altered the hydrologic regimes of these ecosystems (Dodds et al. 2004). Groundwater mining, diversions, and reservoirs have greatly increased habitat intermittency and drying (Falke et al. 2011). Thus, while most Great Plains stream biota is adapted to harsh environmental conditions, the level of anthropogenic disturbance to this habitat is beyond the limit of what many local species can tolerate (Fausch and Bestgen 1997; Samson et al. 2004).

The Arkansas darter (Percidae: *Etheostoma cragini*; Gilbert 1885) is one such Great Plains fish that is threatened by anthropogenic impacts to its stream habitats. Throughout their range, Arkansas darters occur primarily in isolated populations within headwaters of plains tributaries to the Arkansas River (Miller 1984). Once widely distributed in tributaries of the Arkansas River from southwest Missouri to central Colorado (Kuehne and Barbour 1983; Page 1983), Arkansas darters have declined in abundance and now occur in fragmented populations throughout their range, warranting protection in every state in which they occur (Miller 1984; Eberle and Stark 2000; Hargrave and Johnson, 2003; Groce et al. 2012). Due to range-wide

population declines and ongoing threats to its habitat, the Arkansas darter is a candidate for listing under the U.S. Endangered Species Act.

In eastern Colorado, the western-most part of their range, Arkansas darters are currently only found consistently in approximately 10 stronghold sites out of 50 locations at which Arkansas darters have been collected within the last twenty years (Colorado Parks and Wildlife, *unpublished data*). That being said, over the course of 30 years of monitoring by Colorado Parks and Wildlife (CPW), many specific sites are occupied by Arkansas darters some years and unoccupied in others, suggesting that this species exists in one or more metapopulations (Harrison 1991; Labbe and Fausch 2000), which is typical for organisms inhabiting temporally variable environments (Pulliam 1988). In contrast to other plains stream darters such as the johnny darter (*Etheostoma nigrum*) and orangethroat darter (*Etheostoma spectabile*), which are among the most widespread and non-specialized members of their genus (Feminella and Matthews 1984, Smith and Fausch 1997), Arkansas darters are considered habitat specialists, preferring low-gradient, silt-bottomed streams with dense vegetation (Labbe and Fausch 2000). Arkansas darters were found to be more tolerant to high and variable temperatures than the johnny darter, withstanding rapid warming to water temperatures up to 35 °C, suggesting that this species is adapted to variable thermal conditions (Smith and Fausch 1997). However, aquatic ecosystems of the Great Plains have a highly endangered fauna and in particular, plains fishes have experienced steady declines throughout the last several decades (Fausch and Bestgen 1997). The patchy distribution of Arkansas darters in Colorado, coupled with increasing anthropogenic threats to its habitat, and the tenuous status of the species elsewhere, prompted a series of research and management actions geared towards the conservation of this species within Colorado (Labbe and Fausch 2000; Groce et al. 2012).

Previous conservation efforts included an extensive history of translocations and stocking by Colorado Parks and Wildlife (CPW), assessment of taxonomic status based on mitochondrial DNA (Proebstel et al. 1996), mark-recapture methods to estimate demographic parameters (Labbe and Fausch 2000), and occupancy analysis to determine the scale and specific habitat features influencing Arkansas darter site occupancy (Groce et al. 2012). Our study builds on this effort to understand and improve Arkansas darter population dynamics by using a conservation genetics approach, a contribution that sheds new light on understanding the effects of habitat fragmentation on connectivity as well as the vulnerability of these populations in the face of climate change. Specifically, we set out to address three main questions: (1) What are the natural patterns of genetic diversity, effective population size (N_e), and gene flow in the Colorado portion of the species' range?; (2) How does the landscape affect genetic diversity and gene flow? Do the same factors that influence site-occupancy also affect connectivity and population genetic patterns?; and (3) Have historical stocking efforts augmenting natural populations succeeded in contributing to the breeding population? This case study in Colorado highlights an approach that is broadly applicable to stream taxa worldwide that are becoming increasingly vulnerable to the effects of fragmentation and climate change (Helfman 2007).

Methods

Study area and sampling

During the summer of 2010, we sampled 19 sites with the highest probability of Arkansas darter occurrence in Colorado, as determined by a query of historic sites using the CPW Aquatic Data Management System (Fig 2.1). Sites were sampled for Arkansas darters systematically in a 3.25-km reach centered on the point where this species had been collected in previous sampling events. Using dip nets and minnow traps, more than five individuals were found at 12 of the 19

sites (Table 2.1) within four distinct basins (Fountain Creek, Big Sandy Creek, Rush Creek, and the Arkansas River floodplain). Additional sites have been established through translocation (Groce et al. 2012), however, with the exception of one site that was not sampled due to restricted access, the 12 sites included in our study likely represent the extant naturally established Arkansas darter populations in Colorado. Thus, we use 'natural Arkansas darter site' to refer to historic sites that were not started by anthropogenic translocation, even if a subset of these have received supplementation from hatchery fish. Additionally, since we lack *a priori* information about the spatial scale at which Arkansas darters interbreed we use the term site (instead of population) to mean a group of Arkansas darters occurring in the same geographic location, and as the unit of focus for population genetic analyses.

Pelvic fin samples were collected from 29-100 individuals per site, stored in 100% ethanol, and fish were released at their capture location. We also collected fin clips from one additional site that was artificially established from hatchery fish (Hugo Ponds; Fig 2.1) and from Arkansas darter broodstocks (known to originate from Black Squirrel Creek, Horse Creek and Big Sandy Creek) at CPW's Mumma Native Aquatic Species Rearing Facility (NASRF) in Alamosa, Colorado. Hatchery broodstocks were included in a subset of the analyses to assess the extent of hatchery genotypes found in wild populations. In total we sampled 477 Arkansas darters from 12 natural sites and 137 individuals from hatchery broodstock (Table 2.1; Fig 2.1).

Habitat surveys were conducted at each site following the methods of Groce et al. (2012) in order to estimate Arkansas darter occupancy at naturally established sites and to test whether the same landscape variables that influence occupancy also play a role in shaping genetic diversity patterns (Table 2.2). Average depth and water temperatures at the stream bottom were measured at nine points throughout the sample reach. The proportion of the site covered by

vegetation was estimated visually. Two habitat variables were calculated using low-altitude flights: percent wetted area (the proportion of a 10-km reach centered on the historic site having a wetted channel), and available habitat (total length of stream accessible from the historic sampling site at low flow). The latter was determined by measuring the length of wetted habitat upstream from the center point of the reach until a barrier or dry segment was reached, and downstream until a dry segment was reached or the stream entered unsuitable habitat for Arkansas darters (i.e., confluence with a large canal).

Laboratory methods

Total genomic DNA was extracted from fin clips using DNeasy96 tissue protocol (Qiagen, Valencia, CA, USA). Following a screening of published microsatellite primers designed from *Etheostoma* darters, we found seven primers that amplified and were polymorphic in Arkansas darters: Eca10, Eca37, Eca46, Eca48, Eca49, Eca71 (Tonnis 2006) and Etsp224 (Hudman et al. 2008). PCR amplifications were carried out in 25 µl reactions with 13.1 µl H_2O, 3.3 µl 10x ABI Buffer I with added $MgCl_2$, 0.5 µl dNTPs, 2.5 µl of dye-labeled forward primer (10 µM), 2.5 µl of reverse primer (10 µM), 0.1 µl AmpliTaq DNA polymerase, and 3 µl of genomic DNA. All reactions were performed using thermocycling conditions of: 94 °C for 10 min; 45 cycles at 94 °C for 30 sec, 59 °C for 30 sec, 72 °C for 45 sec; and a final extension at 72 °C for 7 min. PCR products were mixed with HIDI formamide and LIZ ladder (500 GeneScan) and read on an ABI 3730 genetic analyzer (Life Sciences Core Laboratories at Cornell University). Fragment sizes were manually confirmed using GENEMARKER® version 1.91 (SoftGenetics, LLC, State College, PA). To ensure genotype accuracy, we included at least two negative controls per extraction and PCR, amplified a known genotype in each reaction, and

re-amplified at least 10% of samples to screen for genotyping and human error. Concordance between runs was high with an error rate of <0.5%.

Characterizing natural patterns of genetic diversity and gene flow

Conformity of genotype proportions to Hardy-Weinberg equilibrium was assessed with exact tests (Guo and Thompson 1992) and linkage disequilibrium was tested across all pairs of loci using GENEPOP version 4.010 (Raymond and Rousset 1995; Rousset et al. 2008). Markov chain parameters for all comparisons used 10,000 dememorization steps, 200 batches, and 10,000 iterations per batch. Microsatellite loci were examined for evidence of null alleles and scoring error due to stutter or large allele dropout using MICROCHECKER version 2.2.3 (van Oosterhout et al. 2006).

Allelic richness and observed and expected heterozygosities were estimated using ARLEQUIN 3.0 (Excoffier et al. 2005). Estimates of private allelic richness were calculated using HP-Rare, after accounting for differences in sample size (Kalinowski 2005). For each site we estimated effective population size (N_e) and 95% credible limits of the estimate via summary statistics and approximate Bayesian computation methods as implemented in ONeSAMP (Tallmon et al. 2008). We tested for evidence of recent population bottlenecks in each of the 12 naturally established sites using the program BOTTLENECK 1.2.02 (Cornuet and Luikart 1996). This analysis is based on the loss of rare alleles predicted in recently bottlenecked populations, which results in heterozygosity excess. We used two models, the infinite alleles model (IAM) and the two-parameter model (TPM). As suggested by Piry et al. (1999) we set the parameters for TPM to 95% single-step mutations and 5% multiple-step mutations, and the variance among multiple steps was set to 12. Based on the number of loci in our dataset, the Wilcoxon signed rank test was used to determine significance of heterozygosity excess.

To characterize partitioning of genetic variation at a broad geographical scale, an analysis of molecular variance (AMOVA; Excoffier et al. 1992) was used as implemented in ARLEQUIN. We grouped sites by basin for an *a priori* test for which hierarchical level (basin or site) explained the highest proportion of genetic variance and therefore represents the most appropriate groupings for management. We calculated differentiation and associated significance among naturally established Arkansas darter sites using 500 permutations and strict Bonferroni correction in ARLEQUIN. A non-significant F_{ST} indicates that those two sites are not statistically differentiated.

We used individual-based clustering analyses in STRUCTURE v 2.3.3 (Pritchard et al. 2000) to determine the number of distinct genetic clusters across all sites. We conducted 10 independent runs for each of a range of possible genetic clusters ($K = 1 – 12$). We used an initial burn-in of 100,000 with an additional 3,000,000 iterations. Correlated allele frequencies and admixture were assumed. The most likely number of genetic clusters was determined using the ΔK method (Evanno et al. 2005) and by calculating the posterior probabilities of each model. We also used this clustering method to test the extent of hatchery genotypes found in naturally established Arkansas darter sites (see below).

Testing the effect of the landscape on genetic diversity and gene flow

In fragmented populations constrained to small tributaries with low dispersal, the quality of ‘at-site’ habitat variables is expected to influence within-site genetic diversity whereas ‘between-site’ variables are expected to affect functional connectivity and genetic differentiation among sites (Murphy et al. 2010). We examined a suite of landscape characteristics hypothesized to affect genetic variation within sites using ‘at-site’ variables and genetic differentiation among sites using ‘between-site’ variables (Table 2.2).

We tested the effects of habitat variables on three within-site genetic diversity indices (allelic richness, effective population size, and expected heterozygosity) using multiple regression in R 2.15.3. First, we examined variance inflation factors and tested for multicollinearity among habitat variables (Graham 2003). Second, a candidate set of linear models that included all possible combinations of 'at-site' landscape variables was constructed because we did not have *a priori* reasons to know which combinations of variables would best explain variation in genetic diversity and we wanted to directly compare to the previous occupancy study (see Groce et al. 2012). We used the R package AICmodavg to rank models based on Akaike's Information Criterion corrected for small sample bias (AICc; Burnham and Anderson 2002). Models with the lowest AICc, and highest Akaike weight were considered to have the best fit with the data.

We used simple Mantel tests (Mantel 1967) and multiple regression on distance matrices (MRM) to test the effect of between-site landscape variables on pairwise genetic differentiation (F_{ST}). While Mantel tests assess the correlation between two matrices, MRM simultaneously examines the effect of a group of explanatory matrices on the response matrix (Legendre and Legendre 1998; Lichstein 2007; Goslee 2010). First, simple mantel tests were performed between the F_{ST} matrix and stream distance (i.e., isolation by distance), percent cultivated land, and percent intermittency matrices. Each landscape variable was quantified for all site pairs along the dendritic stream network in ArcGIS 10.0. Percentages were used for cultivated land and intermittency to control for overall distance between sites. We expected overall stream distance and % intermittency to decrease connectivity between sites (higher F_{ST} values). In the Arkansas River floodplain, extensive irrigation return flows are thought to have elevated the water table, making tributaries in this basin more perennial than they were historically (Groce et

al. 2012). In other parts of southeastern Colorado, groundwater mining is hypothesized to have had the opposite effect, decreasing flows and increasing isolation among tributaries (Miller 1984; Falke et al. 2011), but these areas are less extensively cultivated than the floodplain. Thus, we predicted that sites with a greater percentage of cultivated land between them would have greater connectivity (lower F_{ST} values).

Second, we employed MRM and included all three landscape matrices to determine the relative importance of each landscape variable. For this analysis we used absolute values of intermittency and cultivated land because the model controls for stream distance by including it as a factor. In all analyses the natural logarithm of stream distance was used to linearize the relationship between F_{ST} and distance. Mantel tests and MRM were carried out using the ecodist package in R 12.15.3 (Goslee and Urban 2007). Statistical significance was assessed using 10,000 permutations for both analyses.

Evaluating the success of hatchery genotypes in the wild

Translocation and supplementation are common management actions geared towards aiding the recovery of vulnerable species. However the success of these actions is difficult to determine without the use of genetic tools, and the persistence of hatchery fish and their progeny in the natural environment is not often quantified. CPW has stocked hatchery-reared Arkansas darters to supplement four naturally established sites within the Arkansas River floodplain tributaries (AFT09, AFT10, AFT12, AFT13). To assess the genetic contribution of hatchery genotypes into wild populations of Arkansas darters we first compared F_{ST} values between the hatchery and natural sites that have varying stocking histories (un-stocked, stocked natural site, established by hatchery) and second, conducted an admixture analysis using STRUCTURE v 2.3.3 (Pritchard et al. 2000). For the STRUCTURE analysis, we included only the naturally established

sites that have a history of stocking, samples collected from the NASRF hatchery (HTY14, HTY15, HTY16), and samples from one artificially established site, originating from hatchery broodstock (HGP08). We used the same run parameters and methods for estimating number of distinct genetic clusters (*K*) as our previous analysis. We estimated the admixture coefficient, *q*, by summing the proportion of contribution of the hatchery reference populations to an individual's genotype (Koskinen et al 2002).

Results

Characterizing natural patterns of genetic diversity and gene flow

We found no evidence for linkage disequilibrium between loci and departures from Hardy-Weinberg equilibrium were observed at only 2 out of 76 locus-by-population combinations (FTN01 at Etsp224; LAR10 at Eca37), following sequential Bonferroni correction. Four sites showed evidence for one null allele, however, there were no discernible patterns to suggest that a particular locus consistently showed evidence of null alleles in multiple sites (Table S2.1). The instances in which we found evidence for null alleles are likely due to slight deviations from Hardy-Weinberg proportions. We tested basic within-site genetic parameters such as observed and expected heterozygosity (H_o and H_e) with and without the loci for which null alleles were detected but results were unchanged and this did not affect our conclusions. Thus, we kept all loci in the analyses.

Overall levels of genetic diversity within Arkansas darter sites in Colorado were low (Table 2.3). Microsatellite genotyping revealed a relatively small number of alleles per locus for all sites, ranging from 2.5-5.3, and low expected heterozygosity averaging 0.44 ($\pm$ 0.09). Effective population sizes, estimated for all 12 naturally established sites were small, ranging from 20 - 47 (average $\pm$ STD = 35 $\pm$ 9). The most conservative model (TPM) implemented in

BOTTLENECK did not provide evidence of a recent bottleneck, nor did the allele distribution shape. However, the IAM showed that two sites have recently experienced a loss in heterozygosity (Table 2.3).

Although variation among individuals explained the majority of the total genetic variation (75.6%; p <0.001), analysis of molecular variance provided support for a hierarchical partitioning of genetic variance among basins. Grouping by basin explained more of the remaining genetic variation (14.8%; p <0.001) than variation among sites (9.6%%; p <0.001). High pairwise F_{ST} estimates provided evidence for substantial genetic divergence between most sites (Table 2.4). In general, pairwise F_{ST} estimates were higher among sites in different basins than those within the same basin. The highest average values of among basin F_{ST} were between the Fountain and Big Sandy basins (0.314) and the overall average among basin F_{ST} values was 0.248. Average within-basin estimates of pairwise F_{ST} were substantially lower (0.110) and the only two non-significant estimates of pairwise F_{ST} were between sites RCR06 and RCR07 in the Rush Creek basin (F_{ST} = 0.000) and AFT09 and AFT10 in the Arkansas River floodplain (F_{ST} = 0.007).

The STRUCTURE analysis revealed 7 distinct genetic clusters (K=7; Fig 2.2B), supported by Bayesian posterior probabilities and the ΔK method. In general, genetic clusters were divided among basins with some partitioning of sites within basins. Fountain Creek contained two main clusters: FTN01 is isolated but FTN02 and FTN03 are grouped together. Big Sandy Creek only has one known site (BSY04), which is a distinct cluster. Rush Creek has one main cluster, although while RCR05 is relatively isolated, RCR06 and RCR07 show some introgression with Fountain Creek. The Arkansas River floodplain shows three main genetic clusters. AFT09 and AFT10 are predominantly one group with some introgression with the AFT12 cluster. AFT11

and AFT13 are grouped together and isolated from all other clusters.

Testing the effect of the landscape on genetic diversity and gene flow

Despite overall low levels of genetic diversity within Arkansas darter sites, we found that characteristics associated with Arkansas darter habitat quality had positive relationships with genetic diversity (Fig 2.3). Depth was collinear with several other habitat features, thus we excluded this factor from all models. Our final model set contained 15 models for each of the three response variables. Model selection revealed that available stream habitat and relative amount of connected stream (% wetted) had the strongest positive influence on genetic diversity (Table 2.5). The two top-ranked models, which had approximately equal weights of evidence for all diversity indices, contained either % wetted or available habitat (Fig 2.3). Amount of vegetative structure received 10% of the weight of evidence for predicting allelic richness.

Mantel tests and MRM identified stream distance as the most consistent influence on genetic differentiation among pairs of sites (Table 2.6). Both tests revealed statistically significant positive correlations between stream distance and genetic differentiation, suggesting that isolation by distance is the prevailing factor affecting connectivity in Arkansas darter populations (Fig 2.4). Percent intermittency was significantly and positively related to genetic differentiation in Mantel tests ($r = 0.44$, $P = 0.005$), but total intermittency was not statistically significant in MRM ($P = 0.71$). Cultivated land was not found to be statistically significant in either test (Table 2.6).

Evaluating the success of hatchery genotypes in the wild

We detected some evidence for hatchery introgression into the wild, but hatchery contribution was overall low. Pairwise-F_{ST} values between hatchery and unstocked natural sites were slightly, but not significantly higher than between the hatchery and natural sites that have

received stocking (Fig 2.5). In contrast, F_{ST} values between HGP08 (established from hatchery fish) and hatchery populations were much lower. The STRUCTURE analysis revealed three distinct genetic clusters of hatchery fish, which correspond to the known sites of original capture: Black Squirrel Creek, Horse Creek, and Big Sandy Creek (Fig 2.2D). Since Arkansas darter stocking likely consisted of individuals from multiple hatchery broodstocks, we combined the contribution of all hatchery genotypes and found less than 20% contribution to the total genetic stock in each of the four sites that have received supplementation. In contrast, individuals sample from Hugo Ponds, known to originate from hatchery stock, show almost complete assignment to the hatchery cluster (Fig 2.2D).

Discussion

What are the natural patterns of genetic diversity and gene flow in this species?

Our analysis of microsatellite variation revealed extensive genetic structuring among Arkansas darter sites in southeastern Colorado, suggesting these fish occur in small populations that are highly differentiated from one another. Low allelic richness and expected heterozygosity point to overall low levels of genetic diversity within Arkansas darter sites in southeastern Colorado. Accordingly, effective population sizes within each geographic location are all relatively small (<50). Low levels of genetic variation and small effective population size are both signs that Arkansas darter populations are potentially vulnerable to the negative effects of inbreeding depression. In small populations, an increased probability of mating among relatives can result in the accumulation and fixation of deleterious alleles, leading to reduced fitness (Saccheri et al. 1998; Amos et al. 2001). Additionally, there is evidence linking populations with low genetic variation to a reduced ability to adapt to environmental change (Frankham 1995a; Willi et al. 2006). We did not, however, find strong evidence for recent population bottleneck events (Table

2.3), suggesting that these stronghold Arkansas darter sites have not recently undergone drastic reductions in effective population size.

Skewed sex ratios, variation in reproductive success, and fluctuations in population size can all lead to smaller effective population sizes than census size (Frankham 1995b). The similarity of estimated effective population sizes among sites that varied widely in apparent abundance (as indicated by catch per effort), may be an indication that relatively low N_e arises from the species' reproductive strategy or fluctuations in population sizes over time. Population genetic characterization of other darter species of conservation concern show comparably low diversity estimates and sometimes fail to detect recent signatures of population decrease, indicating that historically small N_e might be common for rare and specialized species in this genus (Fluker et al. 2010, Austin et al. 2011, Sterling et al. 2012). Moreover, deleterious alleles that become exposed through inbreeding may already be purged if effective populations sizes have been historically small (Charlesworth and Charlesworth 1987). Our analysis of microsatellite data cannot determine the cause of small N_e or whether inbreeding depression is occurring, although studies indicate that low N_e increases susceptibility to the negative impacts of inbreeding depression in other taxa (Newman and Pilson 1997).

The geographic distribution of genetic variation of Arkansas darter sites throughout southeastern Colorado suggests a broad division of at least four main groups corresponding to the four basins represented in this analysis: Fountain Creek, Big Sandy Creek, Rush Creek, and the Arkansas River floodplain. Evidence for high levels of genetic divergence among these four groups is provided by higher F_{ST} values among than within basins (Table 2.4) and more of the total genetic variation to be explained among than within basins. These findings are consistent with Proebstel et al. (1996), which found some evidence for historic isolation based on genetic

differentiation in mitochondrial DNA among the three basins included in their study (Fountain Creek, Rush Creek, and Big Sandy Creek). Results from Proebstel et al. (1996) were mixed as they found evidence of both shared and unique haplotypes in different frequencies among basins but overall low variation in mtDNA, however Big Sandy Creek haplotypes were the most divergent, suggesting that this population has been isolated the longest (Proebstel et al. 1996).

At a finer scale, our Bayesian clustering results uncovered seven distinct genetic demes, providing additional support for longer-term isolation of some sites such as Big Sandy Creek (BSY04; Fig 2.2B). For the most part, distinct genetic clusters are found within as opposed to across basins, however the clustering of Fountain Creek and Rush Creek sites is a notable exception (Fig 2.2B). The genetic similarity between these geographically distant sites could be explained by i) historic connectivity, ii) undocumented translocations, or iii) convergence of alleles.

How does the landscape affect genetic diversity and gene flow? Do the same factors that influence site-occupancy also affect connectivity and population genetic patterns?

We found that genetic diversity was positively correlated with localized habitat quality, specifically wetted habitat and longer available stream reaches. Detecting such relationships was surprising given our sample size of 12 sites is low, albeit exhaustive (i.e., with a single exception, all sites most likely to contain Arkansas darters in our study region – Colorado – were sampled). Our results accord with previous occupancy modeling that found available stream habitat and continuous wetted area (along with cool water temperatures) to be the strongest predictors of site occupancy of Arkansas darters (Groce et al. 2012). Additionally, our results corroborate an earlier mark-recapture study that found higher survival in stream reaches with stable habitat refugia (Labbe and Fausch 2000). Few studies have tested whether genetic and demographic

parameters are influenced by the same ecological features (Cosentino et al. 2011), despite the recognition of the importance of integrating these processes (Nunney and Campbell 1993). Jointly, this information can provide managers with goals for habitat restoration that encompass the ability to increase both genetic diversity and abundance.

Given the extinction-recolonization dynamics of historic Arkansas darter locations and similarities to other tributary-bound Great Plains fishes, it is likely that this species exists in a metapopulation context (Labbe and Fausch 2000). Metapopulation viability relies on immigration and colonization of new habitat patches, thus leaving populations that have become isolated from dispersal more vulnerable to extinction. As the number of populations isolated in this manner increases, so does the risk of metapopulation collapse (Hanski 1998). Tests of isolation-by-stream-distance were significant and explained the majority of the variation in genetic differentiation among sites (Table 2.6). Although long-distance dispersal is difficult to quantify, Labbe and Fausch (2000) showed that Arkansas darters are naturally able to disperse and colonize suitable habitat at the reach scale (up to 3 km), and observations of single individuals are occasionally captured in large mainstem rivers (Crockett *pers. observation*). Furthermore, reproductive rate is high as females were shown to spawn more than once per season, and generation time is relatively fast (1 year) (Taber et al. 1986). However, we observed occasional high F_{ST} values between even geographically proximate populations in the same basin (Table 2.4). In conjunction with habitat drying, some potential dispersal corridors are occupied by native or non-native predators, including the Northern pike, which could have major impacts on the fitness of dispersing Arkansas darters (Labbe and Fausch 2000). Thus, we posit that the observed isolation is not likely to be exclusively due to the biology of this specialized

headwaters species, but rather partly due to the patchiness and the degradation of intervening suitable habitat.

We tested two other landscape variables hypothesized to affect genetic differentiation – intermittency and cultivated land. Between some sites, long reaches of intervening stream dry completely in summer and early fall before rewetting by the following spring (Labbe and Fausch 2000). Mantel tests found a significant and positive relationship between percent intermittency and genetic differentiation, suggesting that seasonal drying presents a barrier to gene flow among Arkansas darter sites. Although the MRM found overall distance to be a better predictor of genetic distance than intermittency, there is a clear positive association between percent stream intermittency and F_{ST} (Fig 2.4). Importantly, percent intermittency controls for stream distance, and thus is not simply a by-product of distance between sites. We expected that the amount of cultivated land between Arkansas darter sites could serve as a proxy for water diverted from elsewhere for irrigation purposes, and therefore exhibit a negative relationship with genetic differentiation as increased stream flow might increase connectivity among sites. However, we did not find support for this hypothesis (Table 2.6; Fig 2.4), suggesting that surrounding land use is not necessarily a good proxy for in-stream processes.

The parallel lines of evidence for at-site and between-site effects are concordant with recent theory that stream fish distributions reflect the influence of habitat variables at multiple scales (Labbe and Fausch 2000; Falke and Fausch 2010). Notably, the driest basins (Big Sandy Creek, Fountain Creek, and Rush Creek) which showed the lowest levels of genetic diversity, are concentrated in the arid high plains and tablelands (Fig 2.1) where they are more susceptible to water depletion and increasing fragmentation by groundwater extraction (Gutentag et al. 1984; Krieger et al. 2001; Winter 2007). Additionally, sites with the lowest genetic diversity are higher

in the stream networks and therefore might be expected to have lower genetic diversity due to lower levels of immigration and smaller stream sizes. In contrast, in the Arkansas River floodplain, an elaborate ditch system and accumulation of irrigation return flows has rendered tributaries near the mainstem more perennial than in the past (Groce et al. 2012). We documented both the highest within-site genetic diversity and among-site connectivity in the Arkansas River floodplain.

Has historical supplementation of naturally established sites by stocking successfully contributed to breeding populations?

Sustained efforts have been made by CPW to supplement certain wild Arkansas darter sites with hatchery stock, however, we found marginal evidence that hatchery fish have successfully reproduced and contributed their genes to future generations in the wild. Genetic differentiation between hatchery and all natural sites is high, regardless of whether the site has a history of augmentation (Fig 2.5). The STRUCTURE analysis indicates that on average, hatchery alleles contribute to less than 20% of an individual's genotype (Fig 2.2C), despite stocking having occurred just a year prior to sampling in some cases, which is less than the average lifespan of an Arkansas darter (Taber et al. 1986). The average hatchery contribution was low (16.7%) even in the site that has received three times as many supplemented individuals (AFT12) and for which we have the largest sample size to detect contribution from hatchery stock. However, it is difficult to assess the precise contribution of hatchery genes, as there is inherent error in STRUCTURE assignments (Waples and Gaggiotti 2006). It is additionally difficult to know what the ideal hatchery contribution to a natural population should be in order to minimize the swamping of local alleles but positively contribute to population growth (Hansen et al. 2009).

Arkansas darters stocked from NASRF are no more than a few generations removed from fish collected from the wild, the broodstock is frequently supplemented with wild-caught fish, and spawning pairings are carefully managed to maximize genetic diversity. However, the literature contains abundant evidence that hatchery genotypes often have reduced fitness in the wild (Araki et al. 2008), arising from genetic (Araki et al. 2007; Marie et al. 2010), behavioral (Fleming and Gross 1994), or immunological (Naish et al. 2007) inferiority to wild populations. Recent research on salmonid supplementation programs indicates that substantial declines in fitness for the wild can occur within a single generation of captive breeding, even when inbreeding is ruled out as an explanatory mechanism (Christie et al. 2012). Our results show little genetic signature of hatchery fish in the wild but further studies are necessary to understand the demographic effects of augmenting natural Arkansas darter populations. We suggest modifications to the supplementation regime in the specific management recommendations section below.

Specific management recommendations

Characterize conservation units: The distribution of genetic variation is an important consideration for delineating conservation units (Palsbøll et al. 2007). For example, extant genetic variation is the raw material for short-term evolutionary response to environmental change, such as climate change (Santamaría and Méndez 2012). An evolutionarily significant unit (ESU) is a classification of populations that are isolated to the point that they represent significant evolutionary components of the species and likely have adaptive differences among them (Funk et al. 2012). At a smaller scale, management units (MU) are distinct, demographically independent populations (Funk et al. 2012). Ideally, multiple MUs should be

conserved within each ESU to ensure the long-term persistence of the species, especially in the case of metapopulation dynamics (Hanski and Gilpin 1997).

Both natural and anthropogenic factors likely play a role in shaping the patterns of genetic variation in Colorado populations of Arkansas darters. Evidence for historic isolation among basins as indicated by mtDNA (Proebstel et al. 1996), small but stable effective population sizes as indicated by a lack of recent population bottlenecks (Table 2.3), and higher differentiation among than within basins (Table 2.4) suggests a fair amount of natural neutral genetic structure for this species, much of which is distributed among basins. However, human-induced range restrictions, alterations to the hydrology of plains streams, and severe drought and drying conditions seem to be further isolating an already patchily distributed species. Therefore, although range-wide analyses of adaptive and neutral genetic variation for this species is needed to determine ESUs with confidence, we suggest a tiered level of prioritization in which darter sites within the four historically distinct basins (e.g., Fountain Creek, Big Sandy Creek, Rush Creek, and Arkansas River floodplain) are managed as potential ESUs and seven genetically and demographically independent populations corresponding to unique genetic clusters are managed as MUs (Fig S2.1). Protecting basin-level potential ESUs may be a natural outcome of management to protect MUs provided that management includes protecting the hydrologic processes that sustain them. Moreover, each genetic cluster may harbor valuable genetic variation that could contribute to the adaptive potential of the species, especially in the face of rapid change to Great Plains stream habitat (Davis and Shaw 2001).

Protect and restore habitat: Our landscape genetic results suggest that reducing drying at both local and basin-level scales is the most important factor for improving the quality of Arkansas darter sites and facilitating connection between populations (Fig 2.3; Table 2.6). At the local

scale, restoration efforts should be directed towards securing or restoring stream flow and maintaining permanent refugia by reducing water withdrawals or planting streamside vegetation. At the broadest scale, conservation of this species might require not only protecting the immediate habitat supporting key populations, but also ensuring—for example through easements and private lands programs—that groundwater aquifers and hydrologic dynamics providing connectivity at larger spatial and temporal scales are maintained (Nesler et al. 1999).

Optimize artificial translocation strategy: If populations are isolated to the extent that emigrating individuals fail to colonize new habitats and existing populations are not compensated by immigration, then artificial movements and supplementation may indeed be a vital management strategy. However, our results suggest that current practices might be improved with modifications. First, we recommend evaluating the returns from protection and rehabilitation of naturally established Arkansas darter sites versus creating new sites, given the low proportion of translocation attempts that have created self-sustaining populations (Groce et al. 2012). In light of this result, focusing efforts on protection and restoration of existing sites and increasing connectivity among them might be most fruitful.

Second, we recommend taking further genetic and fitness information into account when designing supplementation action from hatchery broodstock. The guidelines for propagation and translocation outlined in George et al. (2009) suggest prioritizing translocations of natural populations (if sources are naturally abundant) over stocking individuals from a propagation facility in order to minimize disease transmission, domestication, or artificial selection. Additionally, an evolutionary framework for choosing source sites that are most genetically and morphologically similar to the recipient population is widely recommended (Edmands 2007; George et al. 2009). Arkansas darter stocking sources have thus far originated from some of the

most geographically and genetically isolated populations. To minimize potential outbreeding depression, we suggest maintaining broodstock from each of the suggested ESUs and using fish from the same basin for supplementation efforts. Additionally, we recommend experimental studies to test the fitness effects of crossing hatchery and wild Arkansas darters as evidence for negative carry-over effects from wild-born hatchery descendants can reduce overall population fitness (Araki et al. 2009).

Future studies on fitness and adaptive variation: Finally, we encourage additional studies aimed at understanding genetic and adaptive variation across the full range of the Arkansas darter. Although it is widely assumed that neutral genetic diversity is positively related to fitness (Frankham 1995a), the strength of this relationship has yet to be characterized for any *Etheostoma* species.

If change to the Great Plains region continues as expected, understanding the adaptive potential and protecting adaptive variation of the species is crucial (Funk et al. 2012). Identifying adaptive differences among populations could consist of measuring and comparing fitness-related traits, using genetic data for reconstructing wild pedigrees, or conducting reciprocal transplant experiments. Finally, population genomic data could facilitate improved estimates of demographic parameters such as gene flow, effective population size, and population-level admixture, as well as identification of loci that may represent locally adapted genes.

Conclusions

Understanding the factors that influence genetic connectivity among occupied habitats is a major goal for long-term population persistence of stream fish metapopulations (Fagan 2002). Genetic approaches can play an important role for informing complex management decisions,

particularly when combined with demographic information. Increasingly, the interaction among genetic and demographic factors is being recognized and used for reversing the negative impacts of anthropogenic habitat fragmentation (Neuwald and Templeton 2013). The Great Plains is a region of severe water scarcity due the combined effects of natural aridity, intense human competition for the water that does exist, and increasing temperatures and variability in precipitation due to climate change (Dodds et al. 2004). Conserving the fish assemblages and stream biodiversity endemic to this region, therefore, poses a formidable challenge (Milly et al. 2005). The task will likely require a creative combination of continued monitoring, targeted research efforts, and timely and thoughtful management. For example, improving habitat quality by preventing further stream intermittency and restoring larger reaches is critical for maintaining population persistence through demographic and genetic processes. Additionally, designing supplementation programs in which locally adapted species are used to infuse genetically depauperate populations may be necessary to reinforce isolated populations and maintain genetic diversity across the landscape. Further integration between genetic and demographic studies will allow evolutionary ecologists and managers to better understand the mechanisms underlying the distribution, abundance, and adaptive dynamics of stream fishes and other organisms.

Table 2.1 Sample origin, site ID (corresponding to Figure 2.1), sample size (*N*), and sample locations for *Etheostoma cragini* collected for this study. Three hatchery broodstock populations (HTY14-16) and one site that was established from hatchery broodstock (HGP08) are included. All other locations are naturally established Arkansas darter sites.

River basin/ sample origin	Stream	Site ID	*N*	UTM coordinates	
				Easting	Northing
Fountain Creek	Jimmy Camp Creek	FTN01	30	0527131	4281806
Fountain Creek	unnamed tributary	FTN02	31	0523598	4281489
Fountain Creek	unnamed tributary	FTN03	30	0535464	4254691
Big Sandy Creek	Big Sandy Creek	BSY04	30	0568707	4327980
Rush Creek	South Rush Creek	RCR05	32	0605827	4311986
Rush Creek	North Rush Creek	RCR06	59	0631403	4301149
Rush Creek	Rush Creek	RCR07	31	0644201	4294148
Arkansas River	Vista Del Rio Ditch	AFT09	29	0705215	4220664
Arkansas River	West May Valley Ditch	AFT10	30	0709532	4222566
Arkansas River	unnamed slough	AFT11	30	0735300	4220477
Arkansas River	Wild Horse Creek	AFT12	100	0751157	4223931
Arkansas River	Buffalo Creek	AFT13	45	0735595	4225726
Hatchery- Hugo Ponds	Huge State Wildlife Area	HGP08	37	0635912	4310737
NASRF Hatchery	NA	HTY14	30	NA	NA
NASRF Hatchery	NA	HTY15	30	NA	NA
NASRF Hatchery	NA	HTY16	40	NA	NA

Table 2.2 Landscape variables hypothesized to affect at-site genetic diversity or between-site genetic connectivity.

Landscape scale	Variable	Data Source	Mean	SD	Range	Predicted effect	Ecological justification
At-site							
	Depth (m)	Field	0.26	0.14	0.11-0.60	+	Increased space to reproduce (Taber et al. 1986)
	Bottom temperature (°C)	Field	21	5	8-29	-	Cooler temperatures are preferred (Taber et al. 1986; Labbe and Fausch 2000)
	% Vegetated	Field	66	17	45-99	+	Vegetated pools are more likely to be permanent, preferred by darters, and more favorable for survival and growth (Smith and Fausch 1997)
	% Wetted	Fly-over	69	38	1-100	+	Facilitates dispersal (Falke and Fausch 2010)
	Available habitat (km)	Fly-over	3.61	2.99	0.1-9.8	+	Increased space to reproduce (Labbe and Fausch 2000)
Between-site							
	Stream distance (km)	CPW	291	177	6 - 637	-	Dispersal distance
	% Intermittency	CPW	20	19	0-84	-	Seasonal drying could act as a barrier to gene flow (Labbe and Fausch 2000)
	% Cultivated crops	NLCD (2006)	16	11	0-64	+	Agricultural areas are heavily irrigated, raising water tables and increasing stream flow (Falke et al. 2011)

Table 2.3 Average allelic richness (A), private allelic richness (A_p), expected heterozygosity (H_e), and effective population size (N_e) ± 95% confidence interval are reported for *E. cragini* at each site. Significance of population bottlenecks are evaluated from *p*-values from the Wilcoxon-Rank sum tests (1000 replications) of heterozygosity excess under the IAM and TPM model as implemented in program BOTTLENECK. Sites that show significant evidence for a bottleneck are in bold. Analyses of N_e and tests for bottlenecks were only based on naturally established Arkansas darter sites.

River basin/ sample origin	Site ID	A	A_p	H_o	H_e	N_e (95% CI)	Bottleneck test	
							p-value IAM	*p*-value TPM
Fountain Creek	FTN01	3.7	0.32	0.39	0.50	25 (14, 72)	**0.05**	0.28
Fountain Creek	FTN02	3.3	0.04	0.35	0.34	34 (21, 96)	0.66	0.98
Fountain Creek	FTN03	3.5	0.01	0.42	0.43	32 (21, 84)	0.34	0.96
Big Sandy Creek	BSY04	3.6	0.03	0.41	0.45	28 (16, 82)	0.15	0.66
Rush Creek	RCR05	3.1	0.11	0.29	0.36	38 (21, 95)	0.66	0.96
Rush Creek	RCR06	3.2	0.02	0.34	0.31	41 (25, 97)	0.50	0.95
Rush Creek	RCR07	2.5	0.00	0.30	0.34	20 (13, 48)	0.22	0.66
Arkansas River	AFT09	4.9	0.11	0.49	0.53	34 (24, 74)	0.41	0.77
Arkansas River	AFT10	5.3	0.31	0.52	0.60	32 (21, 64)	0.19	0.47
Arkansas River	AFT11	3.9	0.01	0.42	0.47	47 (31, 122)	0.47	0.77
Arkansas River	AFT12	4.6	0.04	0.48	0.51	45 (32, 109)	0.03	0.81
Arkansas River	AFT13	4.3	0.02	0.50	0.52	44 (32, 105)	**0.05**	0.77
Hatchery- Hugo Ponds	HGP08	3.6	0.02	0.48	0.44	NA	NA	NA
NASRF Hatchery	HTY14	3.8	0.04	0.30	0.31	NA	NA	NA
NASRF Hatchery	HTY15	4.3	0.03	0.40	0.46	NA	NA	NA
NASRF Hatchery	HTY16	3.8	0.02	0.29	0.60	NA	NA	NA

Table 2.4 Pairwise genetic differentiation estimates (F_{ST}; lower diagonal) among sampled Arkansas darter sites and hatchery broodstock in southeastern Colorado. Grey shading indicates comparisons between hatchery broodstock and sites that have received hatchery augmentation. Every pairwise comparison is significantly different except the two values in bold. Upper diagonal is the total stream distance (km) between sites. Site abbreviations are defined in Table 2.1.

Site	FTN01	FTN02	FTN03	BSY04	RCR05	RCR06	RCR07	AFT09	AFT10	AFT11	AFT12	AFT13	HGP08	HTY14	HTY15	HTY16
Naturally established sites:																
FTN01		6	38	636	579	537	516	320	317	367	378	372	--	--	--	--
FTN02	0.240		40	638	581	539	517	321	318	368	380	374	--	--	--	--
FTN03	0.160	0.045		598	541	499	478	282	278	329	340	334	--	--	--	--
BSY04	0.320	0.324	0.298		484	441	420	328	325	346	357	351	--	--	--	--
RCR05	0.327	0.294	0.292	0.247		56	63	271	268	289	300	294	--	--	--	--
RCR06	0.320	0.144	0.170	0.308	0.124		21	229	226	247	258	252	--	--	--	--
RCR07	0.270	0.151	0.145	0.276	0.118	**0.000**		208	204	225	237	231	--	--	--	--
AFT09	0.277	0.280	0.218	0.119	0.219	0.272	0.236		6	59	70	64	--	--	--	--
AFT10	0.252	0.270	0.217	0.125	0.198	0.265	0.228	**0.007**		55	67	61	--	--	--	--
AFT11	0.367	0.346	0.337	0.214	0.256	0.345	0.327	0.191	0.155		32	10	--	--	--	--
AFT12	0.215	0.199	0.140	0.132	0.207	0.192	0.170	0.035	0.050	0.202		38	--	--	--	--
AFT13	0.291	0.292	0.272	0.146	0.237	0.304	0.280	0.124	0.104	0.023	0.132		--	--	--	--
Hatchery:																
HGP08	0.227	0.294	0.204	0.323	0.345	0.339	0.276	0.273	0.277	0.399	0.240	0.341		--	--	--
HTY14	0.378	0.480	0.385	0.497	0.479	0.491	0.446	0.435	0.427	0.537	0.375	0.476	0.077		--	--
HTY15	0.332	0.333	0.213	0.410	0.387	0.325	0.283	0.288	0.181	0.413	0.214	0.347	0.309	0.507		--
HTY16	0.232	0.289	0.240	0.123	0.270	0.310	0.250	0.171	0.164	0.279	0.166	0.218	0.134	0.278	0.363	

Table 2.5 Results of model selection for three candidate model sets. Top three ranked models per response variable are shown based on AIC_c values. Delta AIC_c (ΔAIC_c) and model weights (w_i) are given.

Population genetic parameter	**Model Rank**	**Model**	**ΔAIC_c**	**w_i**
Allelic richness	1	% Wetted	0	0.34
	2	Available habitat	0.24	0.3
	3	% Vegetated	2.38	0.1
Effective population size	1	% Wetted	0	0.35
	2	Available habitat	0.62	0.26
	3	% Wetted + Available habitat	2.85	0.08
Expected heterozygosity	1	Available habitat	0	0.35
	2	% Wetted	0.95	0.22
	3	% Wetted + Available habitat	2.14	0.12

Table 2.6 Summary of Mantel tests and multiple regression on distance matrices (MRM) for examining the effect of landscape variables on genetic differentiation between Arkansas darter sites in Colorado. Mantel tests examine the effect of each variable individually whereas MRM examines the effects of all variables simultaneously. Percent intermittency and cultivated crops were used in Mantel tests whereas total values were used in MRM.

	Mantel test		**MRM**			
	r	*P*-value	β	*P*-value (β)	R^2	*P*-value (R^2)
Stream distance	0.66	**0.001**	0.06	**0.01**	0.44	**0.01**
Intermittency	0.44	**0.005**	0.03	0.46		
Cultivated crops	0.07	0.335	0.02	0.78		

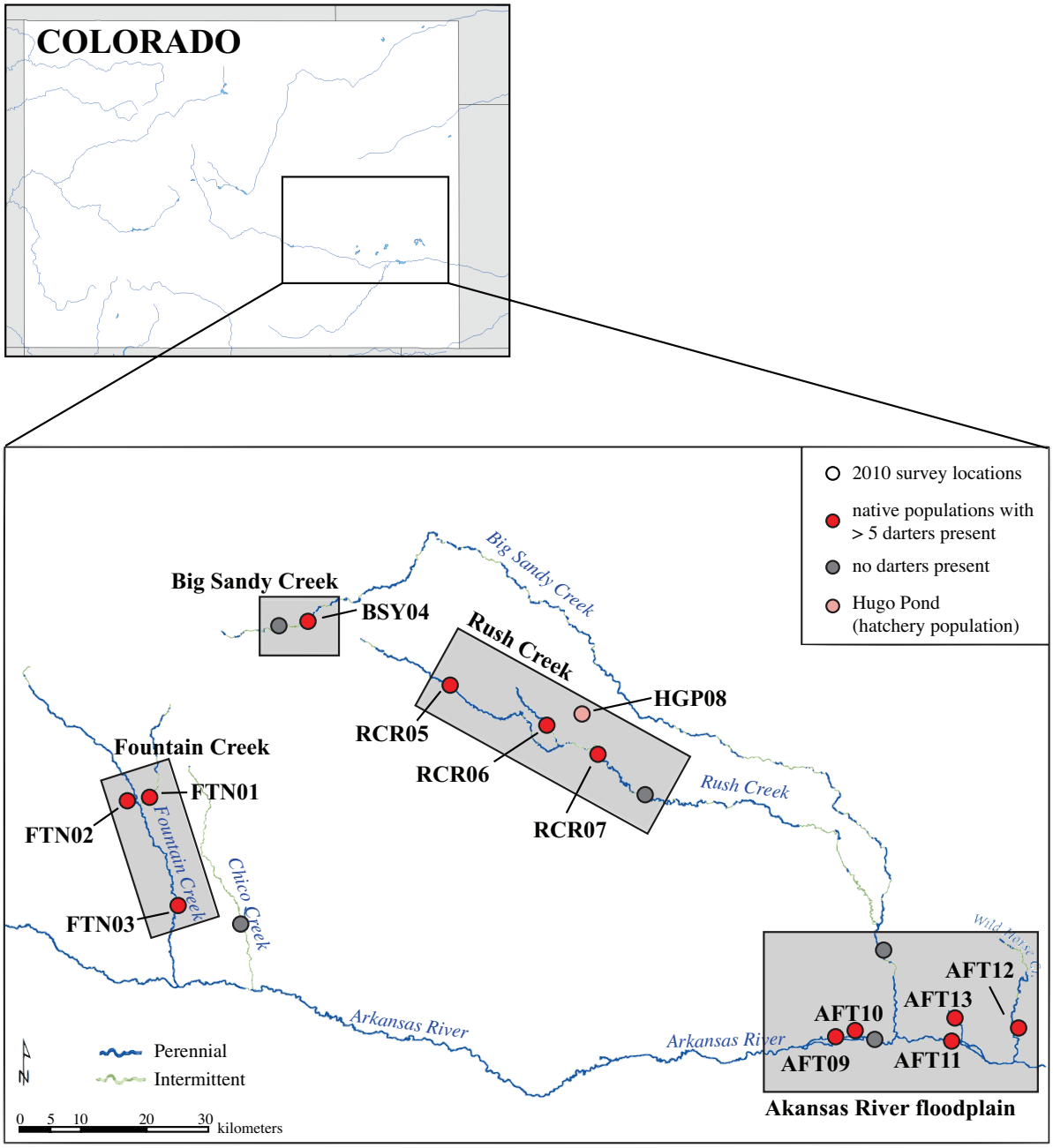
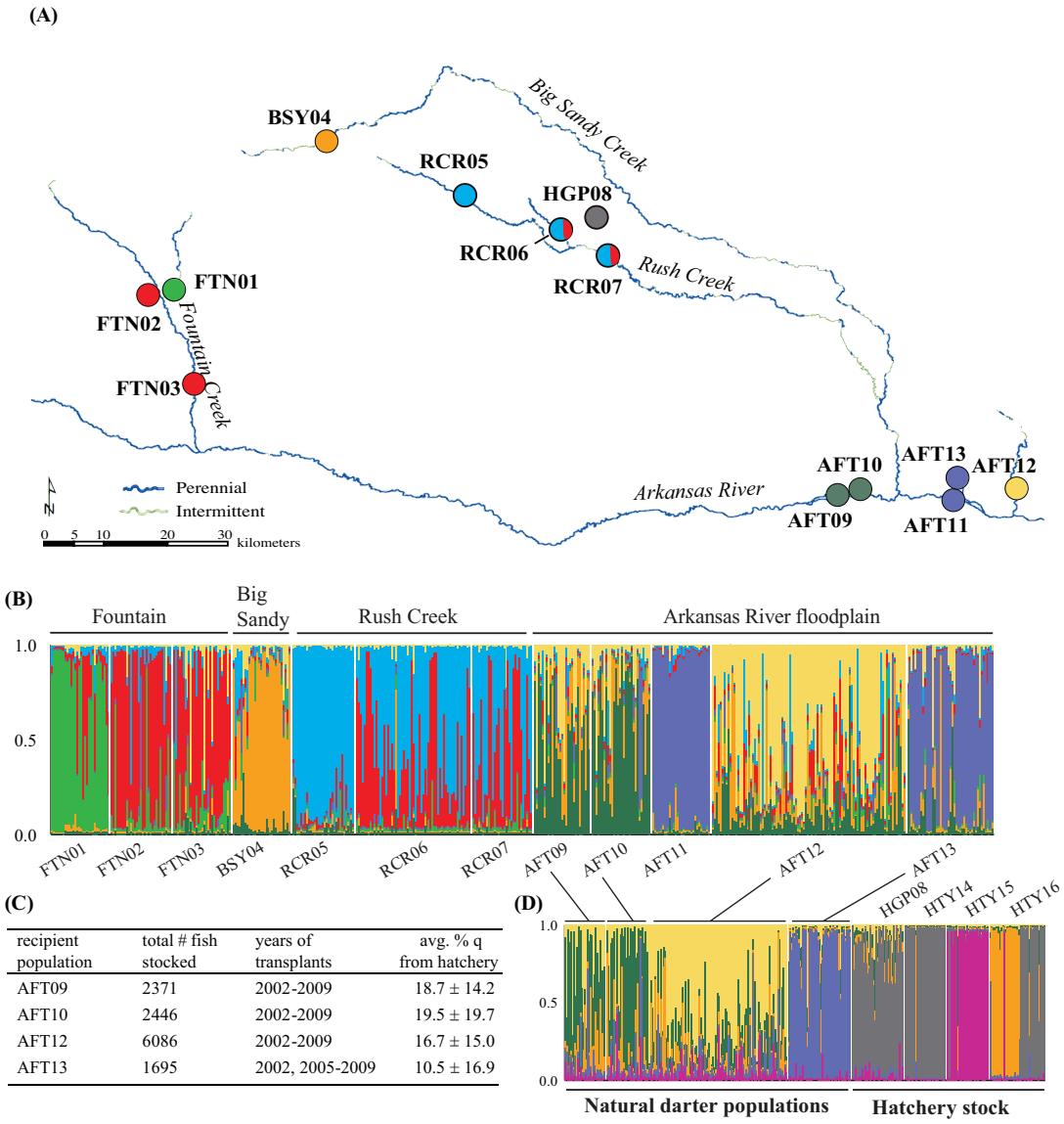


Figure 2.1 Map of Arkansas darter survey locations in 2010, within Colorado. Locations shown by red circles had >5 darters present and were sites included in the microsatellite study. Locations shown by grey circles historically contained Arkansas darters but <5 darters were present in 2010 survey. The location shown by the pink circle is a recent Arkansas darter site established with hatchery stock. Grey boxes indicate the four basins used in the AMOVA. Site ID labels correspond to Site IDs from Table 2.1.



recipient population	total # fish stocked	years of transplants	avg. % q from hatchery
AFT09	2371	2002-2009	18.7 ± 14.2
AFT10	2446	2002-2009	19.5 ± 19.7
AFT12	6086	2002-2009	16.7 ± 15.0
AFT13	1695	2002, 2005-2009	10.5 ± 16.9

Figure 2.2 (a) Map showing the twelve natural Arkansas darter sites and one site started from hatchery stock used in the microsatellite study. Colored circles correspond generally to distinct genetic clusters identified in the STRUCTURE analysis in (b). (b) Results from Bayesian individual clustering with STRUCTURE for $K = 7$. Only individuals from naturally established sites were included in this analysis. Each color corresponds to a distinct genetic cluster and each bar corresponds to the proportion of an individual's genotype assigned to each cluster. (c) Records of natural Arkansas darter sites that have received supplementation from hatchery stock. Table columns refer to site ID, total number of hatchery fish ever stocked, years in which supplementation took place, and percentage of the site's total genetic make-up that was assigned to hatchery signature. (d) Results from Bayesian individual clustering with STRUCTURE for $K = 6$. Included in this analysis were sites that received hatchery supplementation, hatchery broodstock, and one site that originated from hatchery broodstock.

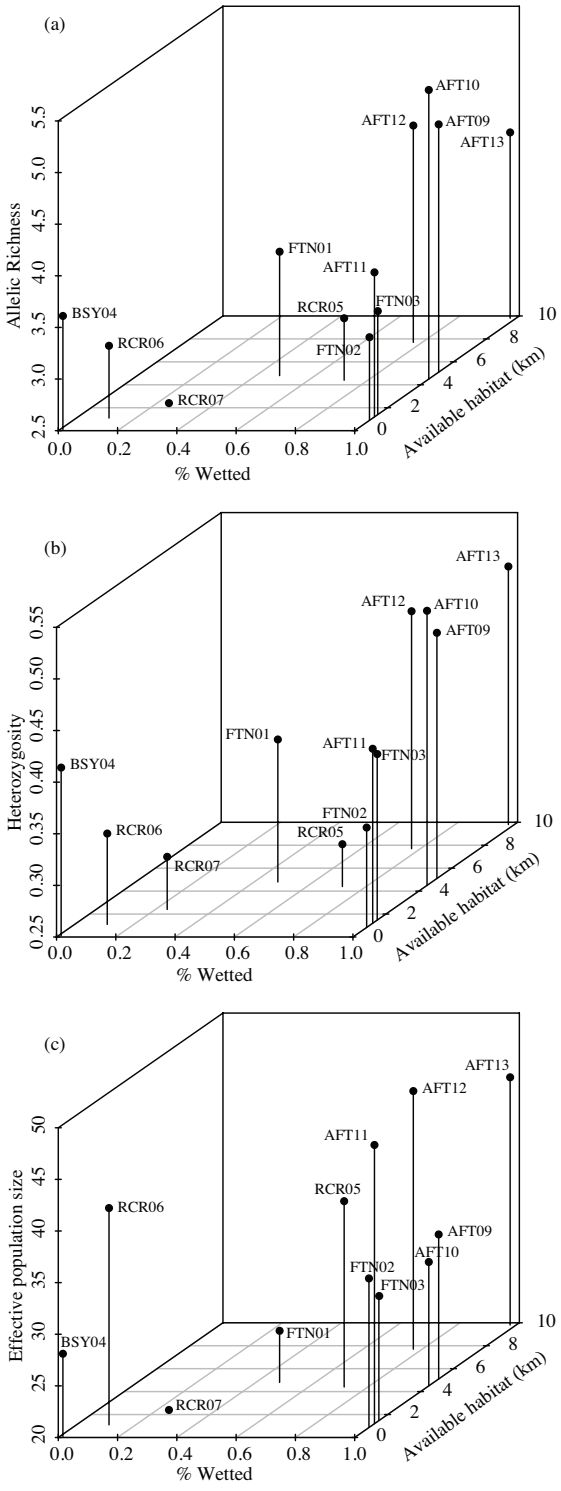


Figure 2.3 Three-way relationships between the two best-supported environmental variables (percent wetted area and available habitat) and at-site genetic diversity indices (a) Allelic richness; (b) Heterozygosity; and (c) Effective population size. Points are labeled by Site IDs that correspond to Table 2.1.

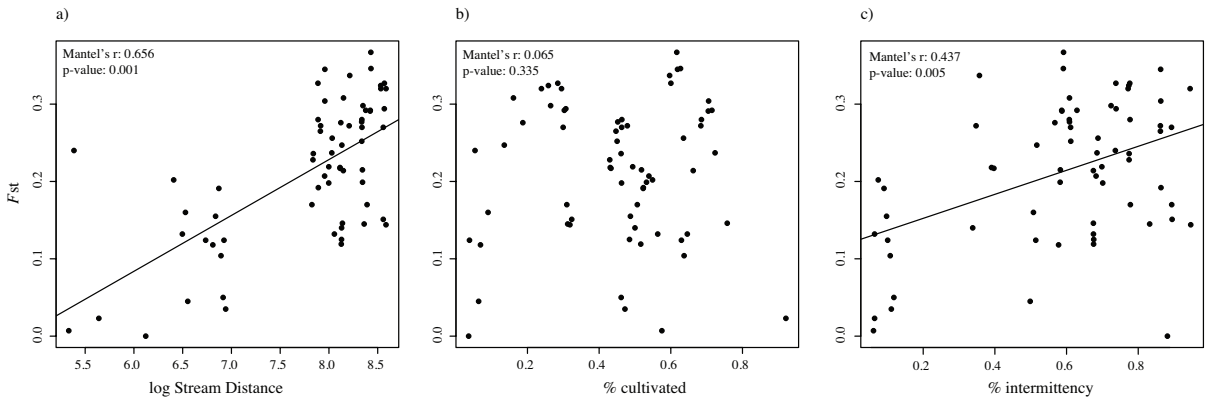


Figure 2.4 Individual relationships between pairwise genetic distance and landscape variables hypothesized to affect connectivity between Arkansas darter sites in Colorado: (a) distance between sites; (b) percent cultivated land between sites; and (c) percent of the stream that is intermittent at some point throughout the year. Results from the Mantel test are shown in upper left corner of each plot.

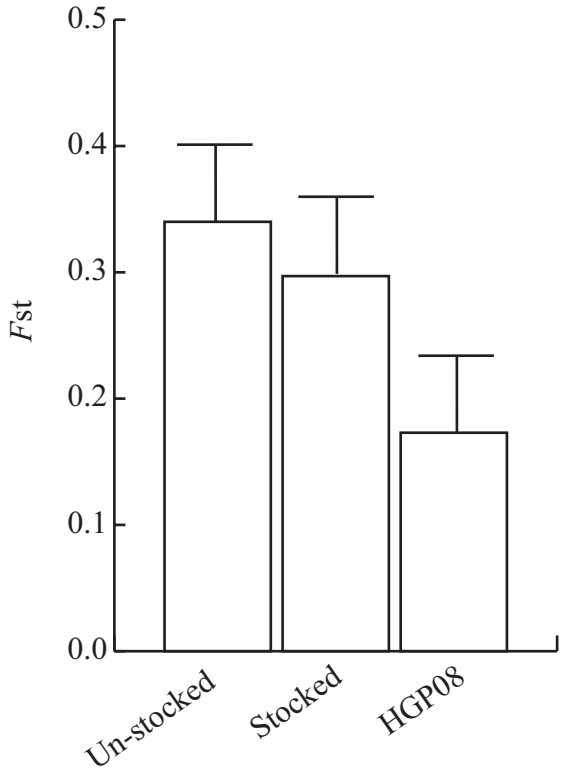


Figure 2.5 Average pairwise-F_{ST} values $\pm$ 95% CI between Arkansas darters in hatchery populations and un-stocked natural sites, stocked natural sites, and a site that was established with hatchery broodstock (HGP08).

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3. LOCALLY ADAPTED TRAITS MAINTAINED IN THE FACE OF HIGH GENE FLOW[2]

Summary

Gene flow between phenotypically divergent populations can disrupt local adaptation or, alternatively, may stimulate adaptive evolution by increasing genetic variation. We capitalized 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 local adapted phenotypes can be maintained despite extensive gene flow from divergent populations.

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 homogenizing 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 homogenization can

[2] Fitzpatrick, S.W., J.C. Gerberich, J. 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.

limit divergence among populations occupying 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 homogenizing 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 hybridization 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), color (Endler 1980), and behavioral (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, guppies originating from high-predation localities were introduced to guppy-free low-predation sites upstream of native guppy populations in six separate streams. While the primary goal of the introduction experiments was to test for rapid adaptive evolution, 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.

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 a scenario 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 3.1). 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 hybridization 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, characterized 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 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 3.2). We sampled introduction and source sites from all introduction experiments and, where possible, up to four incremental sites downstream from the introduction (0 m, 500 m, 1000 m, 5000 m; Fig 3.2; Table 3.1) 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 3.1). 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 3.1). 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*., 2012)

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 3.1). 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 (>14mm) found at a site. All individuals were anesthetized with MS-222, had three scales sampled for genetic analyses, and were photographed on their left side for phenotypic measurements (Fig S3.1). See Appendix 3.1 for standardized photography procedures. Females were euthanized 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.

Characterizing genetic divergence

To confirm high downstream gene flow from introduction sites we characterized genetic variation, connectivity, and population genetic structure within introduction streams at 10 neutral microsatellite loci (Table S3.1). Loci were selected in order to maximize 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 3.1 and Table S3.1.

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 3.1). 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 (color, body shape, and life history) from photographs and field-collected specimens. Polymorphic coloration of male guppies generally represents a local balance between sexual selection (females typically prefer more colorful males; Houde 1997) and predation intensity (more conspicuous males have higher mortality; Weese *et al.* 2010). Male color was assessed with an observer rank approach following Ruell *et al.* (2013), whereby individuals were visually ranked according to relative coloration. This method excels at producing a single comprehensive metric characterizing qualitative differences in overall coloration resulting from the spatial interaction among diverse color elements (i.e., specific color/pattern combinations) and has been used to quantify color 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 one day. Eight observers, ignorant of experimental design, but familiar with Trinidadian guppies, ranked fish for relative coloration based on four criteria: (*1*) number of different colors, (*2*) number of color elements, (*3*) relative intricacy of color elements, and (*4*) relative size and brightness of color elements. Observers assigned each fish a single ranking from 1 (least colorful) to 6 (most colorful). 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 color 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 characterized by eight homologous landmarks and six semi-landmarks digitized with TPSDig2 (Rohlf 2010) from images of each specimen (Figure S3.1). 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 (Mitteroecker & Bookstein 2011). Altogether, the first three PCA axes (PC1, PC2, 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 one 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 modeled 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 normalize the data prior to analysis. All models were carried out with package 'lme4' in R v3.1-108 (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 contrast, 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 1022 individuals (69 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 S3.2), and genotyping error rate was low (<0.05%). Allelic richness and heterozygosity were universally high within recent introductions and showed an increasing downstream trend within sites of old introduction streams (Table S3.2). 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 0.01-0.12 (Fig 3.3A; Table S3.3). In contrast, average level of genetic differentiation between natural sites before or without an upstream introduction was 0.21 and ranged from 0.07-0.27 (Fig 3.3A).

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 3.3B). 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 3.4, Table S3.4). Most traits were significantly affected by predation level, and variation in male color, male size at maturity, and embryo mass matched the predicted adaptive direction (Fig 3.4). Specifically, our results matched expectations that guppies from low-predation environments will be more colorful, 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 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 S3.4). However, the third PC axis was significantly affected by predation in the adaptive direction, with a ventral shift in mouth orientation (higher PC3 score) favored in low-predation environments (Fig 3.4).

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 3.5). 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 3.5A). 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 3.5B).

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 homogenization 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 S3.2). 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 3.3A). 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 3.3B). The genetic uniformity of individuals from introduction sites, the Guanapo 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 S3.3), yet STRUCTURE analyses split all old introduction and source sites into distinct genetic clusters (Fig 3.3B). We also discovered a downstream trend of increasing within-population genetic variation in old introduction streams (Table S3.2), 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 3.4, Fig S3.2). Despite rapid and extensive gene flow from initially maladapted populations, males in low-predation environments tended to be more colorful, 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 favors fewer offspring. If, through higher levels of genetic variation, heterosis, or transgressive segregation, immigrants or hybrids are physiologically able to produce larger embryos (as favored in low-predation environments) but still retain high fecundity, this 'super' phenotype could be selectively favored 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 3.5). 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 (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 S3.5), and results from pre- and post-gene flow common gardens provides evidence for gene flow causing genetically based changes in traits in two of our sites (Handelsman and Fitzpatrick, *unpublished data*; **see Ch.4**). 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 homogenization 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, homogenizing 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 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 S3.2). 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 homogenization 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 immigrant 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 microevolution in the face of climate change and altered patterns of connectivity.

Table 3.1 Site summary

Stream	Age of introduction	# males/females introduced	Site	Coordinates	Predation Level	# males	# females
Turure	1957 'old'	~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'	~100/~100	Native LP		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'	~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'	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'	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'	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 Mainstem			5000 m[1]	N10°41.658' W61°15.836'	high	20	20
			Source[2]	N10°38.402' W61°14.896'	high	20	20

[1]5000 m site for L.Lalaja, Caigual, and Taylor; [2]Source site for Turure, L.Lalaja, Caigual, and Taylor

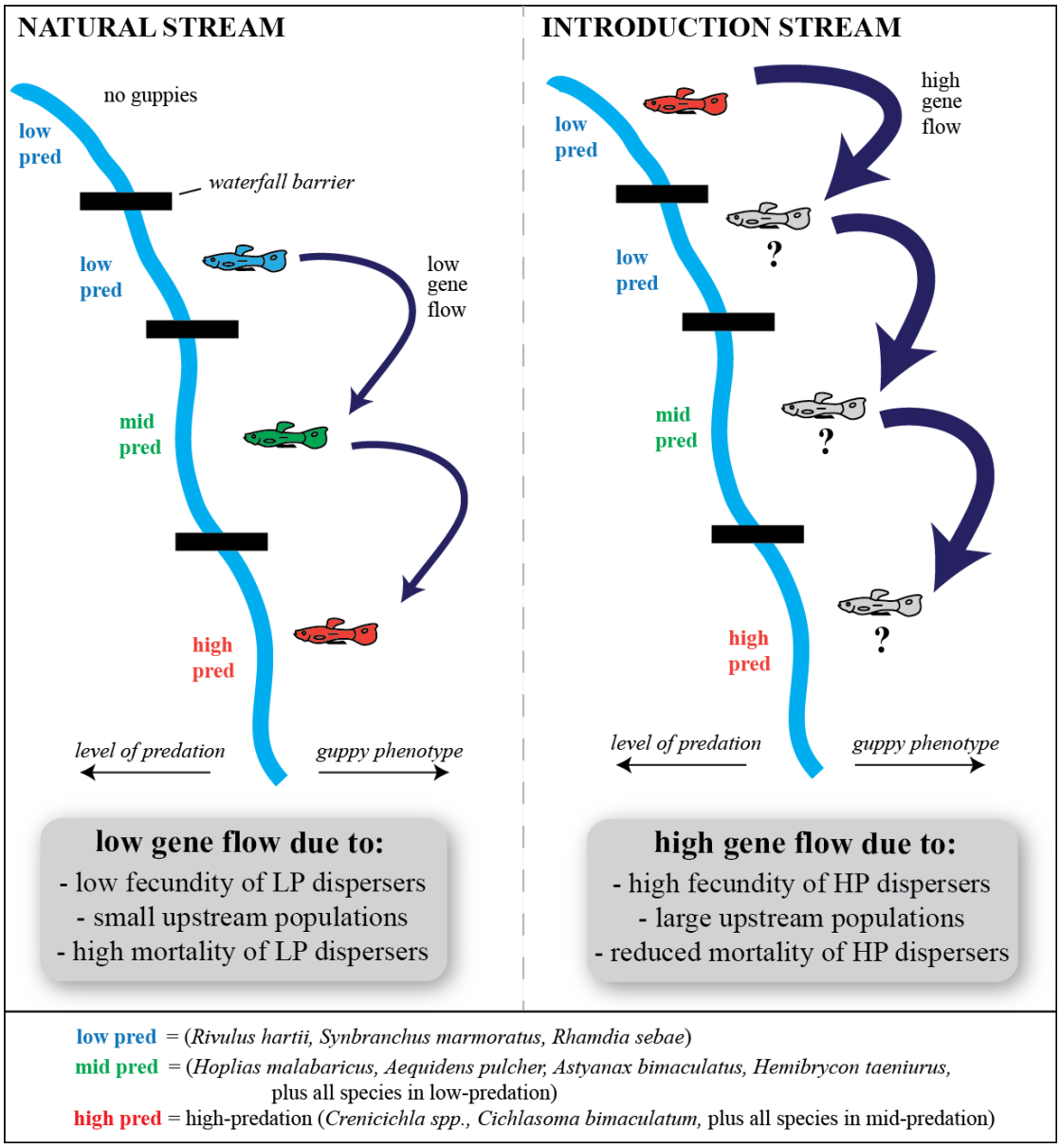

Figure 3.1 Conceptual diagram illustrating the expected differences in amount of gene flow between natural streams and streams with introduced populations. In both hypothetical streams, predation level is color-coded based on the species listed in the bottom key and increases in the downstream direction. Black rectangles indicate waterfall barriers that limit upstream fish dispersal. The color 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 a 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 an introduced stream, guppies from high-predation environments were translocated upstream of naturally occurring low-predation 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 is unknown (indicated by grey fish and question marks).

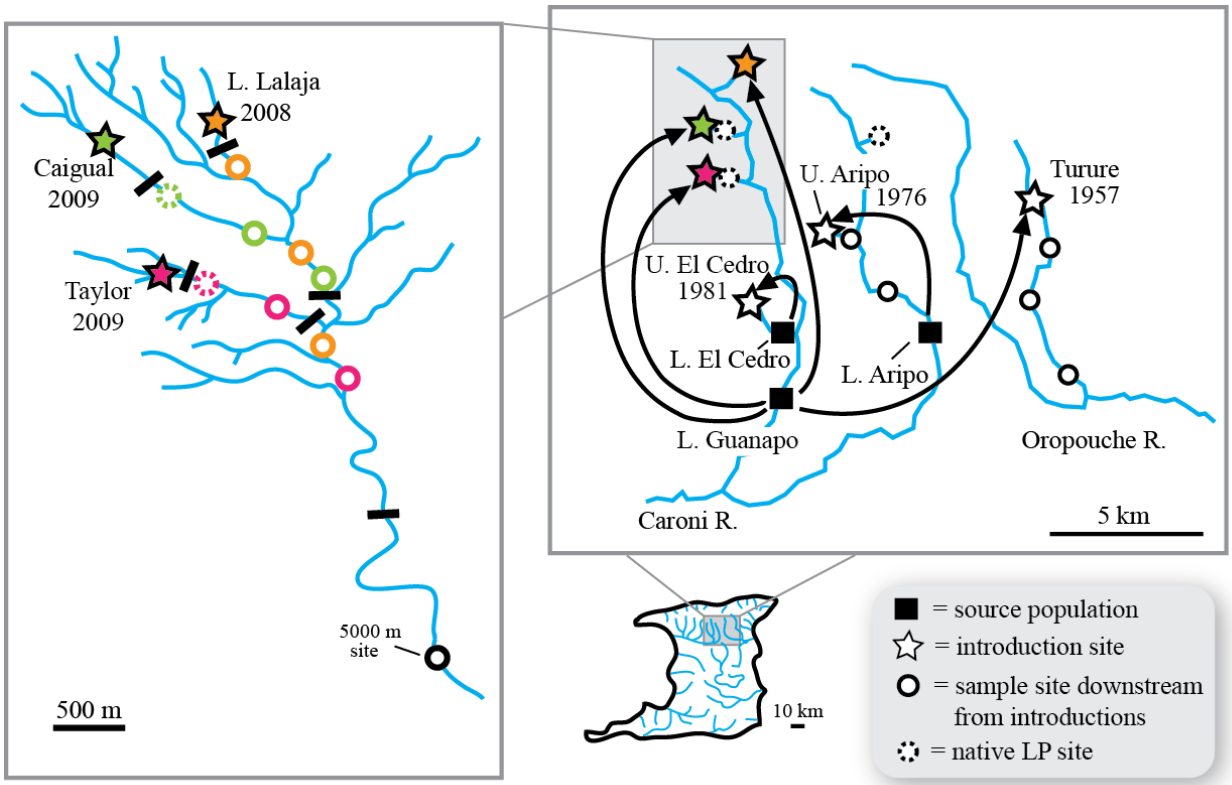

Figure 3.2 Map illustrating sampling scheme for our study. The island of Trinidad is shown at the largest scale, with a grey box indicating where all introductions took place. At the next spatial scale, six introduction scenarios are indicated by black arrows with the names of rivers and year of translocation. Black squares represent source sites and stars represent introduction sites. Colored 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 characterized as low-, mid-, or high-predation based on complexity of fish community (Table 3.1).

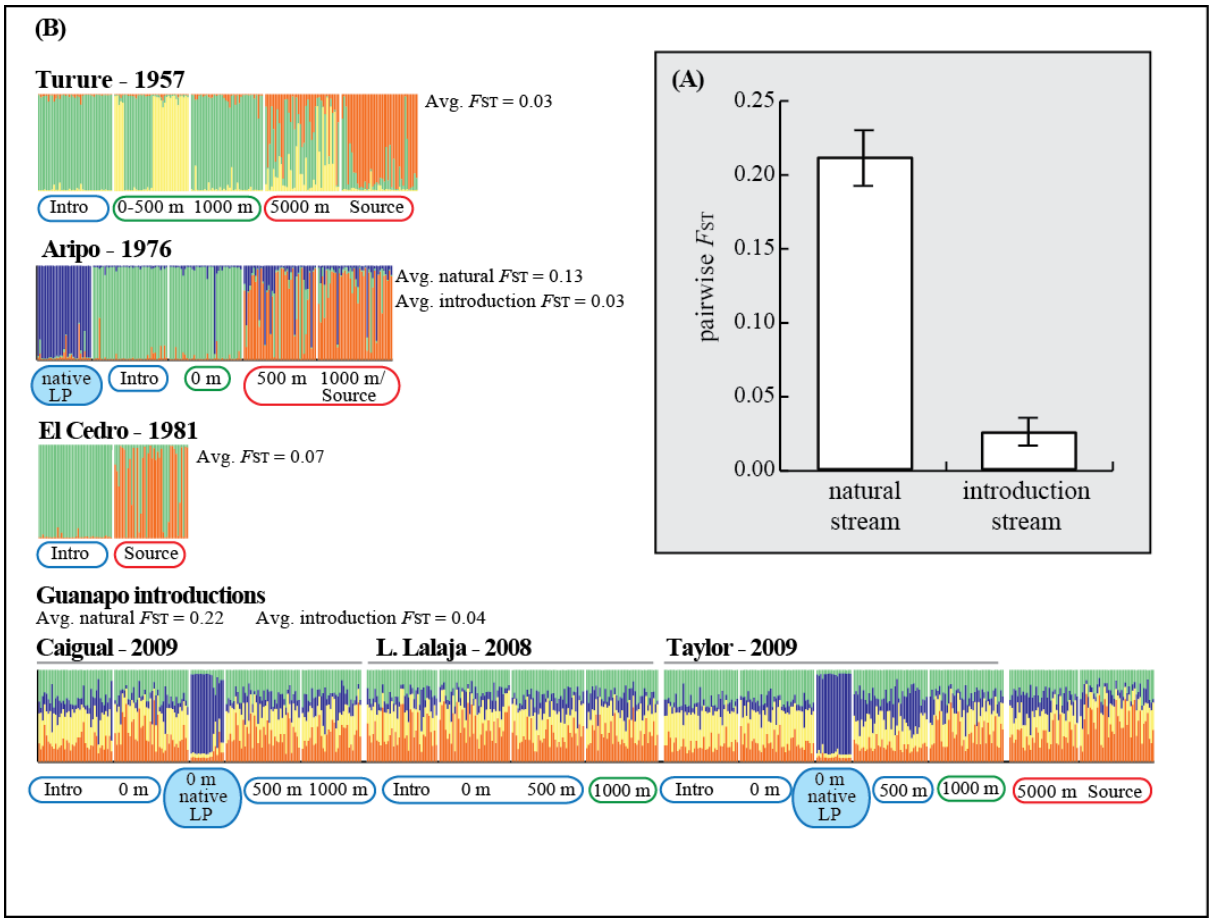

Figure 3.3 (A) Comparison of genetic differentiation (pairwise F_{ST}) among all sites in natural streams versus among all sites in streams after introductions took place. (B) Within stream STRUCTURE plots and average pairwise-F_{ST} values for all six streams that experienced an upstream introduction. Each line in the plots corresponds to an individual with colors 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. Recent introductions are more genetically homogeneous, with the exception of pre-introduction 0 m sites in Taylor and Caigual 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 Figure 3.1. The highest supported number of (k) genetic clusters is shown (see Appendix 3.1).

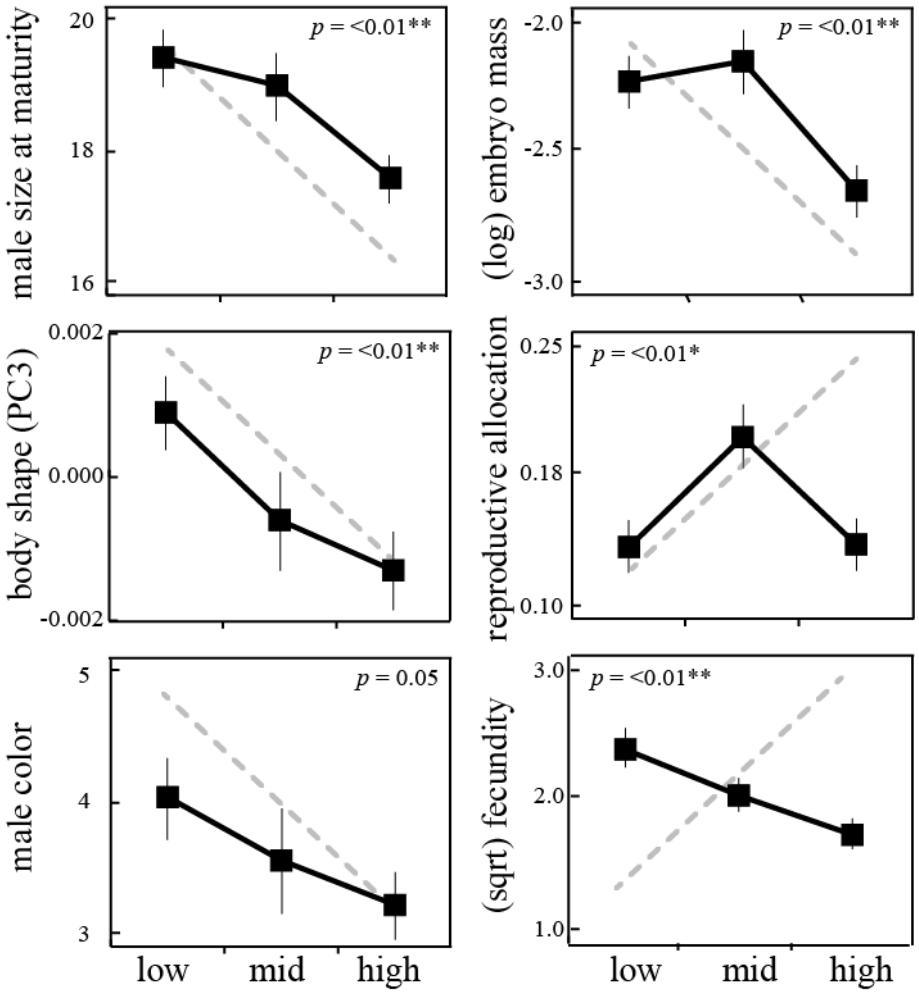

Figure 3.4 Mean values (± SE) of phenotypic traits that vary by predation level based on linear mixed effects models. Dashed grey lines indicate the expected adaptive direction of the trait across the predation gradient based on prior studies, but not the slope or actual trait values. Site was a nested random effect within stream in all models. *p<0.01; **p<0.001

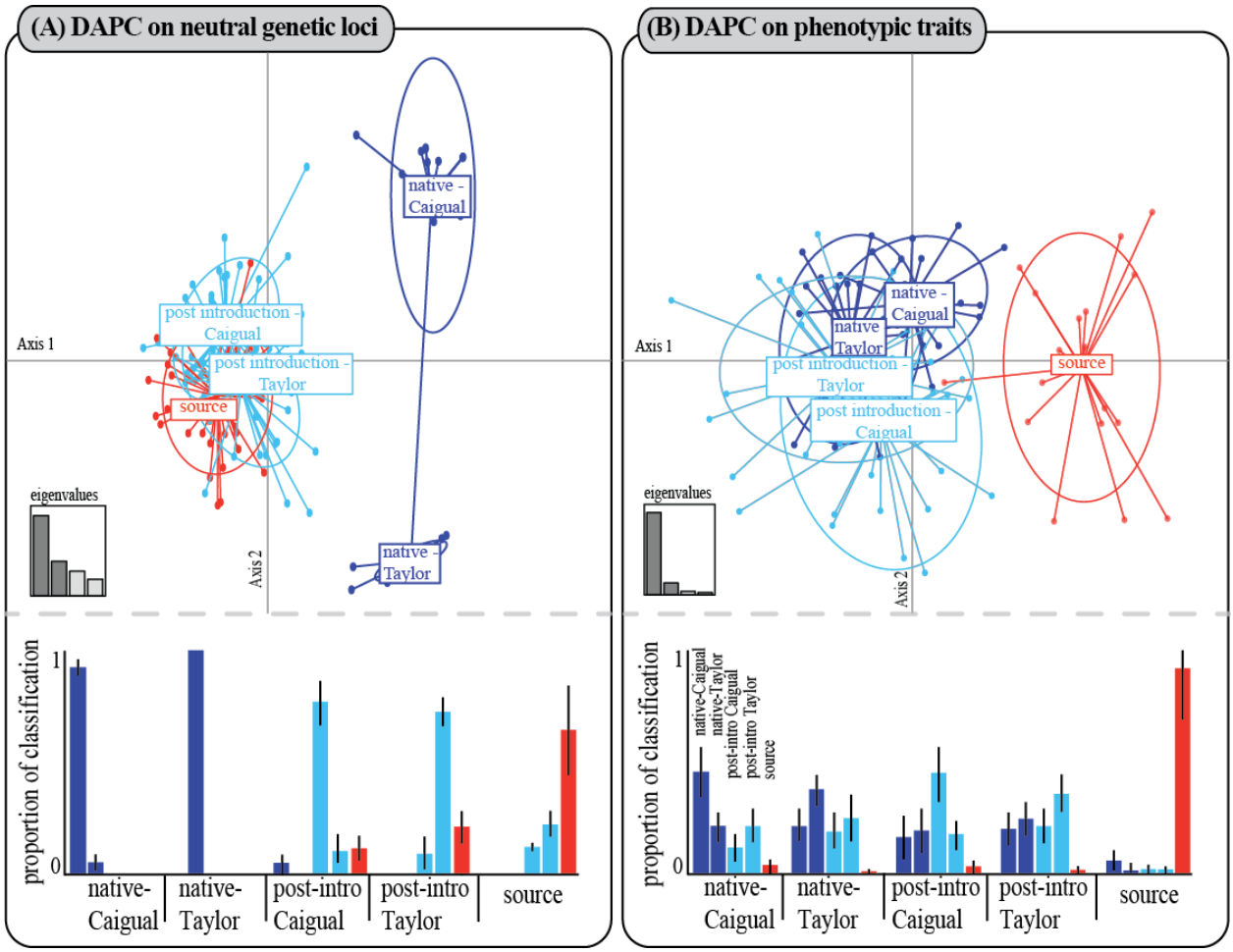

Figure 3.5 Ordination plots and group classification based on discriminant analysis of principal components (DAPC) for neutral genetic loci (A) and phenotypic traits (B). Colors correspond to *a priori* groups based on population origin: native low-predation in purple, the same sites post-introduction 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 labeled for one set of bars in (B). The bottom-left insets show eigenvalues of the four principal components in relative magnitude.

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4. GENE FLOW CONSTRAINS AND FACILITATES GENETICALLY BASED DIVERGENCE IN QUANTITATIVE TRAITS

Summary

Theory predicts that gene flow will decrease phenotypic differences among populations. Correlational studies have in some cases documented a constraining effect of gene flow on phenotypic divergence, but in other cases provide conflicting evidence for the maintenance of local differentiation despite high gene flow. These correlative studies are unable to evaluate how gene flow affects genetically based phenotypic divergence, and whether gene flow constrains adaptive divergence or vice versa. In this study we tested for genetically based changes in a suite of quantitative traits caused by a manipulation of gene flow. Artificial introduction experiments using Trinidadian guppies provided an opportunity to compare two native recipient populations before and ten generations following gene flow from populations originally adapted to a different environment. We measured a suite of fitness-related traits in a common garden before and after gene flow. We interpreted our results in light of *a priori* predictions based on evolutionary theory and extensive background information about guppies and our focal populations. We found that gene flow caused genetically based shifts in most traits, but whether traits shifted in an adaptive direction towards or away from the source depended on the trait and initial conditions of the population. Our results highlight the importance of considering drift in recipient populations and confirm that gene flow does not play a singular role in phenotypic evolution.

Introduction

Evolutionary theory predicts that gene flow should reduce phenotypic divergence among populations by homogenizing allele frequencies at loci affecting traits (Garcia-Ramos and

Kirkpatrick, 1997; Haldane, 1948; Slatkin, 1978). As such, gene flow is often considered a constraining force that limits local adaptation within a species (Lenormand, 2002; Mayr, 1963). Evidence for this in nature is provided by the commonly documented positive relationship between levels of genetic and phenotypic divergence (Calsbeek and Smith, 2003; Hendry and Taylor, 2004; King and Lawson, 1995; Nosil and Crespi, 2004). In addition, experimentally reduced gene flow has been shown to increase phenotypic differentiation (Nosil, 2009; Riechert, 1993). However, conflicting evidence of phenotypic divergence among populations that experience high gene flow suggests that gene flow does not play a purely constraining role in evolution (Fitzpatrick et al., 2015; Hoekstra et al., 2004; Moody et al., 2015; Saint-Laurent et al., 2003). Indeed, 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). Thus, the balance between selection and gene flow may diminish maladaptive phenotypic effects of gene flow on strongly selected traits (Hendry et al., 2001). The interaction between gene flow and inbreeding caused by genetic drift can also cause phenotypic change. For example, gene flow into small, inbred populations can mask deleterious alleles and reduce occurrence of detrimental traits (Keller and Waller, 2002). Due to the complex interactions of gene flow, drift, and selection we have a relatively poor understanding of the extent to which gene flow drives phenotypic evolution in nature (Garant et al., 2007; Guillaume and Whitlock, 2007), yet determining the role that gene flow plays in adaptive diversification is a fundamental goal of evolutionary biology (Bolnick and Nosil, 2007; Ehrlich and Raven, 1969; Endler, 1973; Hendry et al., 2001; Lenormand, 2002; Mayr, 1963; Slatkin, 1987).

There are several reasons it has proven difficult to evaluate how gene flow affects adaptive divergence among natural populations (Garant et al., 2007). 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, and therefore the genetic basis of these traits is unknown, due to a lack of either extensive pedigrees or common-garden experiments (Merilä and Hendry, 2014). But 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 if populations are in the early stages of ecological speciation (Räsänen 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). Thus, populations may become phenotypically divergent in the absence of local adaptation, in which case homogenizing gene flow would not constrain adaptation *per se*. 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*) provided 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 of traits with respect to the environment. Trinidadian guppies are a model system for studying rapid adaptation in the wild (Endler, 1980; Magurran, 2005; Reznick, 1997; Reznick et al., 1990). Divergent selection pressure typically associated with level of predation has resulted in genetically based adaptive differences that have evolved 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). Extensive gene flow from introduced populations has been documented throughout native populations at far downstream distances (Fitzpatrick et al., 2015; Shaw et al., 1992; **see Ch.3**). Despite this high gene flow from originally divergent introduced populations, guppy phenotypes from downstream populations were consistently well matched to their local predation regime (Fitzpatrick et al., 2015; **see Ch.3**), providing further evidence for strong deterministic selection of similar traits in similar environments. Yet, whether this adaptive phenotypic divergence was genetically based or being 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 (Handelsman et al., 2013, 2014; Reznick and Bryga, 1987), 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 existed downstream of introduction sites. We first measured a suite of traits from descendants of wild caught guppies captured in two focal sites prior to gene flow from the upstream introduction experiment, as well as those collected from the introduction source site (Figure 4.1A). We then replicated the common garden assay using guppies captured at the same low predation focal sites approximately 10 guppy generations after gene flow from the upstream introduction experiments

that were conducted as part of a separate study by D. Reznick and colleagues (Travis et al., 2014). 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 (Figure 4.1B; **see Ch. 5**).

We took advantage of strong *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) and invertebrate (Zandona et al., 2011) communities, abiotic characteristics and resource levels (Kohler et al., 2012), and phenotypic variation of guppies (Bassar et al., 2013; Torres-Dowdall et al., 2012a) have been previously characterized for the drainage in our study, providing a 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 due to founder effect as these populations are typically colonized by one or a few individuals (Baillie, 2012; Barson et al., 2009; Crispo et al., 2006). 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 4.1), even compared to other upland populations found throughout Trinidad (Figure 4.2). As expected when populations experience substantial drift, non-parallel phenotypes were previously documented despite the similarity of the environment with respect to predation (Fitzpatrick et al., 2014; Torres-Dowdall et al., 2012a). However, selection could also explain phenotypic non-parallelisms if there were non-predator related environmental differences between these two streams.

We developed *a priori* predictions about how traits would respond to gene flow based on evolutionary theory, the wealth of knowledge previously developed for 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. Under the first hypothesis, as generally predicted by theory, we expected gene flow to cause traits to become more similar to the source population, thereby constraining divergence (Figure 4.3A-C). However, field measurements of traits suggest that locally adapted traits are maintained despite gene flow from an originally divergent source (Fitzpatrick et al., 2015; **see Ch.3**). Thus, if this divergence has a genetic basis and natural selection is strong enough to overcome gene flow, an alternative hypothesis is that divergence is maintained despite gene flow (Figure 4.3D-F). This outcome could further be anticipated, given the expectation that introduced populations are themselves evolving towards a low predation ecotype. However, this study only captures gene flow from the earliest generations of the introduced population.

We also 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 drift could therefore be eroded by gene flow as both populations converge to become more similar to the source (Figure 4.3B). Additionally, if traits did not show initial divergence across predation regime, under the "g*ene flow constrains divergence*" hypothesis, we would expect no change in traits following gene flow (Figure 4.3C). However, under the "*divergence in the face of gene*

flow" hypothesis, if initial non-parallelisms were indeed caused by drift, we might expect a non-parallel response to gene flow and selection resulting in post-gene flow traits that match the expected direction of divergence across the predation regime (solid grey line in Figure 4.3E). But if initial non-parallelisms were due to differences in the selective environment between the neighboring low predation streams, under this hypothesis we would expect the non-parallelism to be maintained (dashed line in Figure 4.3E). Finally, 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 post-gene flow populations (Figure 4.3F). Testing these predictions in a system amenable to experimentation in both wild and laboratory environments provided a novel opportunity to interpret the effects of gene flow on phenotypic evolution of adaptive traits.

Methods

Field sampling & 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, headwater tributaries (Taylor River and Caigual River) and the high predation river (Guanapo) that served as the source for introduction experiments were sampled prior to introductions. Between January and April of 2008, 25–30 males 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 10 guppy generations after high predation guppies from the Guanapo site were introduced into previously guppy-free sites upstream from native Taylor and 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. Details about numbers sampled and population genetic parameters before and after gene flow are provided in Table 4.1.

We conducted two common garden assays using identical lab protocols to estimate 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. We did not include the Guanapo population in 2011 due to concerns that high levels of gene flow from upstream introduction experiments confounded 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 Handelsman et al., 2013; Ruell et al., 2013; Torres-Dowdall et al., 2012). Females were randomly outcrossed with unique males to produce first generation 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

All traits measured in this study 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 Bryga, 1996; Reznick and Endler, 1982). 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 on, as in high predation environments (Bassar et al., 2013). Guppy body shape has also been shown to exhibit 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 (Alexander et al., 2006; Robinson and Wilson, 1995). Increased male coloration evolves in low predation environments in response to strong sexual selection, whereas inconspicuous males are naturally selected for in high predation localities (Brooks and Endler, 2001; Endler, 1980).

To measure male and female life history traits, second-generation (G_2) juveniles were first anesthetized with tricaine methanesulfonate (MS-222) at 29 days and sexed. At the age of 4 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 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 becomes even with the tip of their 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, USA).

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 (Rohlf & 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 (**see Ch.3**). 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). 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 and 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. We also tested for a relationship between these shape axes and centroid size using linear regression. 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. All morphological

analyses were conducted using the 'geomorph' package in R v3.1.3 (Adams and Otárola-Castillo, 2013).

Male coloration at maturity was quantified using traditional outline methods from 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 standardized by body area, *ii*) total number of distinct color elements, and *iii*) total area of orange standardized by 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 ID was included as a fixed effect with stream included as a random effect (following Table 4.1). 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 et al., 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).

Results

Support for the 'gene flow constrains divergence' hypothesis

Six out of the eleven traits we measured before gene flow and again ten generations after the onset of gene flow supported the hypothesis that gene flow homogenizes traits (Figure 4.4A-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 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. Given that we know increased coloration is strongly preferred by female guppies, the apparent homogenization caused by gene flow in these traits could be due to the combined effects of selection and 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. Age at male maturity was also consistent with the *'gene flow constrains divergence*' hypothesis. 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 (Figure 4.4C).

Support for the 'divergence in the face of gene flow' hypothesis

Age at female maturity (Figure 4.5A) 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 the Taylor, but age at maturity shifted later (in the predicted adaptive direction), enough to be considered significantly different from the source. Several other 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 (Figure 4.5B-C). Female body shape axis PC1 and male body shape axis PC2 also exhibited divergence away from the source population. However, we found these shape axes that diverged to be more correlated with body size (female PC1: R^2=0.16, p<0.001; male PC2: R^2=0.24, p<0.001) than the shape axes that were homogenized by gene flow (female PC2: R^2=0.02, p=0.10; male PC1: R^2=0.02, p=0.11). Therefore, it is likely that morphological changes in in female PC1 and male PC2 observed in post-gene flow populations were due to the substantial reductions in size at maturity.

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 10 generations after gene flow from a source population that was originally adapted to a different environment. Gene flow is recognized as one of the classical evolutionary

forces, but its role in shaping phenotypic evolution among natural populations remains controversial (Ehrlich and Raven, 1969; Ellstrand, 2014; Garant et al., 2007; Mayr, 1963; Räsänen and Hendry, 2008). Our study provided a rare opportunity to test how a recent onset of gene flow affected genetically based changes in traits with known adaptive significance, and our results indeed attest to gene flow's "multifarious" effects (Garant et al., 2007).

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 (Figure 4.1B) 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 4.1). 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 probably 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 benthic versus limnetic stickleback (Hendry et al., 2002). But despite these differences, we also observed constraints on adaptive divergence in some traits, similar to previous studies.

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 (Allendorf et al., 2001; Crispo et al., 2011; Currat et al., 2008). 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 small and genetically depauperate populations in order to manage imperiled populations (Aitken and Whitlock, 2013; Carlson et al., 2014; Tallmon et al., 2004; Weeks et al., 2011; Whiteley et al., 2015).

Although we focused our study on univariate trait responses, we also recognize that organisms are integrated and 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). The amount and direction of evolutionary change in quantitative traits depends on underlying genetic architecture and correlations among traits known as the genetic variance-covariance matrix, or the **G**-matrix. Theoretical work shows that gene flow can affect the structure and stability of the **G**-matrix, especially when migration rates are high (Guillaume and Whitlock, 2007), as in our study. Our design was not amenable to comparisons of **G**-matrices; however, bivariate correlations between principal component axes of male body shape revealed genetically based divergence in trait correlations that responded to gene flow (Figure S4.1). These observed changes fit theoretical predictions made by Guillaume and Whitlock (2007). Namely, gene flow caused both populations to experience an overall increase in genetic variation (i.e., overall larger ellipses), and in the Taylor, the major axis of variation was rotated

to reflect an exaggerated expansion in the direction of initial divergence between HP source and native-Taylor (i.e., increased variation along PC1 after gene flow).

Finally, 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; **see Ch.3**). But the extent to which observed phenotypic divergence across the predation gradient is genetically based or being maintained through plasticity cannot be discerned from 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. 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 removed, and the common garden environment was highly controlled. We found that gene flow induced genetically based phenotypic evolution for ten out of eleven traits in our study. Whether these changes shifted in parallel in the two focal populations, and whether traits moved towards or away from the source population depended on the trait and population. We interpret these findings in light of our *a priori* predictions and our understanding of the guppy system to inform the question of how gene flow shapes phenotypic evolution.

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 the majority of traits we measured (Figure 4.4A-F). Although we cannot directly link these traits to fitness in our study, body shape and life history traits that fit the *'gene flow constrains divergence'* pattern (Figure 4.4A-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. However, we cannot distinguish whether early male maturity in the Taylor was constrained by gene flow or whether this life history anomaly (early maturation in a low predation environment) is under selection from non-predator induced sources of mortality (Fitzpatrick et al., 2014).

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., higher 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). One possible explanation for the initially low coloration in native Caigual and Taylor, which is consistent with the extremely low neutral genetic variation observed before gene flow (Table 4.1), is that pre-gene flow populations were limited by a lack of genetic variation to evolve high coloration. Indeed, inbreeding is known to reduce coloration in guppies (Johnson et al., 2010; Van Oosterhout et al., 2003; Sheridan and Pomiankowski, 1997). 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 (Figure 4.2). 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 (Figure 4.4D-F). Furthermore, increased coloration has been shown to be one of the fastest traits to evolve within introduced guppy populations (Endler, 1980; Reznick et al., 2008), and evolution for increased coloration in upstream populations could have caused subsequent gene flow to positively affect this trait.

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 (Figure 4.5A). Age at female maturity therefore fits the prediction under our *'divergence in the face of gene flow'* hypothesis. Delayed female maturity 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 (Seghers, 1973), 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 that we found to be correlated to body size (Figure 4.5B-E), but these traits diverged in the presumed maladaptive direction. We attribute this finding 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 wild (Fitzpatrick et al., 2015; **see Ch.3**). 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 produced the observed patterns in body size. Growth rate and size at maturity have been shown to be highly plastic in guppies (Handelsman et al., 2013; Krause and Liesenjohann, 2012; Torres-Dowdall et al., 2012a). In fact, the source population in our study and other low predation populations have been shown to plastically respond to environmental cues in size and morphology but not age at maturity (Handelsman et al., unpublished data). One theory is that a chronic stress response, such as alteration in cortisol levels (Fischer et al., 2014), could reduce growth. Thus, it is possible that an unknown stressor in the post-gene flow lab environment induced this plastic response.

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 (Garant et al., 2007; Slatkin, 1987). As predicted by theory, we showed that most traits were homogenized by gene flow. However, our results showing an increase in male coloration after gene flow suggest that it does not necessarily constrain adaptation, especially if recipient populations may have experienced high drift. 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 gene flow. Given that many of the traits found to resemble the high predation ecotype in the common garden showed local adaptation in the wild (Fitzpatrick et al., 2015; **see Ch.3**), 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 (Gomulkiewicz et al., 1999), but has the potential to quickly erode local differentiation. Determining the conditions under which gene flow constrains or facilitates phenotypic evolution will contribute to our understanding of adaptive evolution in wild populations.

Table 4.1. 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 10 microsatellite loci as described in Fitzpatrick et al. (2015; **see Ch.3**).

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

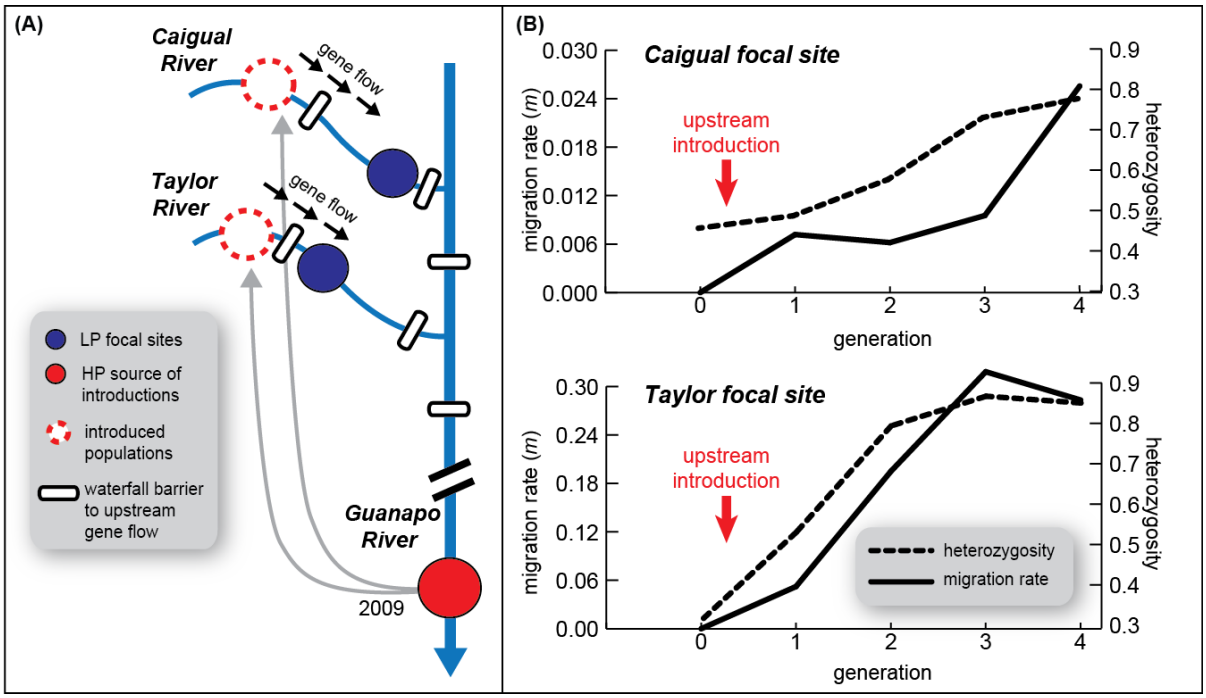

Figure 4.1 (A) Schematic diagram of the introduction scenario that provided the ability to test the effects of gene flow from guppies originating from an adaptively divergent high predation (HP) source population (solid red) into two native low predation (LP) sites (solid purple). Introduction sites (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 and *H* is based on 12 microsatellite loci detailed in Fitzpatrick et al. *unpublished data* (**see Ch.5**).

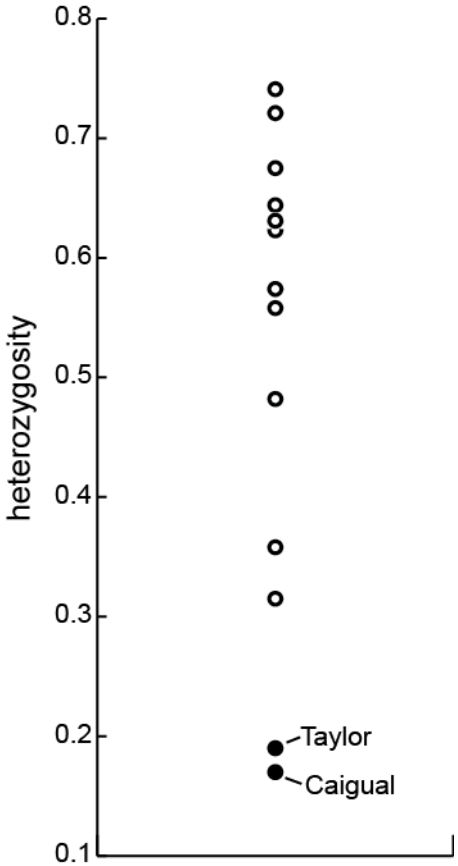

Figure 4.2 Comparison of heterozygosity estimates from 13 low predation guppy populations found in distinct headwater tributaries throughout the Northern Range mountains in Trinidad. All estimates are based on averages across the same ten microsatellite loci described in Fitzpatrick *et al.* 2015 (**see Ch.3**). Filled circles correspond to the native Caigual and Taylor populations that were the focus of this study. Un-filled circles represent other upland sites that were sampled in a range wide population genetics survey (Baillie, 2012).

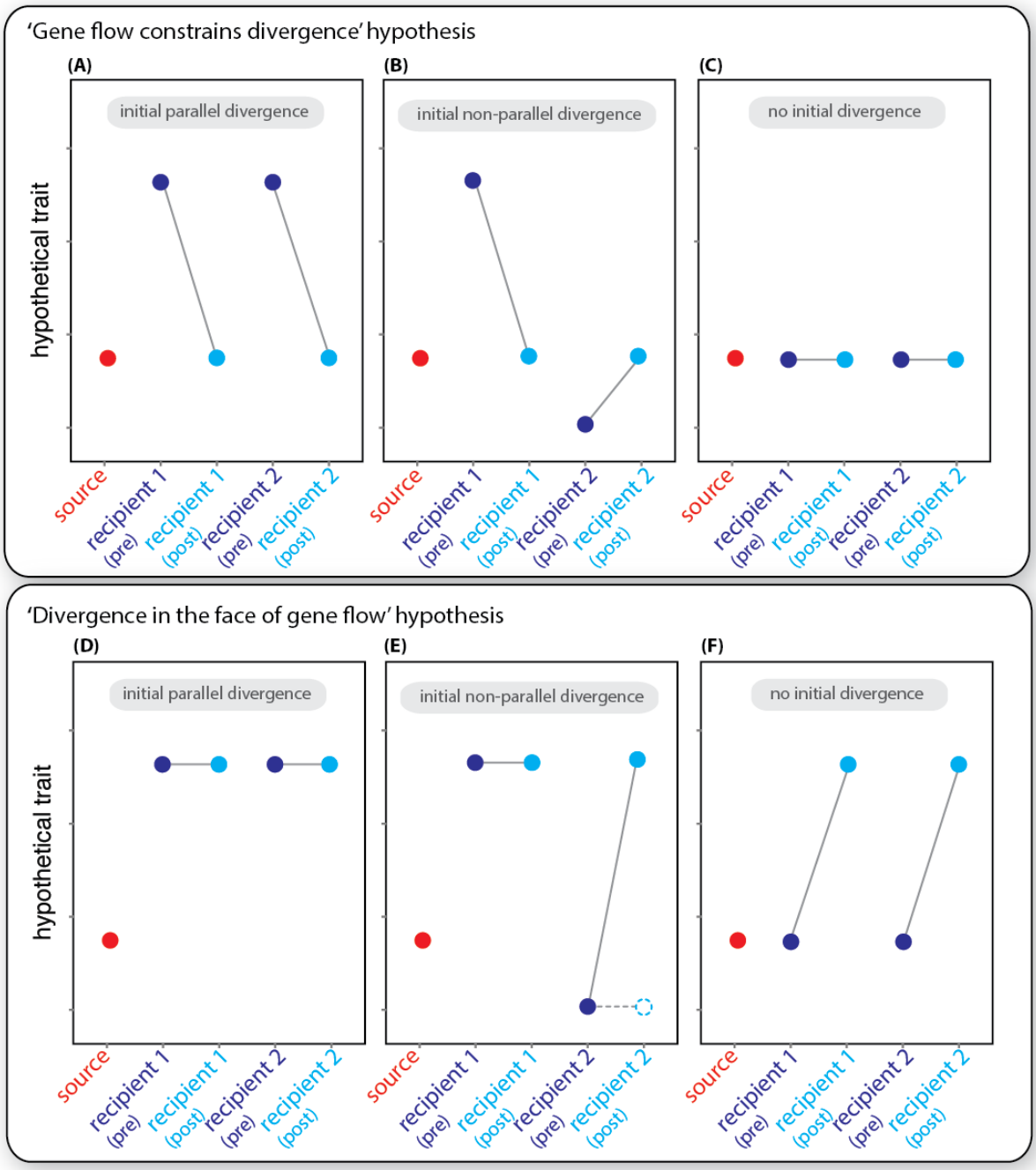

Figure 4.3 Predicted trait responses depending on the role of gene flow and patterns of initial divergence between high predation source and native low predation populations. Under the *'gene flow constrains divergence*' hypothesis, traits will resemble the source population after gene flow **(A-C)**. Under the *'divergence in the face of gene flow'* hypothesis, traits will diverge from the source population after gene flow **(D-F)**, unless selection favors a high predation-like phenotype in the Taylor (dashed line in **E**). Differences among the three predictions under each hypothesis are based on initial patterns of trait variation before gene flow.

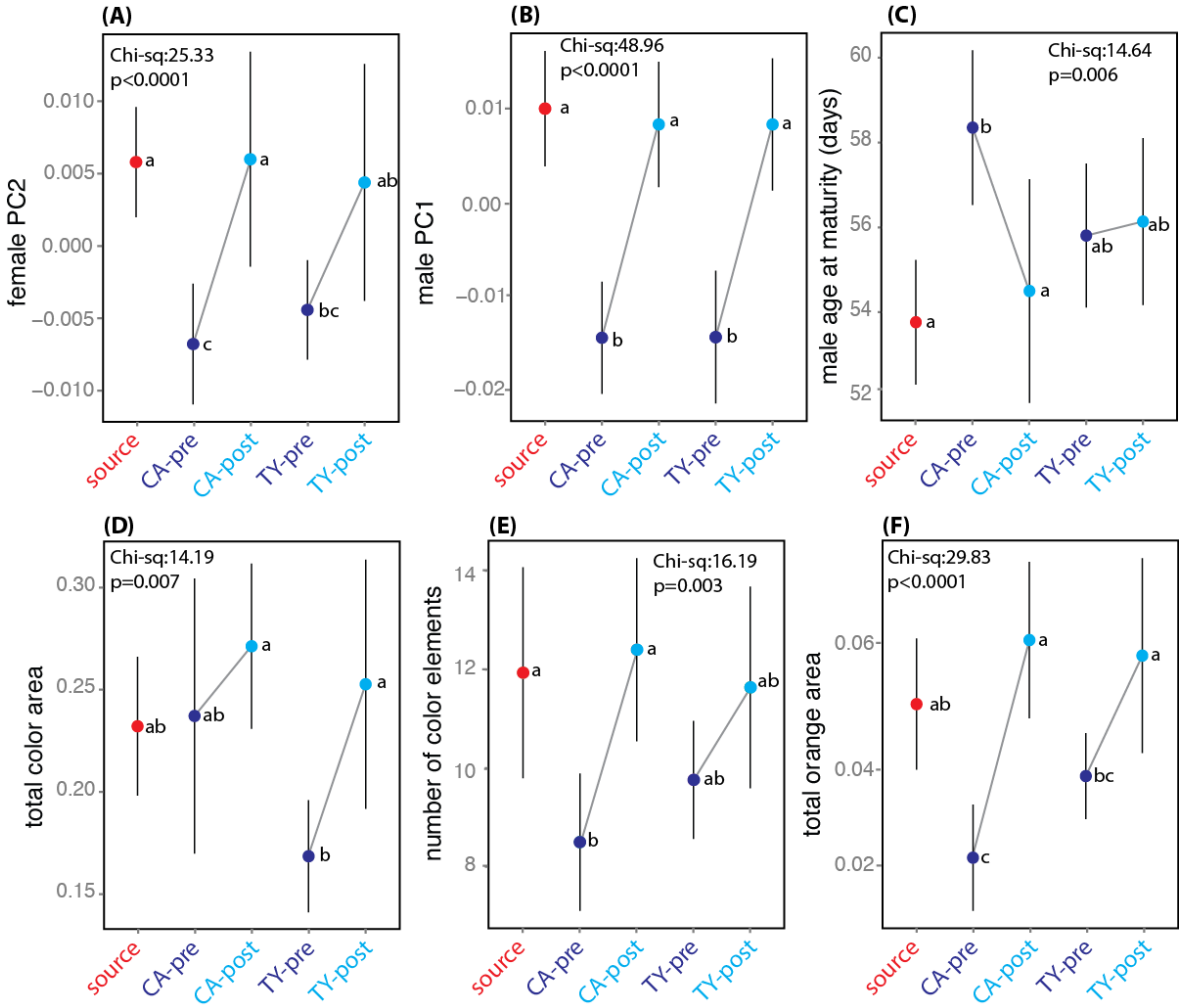

Figure 4.4 Means and 95% confidence intervals for six traits that conformed to the *'gene flow constrains divergence*' hypothesis: **(A)** Female body shape PC2, **(B)** male body shape PC1, **(C)** male age at maturity, **(D)** total color area, **(E)** number of color elements, and **(F)** total orange area. Population IDs on x-axes correspond to population summaries in Table 4.1. Chi-squared statistics correspond to the likelihood ratio test described in the text. Lowercase letters indicate significant differences among populations.

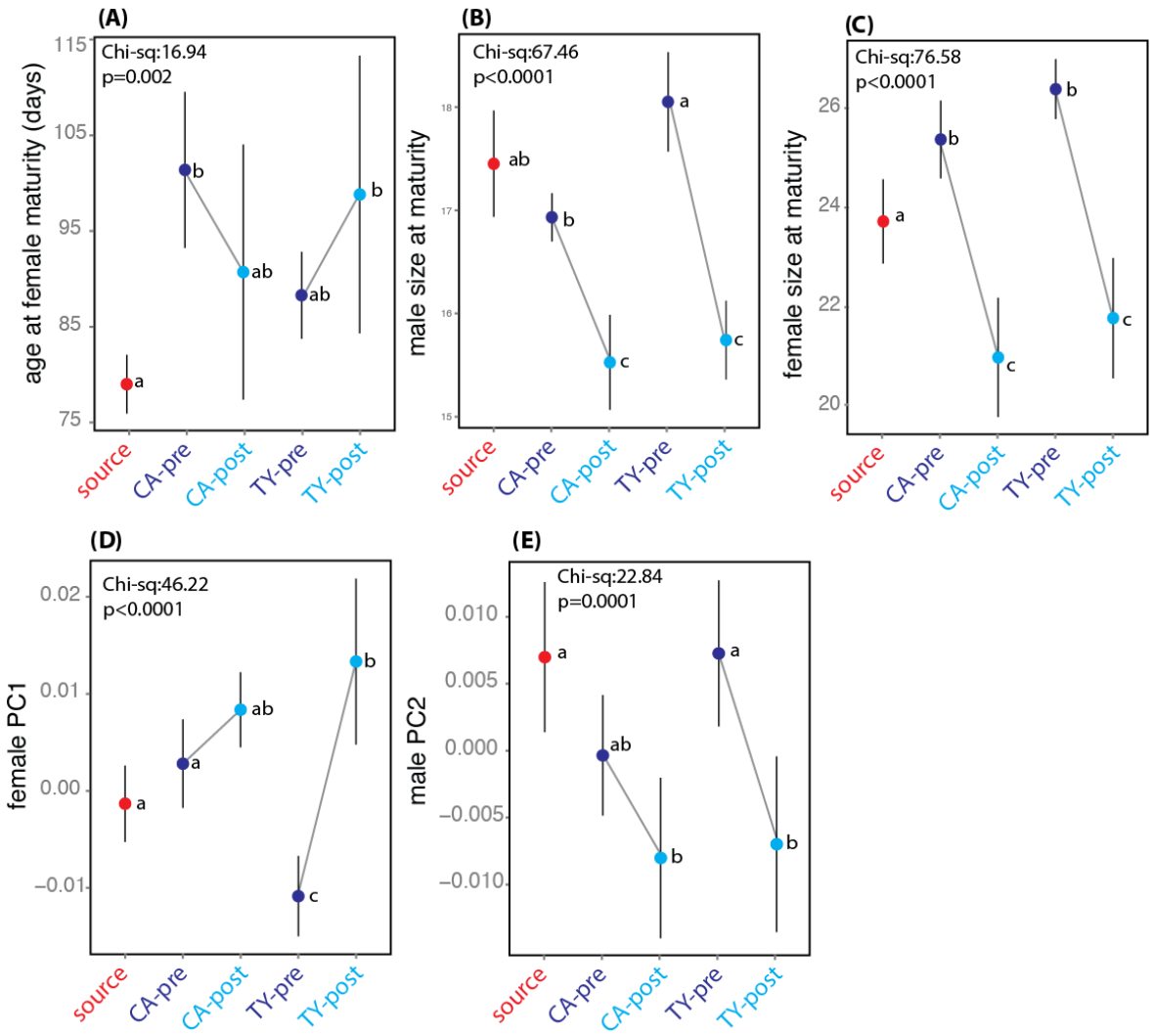

Figure 4.5 Means and 95% confidence intervals for five traits that conformed to the *'divergence in the face of gene flow*' hypothesis: **(A)** age at female maturity, **(B**) male size at maturity, **(C)** female size at maturity, **(D)** female PC1, and **(E)** male PC2. Population IDs on x-axes correspond to population summaries in Table 1. Chi-squared statistics correspond to the likelihood ratio test described in the text. Lowercase letters indicate significant differences among populations.

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5. GENE FLOW FROM AN ADAPTIVELY DIVERGENT SOURCE CAUSES GENETIC RESCUE IN REPLICATE WILD POPULATIONS

Summary

Genetic rescue, an increase in population growth owing to the infusion of new alleles, can increase fitness of small populations, but its use as a management tool is limited by poor understanding of the effects of gene flow on local adaptation and demography. Experimental translocations provide an ideal opportunity to monitor the demographic consequences of gene flow. In this study we take advantage of two experimental introductions of Trinidadian guppies to test the effects of gene flow on downstream native populations. We individually marked guppies from the native populations to monitor population dynamics for 3 months before and 26 months after gene flow. We genotyped all individuals caught during the first 17 months at microsatellite loci to classify individuals by their genetic ancestry: native, immigrant, F_1 hybrid, or F_2 hybrid. Our study documents genetic rescue over 6-8 generations under fully natural conditions. Within both recipient populations, we found substantial and long-term increases in population size, survival, and population growth rate that could be attributed to immigration and gene flow from the introduction sites. Our results suggest that low levels of gene flow, even from a different ecotype, can provide a substantial demographic boost to small populations, which may allow them to withstand environmental stochasticity.

Introduction

The fate of wild populations exposed to environmental variation is determined by an interplay between genetic variation and demography (Lande 1988). Small populations are vulnerable to

the loss of genetic variation due to drift and inbreeding, which in turn may cause population decline and an inability to adapt to changing environments (Keller and Waller 2002; Spielman et al. 2004). A lack of genetic diversity has been implicated in many population and species extinctions (Newman and Pilson 1997; Saccheri et al. 1998; Fagan and Holmes 2006). Given that *de novo* mutations arise too slowly to benefit genetically imperiled populations (Lande 1980), one way to reconnect recently fragmented small populations, or infuse genetic variation into inbred populations, is through managed movement of individuals or gametes (Weeks et al. 2011; Aitken and Whitlock 2013; Carlson et al. 2014). Ideally, gene flow caused by assisted migration would result in "genetic rescue", defined as an increase in population growth owing to the infusion of new alleles (Tallmon et al. 2004). Genetic rescue presents a possible temporary solution, albeit contentious, for curtailing the loss of imperiled populations (Edmands 2007; Whiteley et al. 2015), and has successfully caused the rebound of high profile species like the Florida panther (Johnson et al. 2010b) and the Rocky Mountain bighorn sheep (Hogg et al. 2006). However, use of this management strategy remains controversial and perhaps under-utilized due to concerns that outbreeding depression will cause reduced fitness of offspring between genetically divergent parents (Hufford and Mazer 2003; Frankham et al. 2011).

Predicting the success of genetic rescue as a management tool remains a challenge, largely due to our poor understanding of the fitness effects of gene flow (Garant et al. 2007). Theory predicts that gene flow can boost fitness when recipient populations are small and inbred (Slatkin 1985), but depending on the strength and direction of selection in different environments, excessive gene flow may homogenize populations, constrain local adaptation, and ultimately reduce fitness (Garcia-Ramos and Kirkpatrick 1997). While phenotypic divergence is often reduced between highly connected populations (Lu and Bernatchez 1999; Hendry and Taylor

2004; Nosil and Crespi 2004), some studies have documented adaptive divergence in the face of high gene flow (Fitzpatrick et al. 2015; Moody et al. 2015), suggesting selection may overcome homogenizing effects of gene flow. Thus, we still lack an understanding of the net effects of gene flow on fitness, particularly when immigrants are from a divergent ecotype but the recipient population is small, and thus potentially inbred.

Despite its practical importance, rigorous tests of genetic rescue in wild populations are rare (Whiteley et al. 2015). Most studies are limited to comparing fitness components between locally adapted individuals and early-generation hybrids, and long-term genetic rescue studies are uncommon. Multi-generational studies in the wild are crucial because an increase in individual fitness measured in one or several traits in the lab may not reflect the outcome of gene flow on demography for several reasons. First, successful genetic rescue ultimately depends on population growth rate and not individual fitness. Second, theory predicts that the effects of gene flow will vary over time (Dobzhansky 1948). For example, a study on marine copepods showed that heterosis in F_1 hybrids was followed by a decrease in fitness in later generations due to the breakdown of co-adapted gene complexes (Edmands 1999). Finally, the effects of gene flow on fitness can be very different under laboratory than natural conditions (Armbruster and Reed 2005). In the wild, environmental stress can exacerbate the effects of inbreeding depression and magnify heterosis following gene flow (Keller and Waller 2002). Furthermore, maladapted immigrants may contribute little to the breeding population (Sakai et al. 2001), as often documented when hatchery reared individuals are used to supplement small native populations (Fitzpatrick et al. 2014a; **see Ch.2**).

In this study we take advantage of recent introduction experiments of Trinidadian guppies *Poecilia reticulata* in the wild to overcome the above limitations. Specifically, we tested the

initial and sustained effects of gene flow between populations of guppies locally adapted to streams with different predator regimes. Guppies adapted to predators were introduced upstream of naturally occurring populations in headwater streams lacking most predators. Native populations of guppies isolated in headwater tributaries are typically small and genetically depauperate and thus provide a model for endangered populations that are fragmented and potentially inbred. Artificial translocation experiments were designed by D. Reznick and colleagues to study eco-evolutionary feedbacks in rapidly adapting populations (Travis et al. 2014). Two of these introductions were conducted upstream from small, native populations of guppies and thus we expected unidirectional, downstream gene flow to occur. To test the demographic consequences of this gene flow on native populations, we used genetic sampling paired with capture-mark-recapture monitoring to track changes in population vital rates (survival and population growth) over ~10 generations. This allowed us to assess whether gene flow from a divergent population results in an overall reduction or increase (rescue) in individual vital rates and population growth.

Methods

Experimental set-up in the wild

Trinidadian guppies are a model system in evolutionary ecology because they have provided some of the best evidence for rapid adaptation in response to divergent selection (Reznick et al. 1990; Reznick 1997; Magurran 2005). Waterfall barriers found throughout streams of the Northern Range Mountains of Trinidad limit upstream dispersal and result in simple fish communities in headwater tributaries, with increasing diversity in lower elevation and high-order rivers (Gilliam et al. 1993). Guppies in low elevation streams below waterfalls coexist with a suite of fish that prey on guppies, while most of these predators are excluded from streams at

higher elevations. Throughout independent drainages across Trinidad, guppies in high predation (HP) versus low predation (LP) sites show mostly parallel adaptive differences in life history (Reznick and Endler 1982), behavior (Seghers 1974), color (Endler 1980), and morphology (Alexander et al. 2006). Additionally, guppy populations in upland LP environments tend to be isolated and genetically depauperate (Crispo et al. 2006; Barson et al. 2009; Baillie 2012). Thus, in our system gene flow from an originally maladapted source could either reduce fitness of recipient populations, or increase fitness through demographic and genetic factors.

We began monitoring two native guppy populations of low predation sites in January 2009. Three months later the abovementioned introduction experiment (Travis et al. 2014) was initiated when 150 individuals descended from a high predation locality were introduced into stream reaches upstream of our two study sites that were previously guppy-free (Figure 5.1A). Due to waterfall barriers limiting upstream movement, gene flow was unidirectional from the upstream-introduced populations into our downstream focal sites. At the onset of the upstream experiment, immigrants were genetically distinguishable (Figure 5.1B) and phenotypically divergent (Figure 5.1C; Torres-Dowdall et al. 2012; Fitzpatrick et al. 2015; **see Ch.3&4**) from our study populations.

Monitoring of wild populations

Our study sites were located within the Taylor and Caigual Rivers: two neighboring tropical headwater streams from the Guanapo watershed in the Northern Range Mountains of Trinidad. Stream reaches sampled in the Taylor (240 m long) and the Caigual (80 m long) were chosen because they included the upstream extent of native guppies prior to introductions, and were bound on either end by waterfalls, thereby preventing upstream movement. Due to the location

of waterfall barriers, overall distance between our study sites and the introduction sites differed between streams (Taylor, 5 m; Caigual, 700 m).

Every month from January 2009 to June 2011 (with the exception of April 2009), we recorded every individual captured that was over 14 mm (standard length). We therefore sampled a total of 29 times over 30 months, three of which were before upstream introductions (March 2009). Unmarked individuals were given a unique mark for future identification. Guppies were caught using a combination of butterfly nets, hand nets, and minnow traps. We recorded the location of all pools and riffles within the streams in order for fish to be returned to their precise site of capture. Fish were transported to the lab in Nalgene® bottles filled with stream water and held in aerated tanks separated by location and sex. Before processing, fish were anesthetized with a dilute solution of MS-222 to allow individuals to be marked and photographed. Guppies were marked under a dissecting microscope with visible implant elastomer tags (Northwest Marine Technologies, Inc.) injected subcutaneously. Each fish was given a unique combination of marks using two or three out of eight discrete marking sites, and twelve possible colors. Concurrently, an identical capture-mark-recapture protocol was conducted in upstream introduction sites (López-Sepulcre et al. 2013; Travis et al. 2014). The two studies used non-overlapping marking codes so guppies entering our focal sites from the introduction sites could be individually identified as immigrants. However, unmarked immigrants such as juveniles could also enter our focal sites. Three scales were removed from all new (unmarked) recruits each month and dried for DNA extraction. All fish were returned to their capture site one to two days after initial capture. Previous capture-mark-recapture studies on guppies have demonstrated high recapture probabilities, high mark retention, and low marking mortality using these methods (Reznick et al. 1996).

In total we uniquely marked and monitored 9590 individual guppies throughout 29 capture events (months) between 2009 and 2011. Of these, 4710 were captured in Taylor and 4880 were captured in Caigual. We recaptured 88 individuals in Taylor and seven in Caigual that had originally been marked as part of the upstream introduction experiment (Travis et al. 2014), and thus were confirmed immigrants.

Microsatellite genotyping and genetic analyses

Genetic analyses were conducted on all individuals from both streams captured during the first 17 (out of 29) months of our study. Although we were limited to 17 months of genetic monitoring due to time and resources, this timeframe captured two consecutive wet and dry seasons and ~3-4 guppy generations. We extracted genomic DNA from scale samples using Gentra Puregene Tissue Kits. Individuals were genotyped at 12 microsatellite markers developed for this study (Table S5.1). Microsatellite development and checks for neutrality are described in supplementary Appendix I. We amplified loci using Qiagen Type-It Microsatellite Multiplex PCR kits with reactions carried out following the manufacturer's recommended conditions. PCR products combined with HiDi formamide and LIZ size standard (500 GeneScan) were read on an ABI 3730xl automated sequencer (Life Sciences Core Laboratories at Cornell University). Microsatellites were visually scored using the microsatellite plug-in with GENEIOUS 7.1.7 (Kearse et al. 2012). We scored two positive controls and one negative control on each plate and found low genotyping error rate (<0.5%). In total we genotyped 3298 guppies (1807 from Taylor and 1491 from Caigual) at 12 microsatellite loci.

We evaluated changes in genetic diversity over time by binning individuals by stream and month recruited (i.e., month of first capture). We calculated heterozygosity using ARLEQUIN 3.0 (Excoffier et al. 2005) and allelic richness in the 'hierfstat' package in R

(Goudet 2005). We then used the Bayesian model-based approach implemented in NEWHYBRIDS v.1.1 (Anderson and Thompson 2002) to assign each individual to one of six genotype frequency classes: pure native, pure immigrant, F_1 hybrid, F_2 hybrid, F_1 x native backcross, F_1 x immigrant backcross. We assessed the power of NEWHYBRIDS to correctly assign individuals to genotypic classes by generating datasets of 600 simulated individuals per population using HYBRIDLAB 1.0 (Nielsen et al. 2006; see Appendix 5.1). We analyzed the simulated datasets using NEWHYBRIDS and identified posterior probability thresholds that maximized efficiency and accuracy scores (Figure S5.1) following the approach of Vähä and Primmer (2006). Optimized thresholds were then applied to the real dataset to determine each individual's genotypic class. Individuals known to have pure native genotypes (i.e., those sampled before the onset of gene flow) and a subset of those with pure immigrant genotypes (i.e., those captured with elastomer codes from introduction sites) were used as reference samples for allele frequency priors. Analyses were run using default settings for 100,000 MCMC iterations with the first 10,000 discarded as burn-in. We used Jeffreys-type priors for allele frequencies and mixing proportions. Numerous MCMC runs beginning from random starting points confirmed consistent convergence. Of 3298 genotyped individuals, 3173 were classified into genetic ancestry groups with high certainty by NEWHYBRIDS. We binned individuals with F_2 and F_1 x native/immigrant backcross categorization into a single group (referred hereafter as F_2) due to small per-month sample sizes of each of these categories on their own.

Demographic modeling

Individual capture-mark-recapture data allowed us to estimate survival while accounting for capture probability. Apparent survival (ϕ) was estimated by fitting a Cormack-Jolly-Seber (CJS) model to individual capture histories (Cormack, R.M. 1964; Jolly 1965; Seber 1965). Population

growth rate (λ) was estimated using the Pradel model (Pradel 1996), which fits a second CJS model to the individual histories reversed in time (such that the estimate of survival can be interpreted as an estimate of recruitment). All mark-recapture analyses were carried out using Program MARK v.8.0 (White and Burnham 1999). Variation in detection probability (p) was modeled with stream by month interactions in all models (described in Appendix 5.1).

We carried out two sets of mark-recapture analyses. The first set was aimed at testing for temporal changes in vital rates through time as an indicator of overall population rescue. For example, a steady decrease in monthly survival rate and/or population growth rate over time after the onset of gene flow would be consistent with a negative effect of outbreeding depression, whereas an increase in these parameters over time might suggest demographic rescue, genetic rescue, or both. For this analysis we included all 29 months of capture-mark-recapture data. The most complex models included an interaction between sex, stream, and month on survival (ϕ). This was compared to all possible model simplifications including all two-way interactions, single factors, and the constant model. The same approach was repeated for population growth rate (λ). We used a maximum likelihood approach to fit the models and compared among them using Akaike's Information Criterion adjusted for sample size AICc and AICc weights (Burnham and Anderson 2002).

A second set of models was designed to test the role of gene flow on population vital rates to distinguish between demographic and genetic rescue. If demographic rescue were solely responsible for population growth, we would expect equivalent vital rates between native and hybrid groups. However, if genetic rescue contributed to population growth, we would expect hybrids to show higher relative fitness than native fish. Using capture histories from individuals genotyped during the first 17 months of the study, we grouped individuals by stream, sex, and

genetic classification from the NEWHYBRIDS analysis described above (native, immigrant, F_1 hybrid, F_2 hybrid). Individuals with unknown genetic ancestry (N=125 out of 3,298 fish) were excluded from these analyses. We did not include time variation in these models due to small sample sizes for some genetic classes per month and because our primary goal here was to directly test overall impacts of genetic ancestry on population vital rates. The most complicated model included three-way interactions of stream, sex, and genetic ancestry on survival (ϕ) or population growth rate (λ). All model simplifications were included in the model set and compared using AICc.

In both analyses we obtained maximum likelihood estimates of parameters from the best-supported models. We tested for overdispersion using the median-ĉ method (White and Burnham 1999), and found that there was very little (ĉ=1.36, 95%CI=1.29-1.42). Detection probability was high in both streams with averaged monthly estimates in Taylor as 0.83 and 0.86 in Caigual (Table S5.2; Figure S5.2). Our high detection probabilities allowed precise estimation of parameters of biological interest (survival and population growth rate), and suggest that total number of fish captured each month provides a good proxy for overall population size.

Results

Gene flow increased genetic diversity

In the months prior to upstream introductions, genetic diversity (heterozygosity and allelic richness) was extremely low within native focal sites of both streams (Figure 5.2). However, monthly averages of genetic diversity increased in both streams following the upstream introduction, consistent with the timing of immigration from the introduction sites. Taylor started with slightly lower levels of heterozygosity and subsequently experienced the most dramatic

increase in genetic diversity over time, consistent with the larger number of confirmed marked immigrants detected in this stream.

Gene flow increased population size

Despite substantial seasonal fluctuations, both streams experienced a dramatic increase in population size throughout the course of our study (Figure 5.3). Before gene flow we captured fewer than 100 individuals in each stream. By the end of the study the Taylor population reached its highest size of 1035 individuals. The Caigual population reached its highest size in July 2010 (1075), and we captured 914 guppies on our last sampling occasion. Genetic classifications revealed temporal differences in population dynamics of the different genetic groups in each stream (Figure 5.3). Following increases in population size in May and June 2009, the number of pure native genotypes declined in both streams and almost disappeared from the Taylor population by the end of our genetic monitoring. Concurrently, immigrant genotypes increased to become a large portion of the population in Taylor while F_1 and F_2 hybrids contributed the bulk of the population by May 2010 in Caigual.

Gene flow influenced vital rates

In our first analysis that included all captured individuals, the full model that included sex, stream, and time interactions was clearly superior, with 100% of the weight of evidence for both apparent survival and population growth rate (Table S5.3). This provides strong support for sex and stream-specific temporal changes in vital rates (Figure 5.4A; Figure 5.5A). Seasonal dynamics seem to dominate temporal variation in these parameters since survival and lambda tend to be lowest during rainy season months (June-December) when resources are low and floods may wash fish downstream (Reznick 1989). However, both males and females in Taylor

showed a steady increase in monthly survival over the second year of our study (Figure 5.4A), suggesting that gene flow might have increased this vital rate in Taylor.

We found strong support for genetic ancestry explaining variation in survival (ϕ) and population growth rate (λ) in our second set of analyses that only included capture histories from genotyped individuals. The full model (interaction between sex, stream, and genetic ancestry) was the top model for both parameters with 100% of the weight of evidence (Table S5.4). In Taylor, immigrants of both sexes had the highest survival, while female immigrants had highest survival and male immigrants had lowest survival in Caigual (Figure 5.4B). However, uncertainty in immigrant survival rates was large for Caigual, owing to the low number of immigrants captured. Instead, F_1 and F_2 hybrids in Caigual had consistently highest survival across both sexes.

Population growth rates less than one indicate a declining population. Notably, native males and females were below this threshold in both streams (Figure 5.5B). In Taylor, immigrants and F_2 hybrids had population growth rates above one, and in Caigual, immigrants and both F_1 and F_2 hybrids had increasing populations.

Discussion

We documented substantial positive effects on population fitness that can be attributed in part to gene flow (i.e., genetic rescue) in two natural populations. Immigration and subsequent hybridization with genetically and phenotypically divergent individuals led to an overall increase in within-population genetic variation, abundance, and population vital rates, though dynamic differences were observed between streams, sexes, and over time. Our results provide a detailed replicated picture of how genetic rescue operates in the wild, and add to increasing evidence that intraspecific gene flow can be beneficial, even when immigrants are adaptively divergent.

Evidence for genetic rescue

Prior to the onset of gene flow, the two native populations in our study were small and genetically depauperate. By the end of our genetic monitoring, spanning 17 months and ~3-4 guppy generations, within-population genetic diversity had more than doubled (Figure 5.2). By the end of the full capture-mark-recapture study that spanned 29 months and ~6-8 guppy generations, population sizes in both streams experienced a 10-fold increase (Figure 5.3). Observed increases in population size resulted from a combination of demographic and genetic factors.

Genotyping each individual allowed us to distinguish between demographic and genetic rescue. If the increases in population size were caused only by immigrants and their pure 'immigrant genotype' offspring, demographic but not genetic rescue would be invoked (Brown and Kodric-Brown 1977). Indeed, the demographic contribution of immigrants is considerable, especially in the Taylor where this genotype makes up more than half of the population by May 2010. Predominance of immigrant genotypes in the Taylor is likely a result of high migration rates due to the close proximity of focal and introduction sites in this stream, whereas almost 700 m separate these sites in Caigual. But we also found that hybrids contributed substantially to increases in population size in both streams (Figure 5.3). Estimates of vital rates based on genetic groups revealed that hybrids and immigrants had higher survival and static or positive population growth rates above one, whereas natives had consistently lowest survival and declining population growth rates (λ<1; Figures 5.4 and 5.5). To summarize, the occurrence of genetic rescue is evidenced by the sustained increase in population size and vital rates that can be attributed, at least in part, to the success of the hybrids.

The variation in vital rates that we observed between sexes, streams, and over time is consistent with patterns previously observed in guppies. First, female guppies tend to have higher survival than males (López-Sepulcre et al. 2013; Fitzpatrick et al. 2014b). Second, variation in abiotic and biotic factors can cause differences in guppy demography even between neighboring streams (Fitzpatrick et al. 2014b). Finally, guppy population sizes in headwater streams fluctuate temporally based on seasonal factors that impact resources and stream flow (Reznick 1989; Grether et al. 2001). Our study began in January, which is typically the start of the dry season in Trinidad, and when guppy population sizes are at their smallest as they have not yet recovered from wet season conditions (Reznick 1989). Indeed, our results show typical seasonal patterns of decreased population size throughout the wet season (June-December), followed by a recovery during the dry season (January-May). Despite these multiple sources of variation, we found consistent increases in population size throughout our study (Figure 5.3). Even if starting population sizes likely represent the smallest of the year, our study spans two subsequent wet season cycles in which populations remained well-above initial sizes. Additionally, maximum dry season population sizes in 2010 and 2011 were approximately double what they were in 2009 when populations were made up of mostly native individuals.

Factors that led to rescue over outbreeding depression

Understanding the conditions that underlie opposing fitness outcomes in response to gene flow is a major unresolved problem in evolutionary (Lenormand 2002; Garant et al. 2007) and conservation biology (Edmands 2007). The probability of outbreeding depression is generally determined by the time-since-isolation of immigrant and recipient populations, the magnitude of environmental differences and resulting level of adaptive divergence between populations, and the level of inbreeding in the recipient population (Frankham et al. 2011). For example, crossing

populations with fixed chromosomal differences or those that have been geographically isolated for millions of years is likely to result in outbreeding depression caused by the evolution of postzygotic reproductive barriers such as Dobzhansky-Muller incompatibilities (Edmands 1999; Coyne and Orr 2004). But at lesser extremes, the extent to which gene flow between adaptively divergent populations reduces overall fitness remains a grey area (Garant et al. 2007). Our study lends insight into this question, in part because of the wealth of natural history and genetic information already known about the Trinidadian guppy system.

We know, for example, that adaptively divergent guppy populations are not reproductively isolated (Crispo et al. 2006). Features of the guppy mating system such as female preference for novel male color patterns (Eakley and Houde 2004) and forced copulation by males (Evans et al. 2003) limit the development of prezygotic reproductive barriers (Labonne and Hendry 2010). And, although selection against migrants is strong when guppies adapted to low predation environments are washed downstream or disperse into high-predation environments (Weese et al. 2011), a low level of downstream gene flow does occur (Barson et al. 2009), which likely prevents accumulation of post-zygotic reproductive isolation. The introduced populations that provided the source of gene flow in our study, though phenotypically and genetically distinct to a degree, originated from a high predation locality in the same drainage as the recipient populations (Figure 5.1A) and have experienced low levels of unidirectional downstream gene flow on a contemporary timeframe (Barson et al. 2009). Thus, we would not expect these populations to have evolved post-zygotic reproductive barriers, and general lack of reproductive isolation detected in this species might make them more likely to experience genetic rescue.

Conditions of native recipient populations also likely contributed to the observed response to gene flow. Headwater riverine fish populations often exhibit high levels of local inbreeding due to small population sizes and geographic isolation (Fagan 2002). In general, upland guppy populations in low predation environments have reduced genetic variation (Crispo et al. 2006; Barson et al. 2009), and inbreeding is known to reduce fitness in guppies (Johnson et al. 2010a). 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 when compared to other low predation guppy populations throughout Trinidad (Baillie 2012). In addition, the native focal populations showed signs of potential inbreeding depression such as poor health in Taylor (Fitzpatrick et al. 2014b) and overall reduced male coloration compared to guppies from other low predation sites (**see Ch.4**). Therefore, fitness benefits from mating with unrelated, immigrant individuals may have been particularly strong if the native populations indeed had a high genetic load (Keller and Waller 2002). Even if immigrants were maladaptive for some traits, natural selection acting on the influx of genetic variation following gene flow could increase absolute fitness (Carlson et al. 2014). Indeed, heterosis or adaptive evolution may have caused the high overall rates of population growth observed in F_2 hybrids.

Characteristics of the immigrants such as certain life history traits and large effective population size may have also played a role in determining the demographic success of this group. Guppies adapted to high predation environments typically exhibit a fast life history, maturing at a younger age and producing larger broods during shorter intervals than guppies adapted to low predation environments (Reznick et al. 1990; Torres-Dowdall et al. 2012). Thus, high population growth rates of immigrants and hybrids could result from exhibiting a faster life history than native low predation populations. The demographic components that contribute to

the overall population growth rate (λ) parameter can be parsed into the relative contribution of survival and recruitment (Pradel 1996; Nichols et al. 2000). Recruits are new individuals that enter the population through reproduction and/or immigration. As expected given the high fecundity and fast life history of high predation guppies, differences in recruitment rates between immigrants/hybrids and native guppies drive the overall differences in population growth rate (Figure S3). Recent work has further shown that the fitness of the high predation phenotype is superior, even in a low predation environment, when populations are at low densities (Bassar et al. 2013). If the native populations we studied were indeed inbred, they may have existed at lower densities than what is typical for these environments, causing them to be more easily invaded by the high predation phenotype. Thus, competitive dynamics likely played an important role, and we don't necessarily interpret the decline of the native genotype representative of their trajectory had they not been exposed to competition with hybrid and immigrants.

Conservation relevance of genetic rescue in guppies

Our detailed characterization of genetic rescue in Trinidadian guppies helps fill important gaps for understanding how gene flow could be used to manage imperiled populations and species. Frankham et al. (2011) provides a flow chart of recommendations for avoiding outbreeding depression, but factors such as whether "substantial environmental differences" exist present major remaining uncertainties. In our system, predation level and resource availability are primary drivers of local adaptation in guppies (Reznick et al. 1996). The populations brought into contact by the introduction experiments were phenotypically adapted to opposite ends of these ecological gradients (Torres-Dowdall et al. 2012). Yet our results suggest that adaptive divergence does not necessarily prevent fitness benefits from gene flow.

Our study also illustrated how different rates of migration and gene flow can lead to drastic differences in genetic composition of the population. Over the first 17 months, Taylor received an average of 182 migrants per generation, while Caigual received an average of four migrants per generation. Overall, both streams experienced substantial and sustained increases in population size, regardless of migration rate. However, from a conservation standpoint, the lower migration rate in the Caigual led to a more ideal outcome where increases in population size were mostly due to success of the hybrids and pure native genotypes were maintained in the population. In contrast, high migration into the Taylor led to a near extinction of the pure native genotype, which may have led to the loss of potentially important local alleles. Determining the appropriate level of gene flow to prevent inbreeding without swamping local adaptation is a high priority goal for conservation biologists. The classic rule of thumb is one-migrant-per-generation (Spieth 1974; Mills and Allendorf 1996), yet complexities inherent to natural populations can undermine the usefulness of this rule (Vucetich and Waite 2000; Wang 2004). For example, assumptions of equal selective advantage among genotypes, similar demographic attributes among immigrants and residents, and census sizes equal to effective population sizes are typically violated in imperiled natural populations (Mills and Allendorf 1996). In our case, an understanding of the environment (i.e., immigrants are likely to survive, given the low predation) and fast life history of immigrants (i.e., immigrants are likely to have higher fecundity than natives) might have led us to the *a priori* conclusion that few migrants per generation (<10) would be sufficient to induce genetic rescue, as confirmed by the results from the Caigual.

Concluding remarks

Understanding the genetic factors that underlie demographic responses will improve our ability to manage connectivity and maintain healthy populations in the wild. The scenario we studied,

where immigrants are adaptively divergent and the resident population has low genetic diversity mimics a common situation faced by managers deciding whether to augment endangered populations. Although many questions remain, our results suggest that adaptive divergence should not, in itself, preclude the use of assisted gene flow for inducing fitness benefits, and also that low levels of migration can result in genetic rescue without losing the native genetic signature. Ultimately, sufficient habitat is necessary for long-term persistence, but genetic rescue may provide a demographic buffer that allows populations to persist through environmental disturbances, as well as the genetic variation needed to adapt to a changing world.

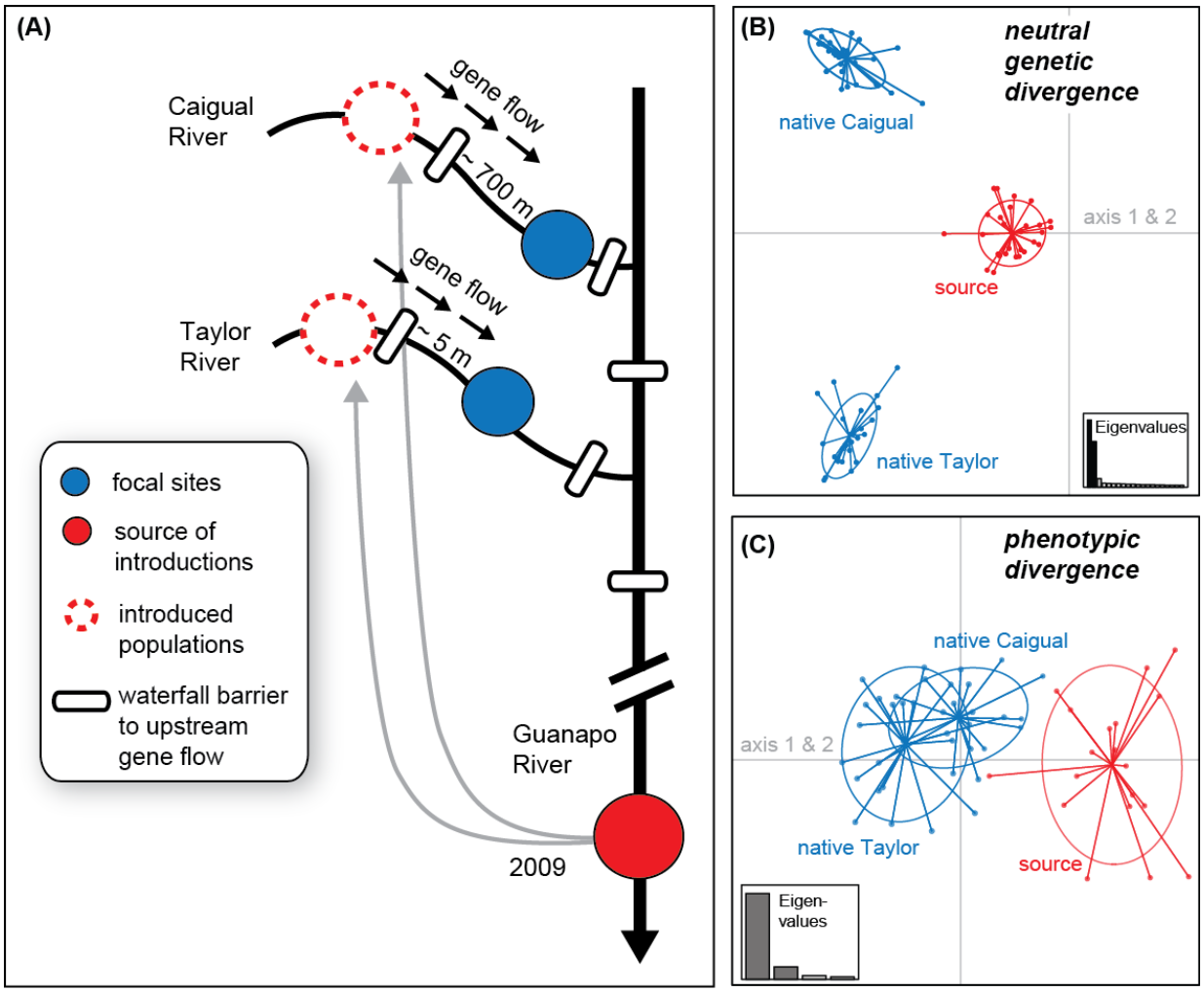

Figure 5.1 (A) Schematic of the introduction scenario that allowed us to test the effects of gene flow from guppies that originated from an adaptively divergent source population (red) into two native populations (blue). **(B)** Principal components analyses using microsatellite data highlights initial genetic divergence between the native populations (blue) and the source of the introductions (red). **(C)** Principal component analyses using phenotypic traits highlights initial phenotypic divergence between native populations and the source of the introductions. Traits included in this analysis were male life history and body shape traits from data published in Fitzpatrick *et al.* 2015 (**see Ch.3**).

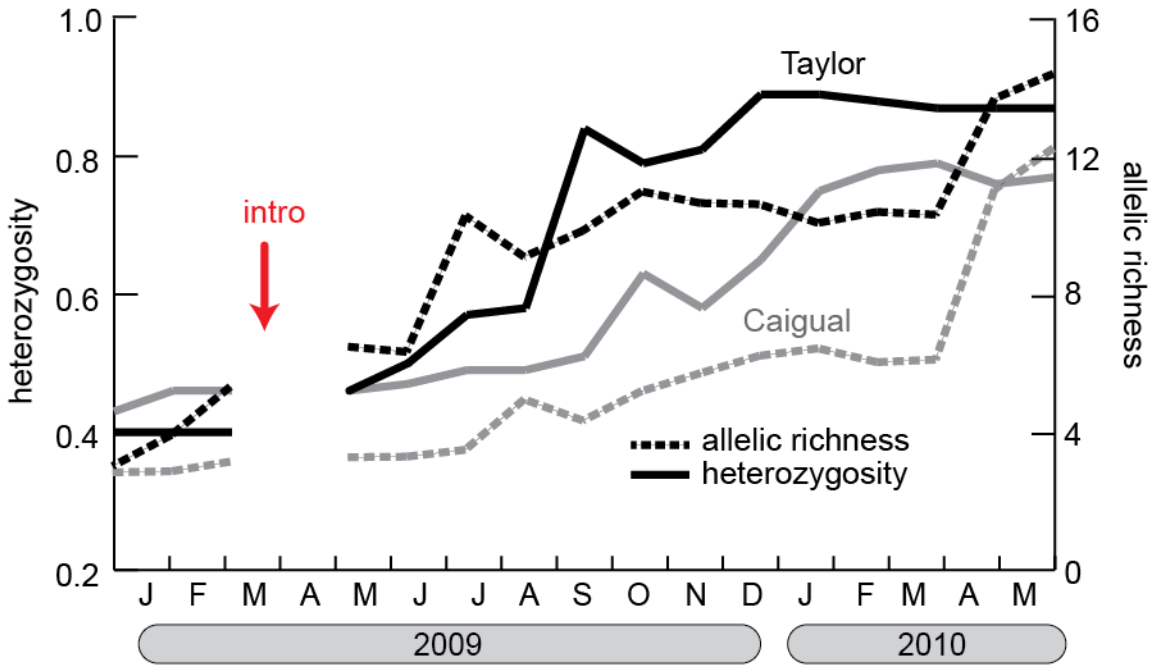

Figure 5.2 Temporal changes in within-population genetic diversity following the introductions upstream that occurred in March 2009, as indicated by the red arrow. Solid lines correspond to heterozygosity (scale on left vertical axis) and dashed lines correspond to allelic richness (scale on right vertical axis). Genetic diversity indices were calculated using genotypes from all individuals caught in a given month.

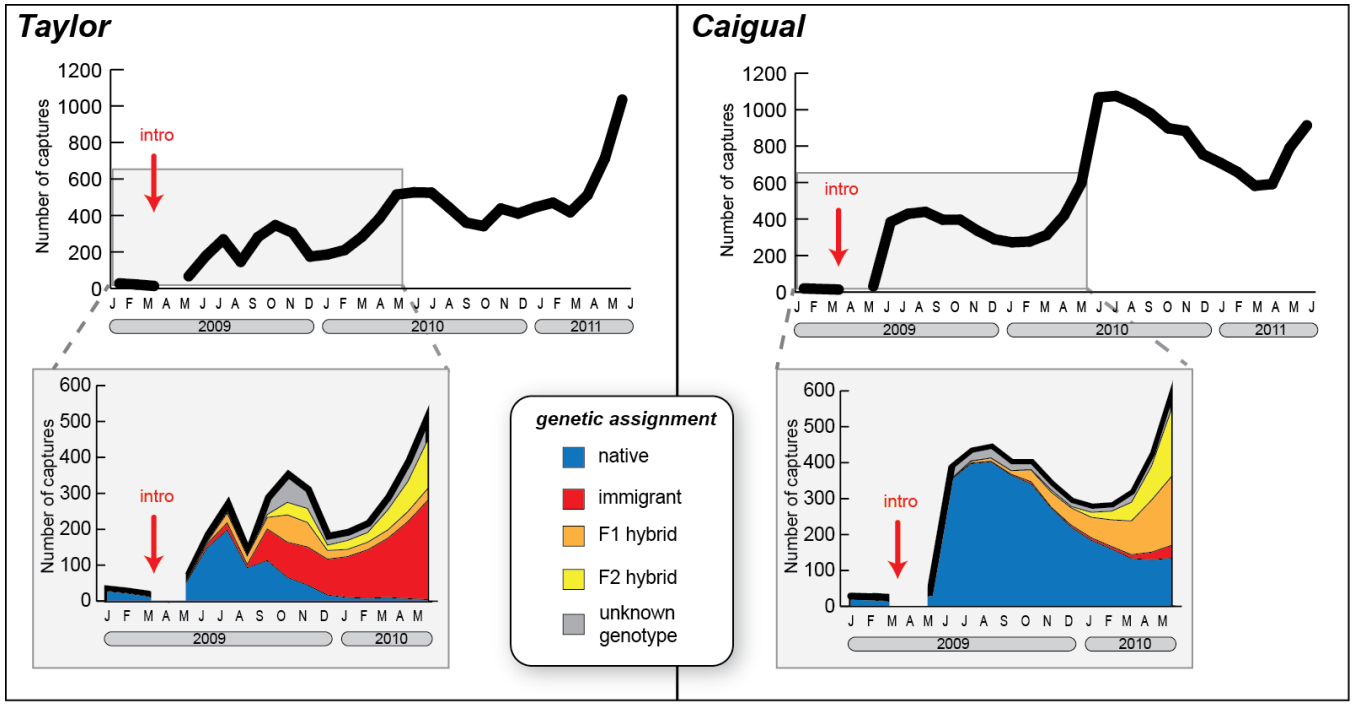

Figure 5.3 Thick black lines indicate total number of guppies > 14mm captured in each stream over time. Grey boxes correspond to the timeframe in which every individual was genotyped at microsatellite loci for classification into genetic ancestry groups. Colors show the number of individuals in each genetic group caught each month.

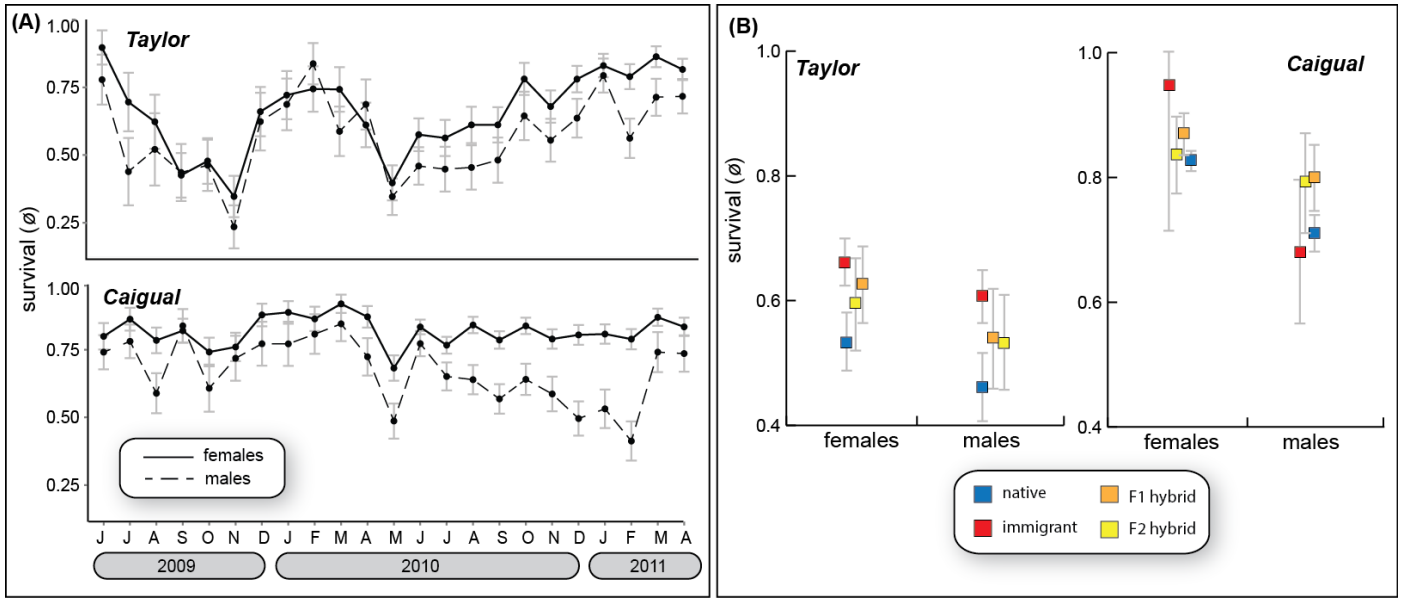

Figure 5.4 (A) Monthly estimates of survival throughout the entire duration of study. **(B)** Genetic classification estimates of survival based on 17 months of mark-recapture data. All estimates are based on best-supported capture-mark-recapture models (see Tables S5.2 and S5.3).

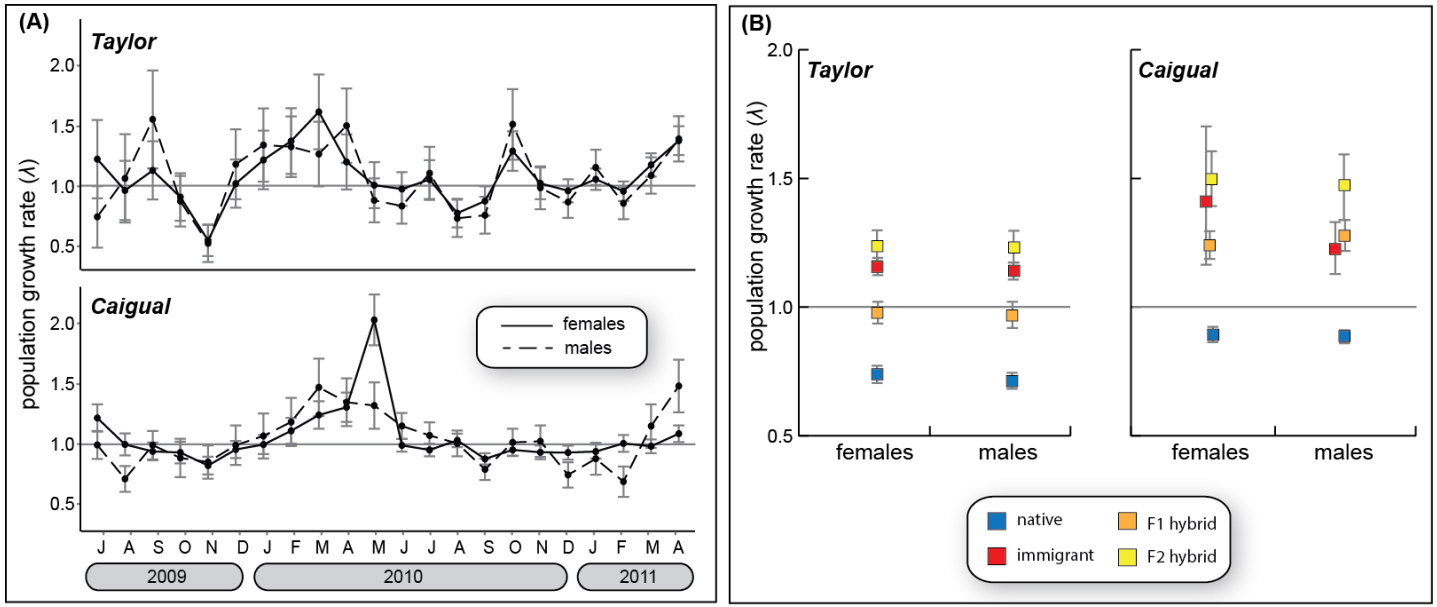

Figure 5.5 (A) Estimates of population growth rate throughout the entire duration of study. **(B)** Genetic classification estimates of population growth rate based on 17 months of mark-recapture data. All estimates are based on best-supported capture-mark-recapture models (see Tables S5.2 and S5.3).

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APPENDIX 2.1

Table S2.1 Per locus population statistics for 7 microsatellite loci used for genetic analyses.

Locus	*Etheostoma* sp. developed for	motif	*n* typed	*n* alleles	evidence for null alleles?	H_o	H_e
Eca10[1]	*E. caeruleum*	GATA	573	5	Yes (AFT12)	0.41	0.60
Eca37[1]	*E. caeruleum*	GATA	558	7	No	0.18	0.27
Eca46[1]	*E. caeruleum*	TAGA	607	6	Yes (RCR05)	0.55	0.70
Eca48[1]	*E. caeruleum*	TAGA	594	10	No	0.48	0.68
Eca49[1]	*E. caeruleum*	GATA	611	10	No	0.48	0.76
Eca71[1]	*E. caeruleum*	TAGA	554	8	Yes (RCR07)	0.20	0.38
Etsp224[2]	*E. spectabile*	TC	598	7	Yes (FTN01)	0.35	0.45

[1]Tonnis 2006; [2]Hudman et al. 2008

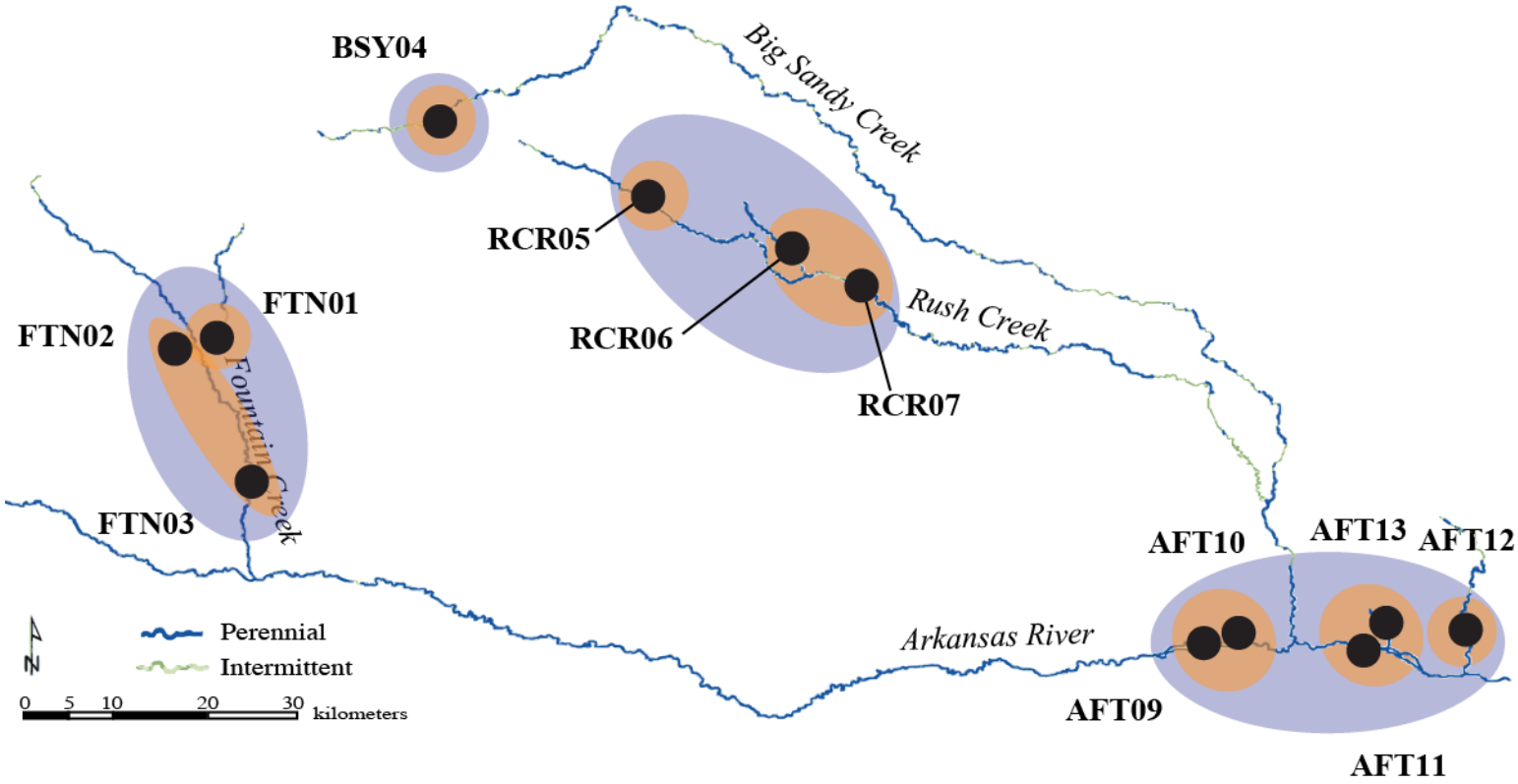

Figure S2.1 Sampling sites for Arkansas darters in Colorado with suggested management delineations discussed in the specific management recommendations section. Purple ovals designate four potential distinct evolutionary significant units. Orange ovals designate eight localized management units indicated by the STRUCTURE analysis.

APPENDIX 3.1

Photography methods

Photographs were taken with a Canon E0S Rebel T3 digital SLR camera, equipped with a Canon EFS 60mm macro lens mounted on a tripod. Tripod height was adjusted to yield a 12-cm field of view that was sufficient to eliminate any parallax within the lens area occupied by a guppy. The illumination in photographs was held constant by using a single camera with no flash, and lighting with two full-spectrum fluorescent lights that were permanently fixed on either side of the camera. All images were captured at a single location with a constant level of ambient light. To standardize fish position and expose homologous landmarks for morphology, a fine-tipped wetted paintbrush was used to straighten the specimen and spread the fins (Figure S3.1). A ruler was placed in each picture to set a scale in each image.

Microsatellite methods

Genomic DNA was extracted from scale samples using Gentra Puregene Tissue Kits and amplified using the Qiagen Type-It Microsatellite Multiplex PCR kit. PCR reactions were carried out following the manufacturer's recommended conditions and sent to the Cornell University Biotechnology Resource Center for fragment analysis on an ABI 3730xl automated sequencer. Microsatellites were visually scored using GENEMARKER software (Softgenetics, LLC, State College, PA, USA). To ensure genotyping accuracy, we included one negative and two positive controls per 96-well extraction and PCR plate. We tested for presence of null alleles using MICROCHECKER (van Oosterhout *et al.* 2006). Tests for significant linkage disequilibrium between all pairs of loci and Hardy-Weinberg equilibrium at each site were performed using GENEPOP 4.0 (Rousset *et al.* 2008). Allelic richness per site and expected and observed

heterozygosity were calculated in FSTAT v2.9.3.2 (Goudet 1995). We found no evidence of null alleles or deviations from Hardy-Weinberg equilibrium after Bonferroni correction.

Methods for STRUCTURE analyses

All STRUCTURE analyses were conducted by running 10 independent replicates for each k and used a burn-in period of 10 000 steps followed by 500 000 Markov Chain Monte Carlo (MCMC) replicates. To determine the best number of clusters we inspected likelihood values and calculated the Δk statistic (Evanno *et al.* 2005).

Table S1. Per locus microsatellite information.

Locus	Source	Repeat Motif	Primer Sequence (5' - 3')
Pret 80	Becher *et al.* 2002	$(GT)_7TGG(GT)_3GC(GT)_{15}$	F: GTACGAACTCTCTCGCAA R: TGTGGTTTAGGTTGGACTGGG
Pre9	Patterson *et al.* 2005	$(CAGA)_{13}$	F: TTGCAAGTCAGTTGATGGTTG R: TGCCCTAGGGATGAGAAAAG
Pre15	Patterson *et al.* 2005	$(GATG)_{16}$	F: CTGAGGGACCAGGATGTTAAG R: CCATAAACACGCAAACCAAC
Pre26	Patterson *et al.* 2005	$(GATG)_{19}$	F: GCTGACCCCAGAAAAGTGG R: TGGGACTTTCATGAGACTTGG
Pre-G145	Shen *et al.* 2007	$(GT)_{11}$	F: TCTCCAAACCTCCCCTGTA R: GACGAGCCTCTGCTTCTTC
Pre-G289	Shen *et al.* 2007	$(TC)_{16}$	F: ATTGGGATTGATGAGGTG R: GTGTTCCAGCAGGTCAGT
Pret27	Watanabe *et al.* 2003	$(GT)_{53}$	F: CACACGGGCTCTCATTTTT R: CTGTGTTTGTGTTCGGTCGTA
Pret28	Watanabe *et al.* 2003	$(GT)_{32}$	F: ACATCGGCGTCCTCACCT R: GGGGGTTGAAACACATCCA
Pret38	Watanabe *et al.* 2003	$(GT)_{19}$	F: AGGGAAAAGGAAAGAAAGAA R: CGAACAAGCCCAAATCTA
Pret46	Watanabe *et al.* 2003	$(CA)_{27}$	F: AACCCTAATGACTCCCAACA R: CGACCCACCAGTAATCCAA

Table S3.2 Total number of fish genotyped (*n*), number of alleles (*A*), and expected heterozygosity (H_e) for each site.

Stream	Age of introduction	Site	*n*	*A*	H_e
Turure	1957 'old'	Introduction	40	7.4	0.61
		0-500m	40	8.7	0.69
		1000m	38	9.0	0.66
		5000m	40	13.1	0.78
Aripo	1976 'old'	Native LP	39	8.6	0.56
		Introduction	40	6.0	0.53
		0m	39	7.1	0.60
		500m	40	10.0	0.64
		1000m/Source	40	12.7	0.73
El Cedro	1981 'old'	Introduction	40	3.0	0.37
		Source	40	6.6	0.61
Lower Lalaja	2008 'recent'	Introduction	39	9.5	0.72
		0m	38	11.0	0.74
		500m	40	10.6	0.73
		1000m	39	11.1	0.71
Caigual	2009 'recent'	Introduction	40	8.3	0.71
		0m – Pre Intro	19	2.2	0.17
		0m	40	10.7	0.72
		500m	40	9.6	0.70
		1000m	40	9.8	0.70
Taylor	2009 'recent'	Introduction	40	7.8	0.71
		0m – Pre Intro	18	1.9	0.19
		0m	40	8.4	0.69
		500m	40	8.4	0.62
		1000m	40	10.9	0.71
Guanapo Mainstem		5000m	40	9.3	0.62
		Source	40	11.6	0.77

Table S3.3 Pairwise-F_{ST} values for all sites in all streams. Lower triangle is pairwise matrix of F_{ST} values and upper triangle contains associated p-values.

	Aripo - Intro	Aripo - 0 m	Aripo - 500 m	Aripo - 1000 m	Aripo - Native LP	Caigual - Intro	Caigual - 0 m	Caigual - 500 m	Caigual - 1000 m	Guanapo - 5000 m	El Cedro - Intro	El Cedro - Source	Guanapo - Source
Aripo - Intro	--	0.303	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Aripo - 0 m	0.00	--	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Aripo - 500 m	0.07	0.07	--	0.077	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Aripo - 1000 m	0.06	0.06	0.01	--	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Aripo - Native LP	0.18	0.17	0.07	0.11	--	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Caigual - Intro	0.26	0.24	0.16	0.20	0.25	--	0.002	0.061	0.058	0.001	0.001	0.001	0.001
Caigual - 0 m	0.25	0.23	0.15	0.19	0.23	0.02	--	0.075	0.012	0.001	0.001	0.001	0.001
Caigual - 500 m	0.23	0.21	0.14	0.16	0.22	0.01	0.01	--	0.087	0.001	0.001	0.001	0.001
Caigual - 1000 m	0.25	0.23	0.16	0.19	0.25	0.01	0.01	0.01	--	0.003	0.001	0.001	0.003
Guanapo - 5000 m	0.26	0.25	0.18	0.21	0.27	0.03	0.05	0.03	0.02	--	0.001	0.001	0.001
El Cedro - Intro	0.53	0.52	0.46	0.47	0.52	0.37	0.37	0.36	0.38	0.43	--	0.001	0.001
El Cedro - Source	0.36	0.35	0.28	0.29	0.35	0.14	0.14	0.13	0.14	0.19	0.12	--	0.001
Guanapo - Source	0.22	0.21	0.14	0.16	0.23	0.02	0.03	0.02	0.02	0.03	0.33	0.11	--
L.Lalaja - Intro	0.24	0.22	0.15	0.18	0.25	0.02	0.04	0.02	0.02	0.03	0.38	0.14	0.01
L.Lalaja - 0 m	0.25	0.23	0.16	0.18	0.23	0.02	0.03	0.02	0.01	0.02	0.37	0.13	0.01
L.Lalaja - 500 m	0.23	0.21	0.13	0.16	0.22	0.01	0.01	0.00	0.00	0.01	0.37	0.14	0.01
L.Lalaja - 1000 m	0.25	0.23	0.16	0.19	0.24	0.01	0.02	0.01	0.00	0.01	0.39	0.15	0.02
Turure - Intro	0.33	0.31	0.21	0.26	0.32	0.06	0.06	0.08	0.08	0.08	0.47	0.22	0.09
Turure - 0-500 m	0.24	0.22	0.16	0.18	0.25	0.05	0.04	0.05	0.06	0.07	0.43	0.20	0.06
Turure - 1000 m	0.27	0.25	0.19	0.21	0.28	0.04	0.03	0.05	0.05	0.06	0.40	0.17	0.04
Turure - 5000 m	0.22	0.20	0.14	0.15	0.22	0.03	0.04	0.03	0.03	0.04	0.36	0.15	0.02
Taylor - Intro	0.23	0.21	0.15	0.18	0.24	0.03	0.03	0.03	0.01	0.04	0.34	0.12	0.02
Taylor - 0 m	0.25	0.23	0.16	0.19	0.27	0.03	0.04	0.03	0.01	0.04	0.34	0.12	0.02
Taylor - 500 m	0.31	0.28	0.20	0.25	0.28	0.03	0.04	0.03	0.02	0.03	0.44	0.18	0.05
Taylor - 1000 m	0.27	0.25	0.18	0.21	0.26	0.02	0.03	0.02	0.01	0.01	0.38	0.14	0.01
native Caigual - 0 m	0.53	0.51	0.42	0.47	0.51	0.20	0.25	0.22	0.19	0.18	0.65	0.40	0.25
native Taylor - 0 m	0.55	0.53	0.44	0.48	0.54	0.24	0.28	0.26	0.23	0.20	0.67	0.41	0.27

	L.Lalaja - Intro	L.Lalaja - 0 m	L.Lalaja - 500 m	L.Lalaja - 1000 m	Turure - Intro	Turure - 0-500 m	Turure - 1000 m	Turure - 5000 m	Taylor - Intro	Taylor - 0 m	Taylor - 500 m	Taylor - 1000 m	native Caigual 0 m	native Taylor 0 m
Aripo - Intro	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Aripo - 0 m	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Aripo - 500 m	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Aripo - 1000 m	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Aripo - Native LP	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Caigual - Intro	0.001	0.002	0.026	0.007	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.001	0.001
Caigual - 0 m	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Caigual - 500 m	0.002	0.001	0.168	0.04	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Caigual - 1000 m	0.002	0.113	0.214	0.294	0.001	0.001	0.001	0.001	0.003	0.008	0.001	0.066	0.001	0.001
Guanapo - 5000 m	0.001	0.009	0.008	0.026	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.111	0.001	0.001
El Cedro - Intro	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
El Cedro - Source	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Guanapo - Source	0.003	0.01	0.007	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.001	0.001
L.Lalaja - Intro	--	0.328	0.054	0.204	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.029	0.001	0.001
L.Lalaja - 0 m	0.00	--	0.007	0.187	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.262	0.001	0.001
L.Lalaja - 500 m	0.01	0.01	--	0.348	0.001	0.001	0.001	0.001	0.004	0.001	0.001	0.02	0.001	0.001
L.Lalaja - 1000 m	0.00	0.00	0.00	--	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.697	0.001	0.001
Turure - Intro	0.09	0.10	0.05	0.07	--	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Turure - 0-500 m	0.05	0.06	0.04	0.04	0.04	--	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Turure - 1000 m	0.05	0.06	0.03	0.04	0.03	0.02	--	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Turure - 5000 m	0.03	0.03	0.02	0.02	0.06	0.02	0.02	--	0.001	0.001	0.001	0.001	0.001	0.001
Taylor - Intro	0.02	0.03	0.01	0.02	0.09	0.06	0.05	0.03	--	0.116	0.001	0.001	0.001	0.001
Taylor - 0 m	0.01	0.02	0.01	0.02	0.11	0.08	0.06	0.04	0.01	--	0.001	0.003	0.001	0.001
Taylor - 500 m	0.04	0.03	0.03	0.02	0.10	0.09	0.08	0.07	0.05	0.04	--	0.001	0.001	0.001
Taylor - 1000 m	0.01	0.00	0.01	0.00	0.09	0.06	0.05	0.03	0.02	0.02	0.03	--	0.001	0.001
native Caigual 0 m	0.24	0.21	0.20	0.19	0.28	0.28	0.28	0.25	0.23	0.23	0.16	0.19	--	0.001
native Taylor 0 m	0.26	0.23	0.23	0.21	0.33	0.31	0.32	0.28	0.26	0.25	0.17	0.21	0.20	--

Table S3.4 Results of linear mixed models for the effects of predation level on eight phenotypic fitness-related traits in Trinidadian guppies. Test results were obtained with the likelihood ratio test against a null model (excluding fixed effect). Site was a nested random effect within stream in all models.

Trait	Fixed effect	d.f.	AIC	log Lik	L. ratio	*p*-value
Male size	*Null model*	4	1663	-827		
	Predation	6	1652	-820	14.1	<0.01**
Male color	*Null model*	4	1517	-754		
	Predation	6	1514	-751	6.1	0.05
Male shape (PC1)	*Null model*	4	-2860	1434		
	Predation	6	-2858	1435	1.3	0.5108
Male shape (PC2)	*Null model*	4	-3661	1834		
	Predation	6	-3657	1835	0.70	0.7211
Male shape (PC3)	*Null model*	4	-4025	2017		
	Predation	6	-4037	2024	15.9	<0.01**
Reproductive allocation	*Null model*	4	-1057	532		
	Predation	6	-1064	538	11.3	<0.01*
Embryo mass	*Null model*	4	407	-200		
	Predation	6	396	-192	15.8	<0.01**
Fecundity	*Null model*	4	404	-198		
	Predation	6	371	-179	37.14	<0.01**

*p<0.01; **p<0.001

Table S3.5 Previous studies documenting a genetic basis to traits in Trinidadian guppies

Trait	Citation
Color	Endler 1980; Brooks & Endler 2001; Tripathi *et al.* 2009;Handelsman & Fitzpatrick, *unpublished*
Male life history (age and size at maturity)	Reznick 1982; Reznick & Bryga 1996; Torres-Dowdall *et al.* 2012; Handelsman & Fitzpatrick, *unpublished*
Female life history (age and size at first parturition, interbrood interval, fecundity, reproductive allocation)	Reznick 1982; Reznick & Bryga 1996; Torres-Dowdall *et al.* 2012a; Handelsman & Fitzpatrick, unpublished
Body shape/swimming performance	O'Steen *et al.* 2002; Ghalambor *et al.* 2004; Torres-Dowdall *et al.* 2012a; Handelsman & Fitzpatrick, *unpublished*

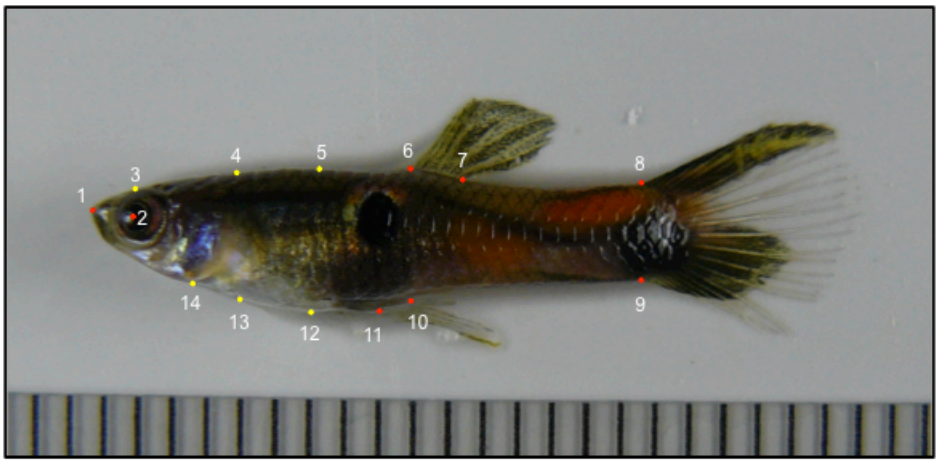

Fig S3.1 Fourteen homologous landmarks used for geometric morphometric analyses to quantify male body shape. Red dots are fixed landmarks whereas yellow dots are semi-sliding along the curve.

Fig S3.3a-f Boxplot summaries for all traits in all sites. Guanapo mainstem populations (pink, far-right) include the 5000m site for Lower Lalaja, Caigual, and Taylor and the source site for Lower Lalaja, Caigual, and Taylor, and Turure. X-axes site labels are color coded by predation level. Central lines represent median values, top and bottom extents of the boxes represent 25th and 75th percentiles, vertical lines extend to the 5th and 9th percentiles, and black dots represent outlier individuals.

Fig S3.3a

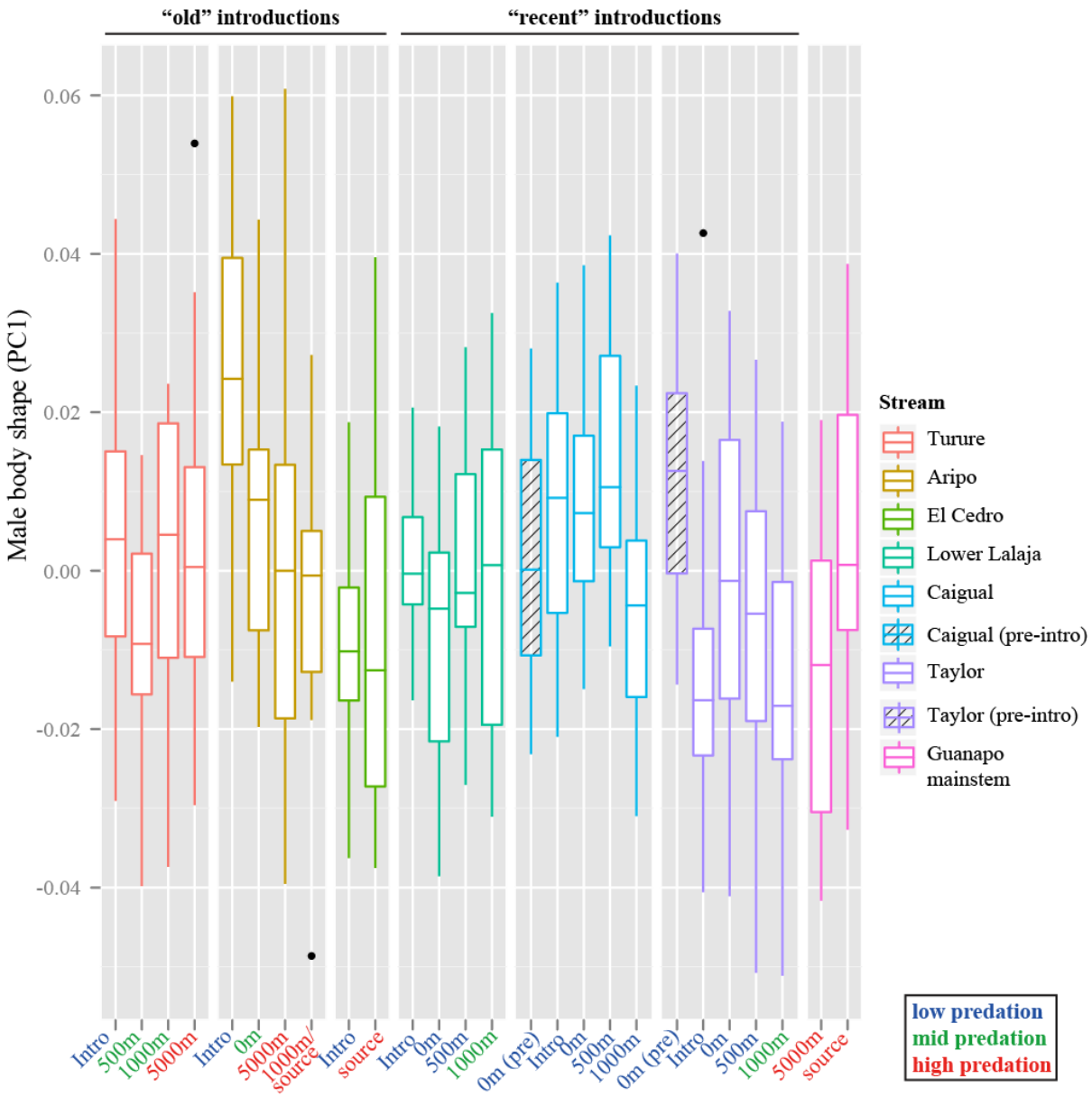
"old" introductions
"recent" introductions
Male body shape (PC1)
0.06
0.04
0.02
0.00
-0.02
-0.04
Intro
500m
1000m
5000m
Intro
0m
5000m
1000m/
source
Intro
source
Intro
0m
500m
1000m
0m (pre)
Intro
0m
500m
1000m
0m (pre)
Intro
0m
500m
1000m
5000m
source
Stream
Turure
Aripo
El Cedro
Lower Lalaja
Caigual
Caigual (pre-intro)
Taylor
Taylor (pre-intro)
Guanapo
mainstem
low predation
mid predation
high predation

Fig S3.3b

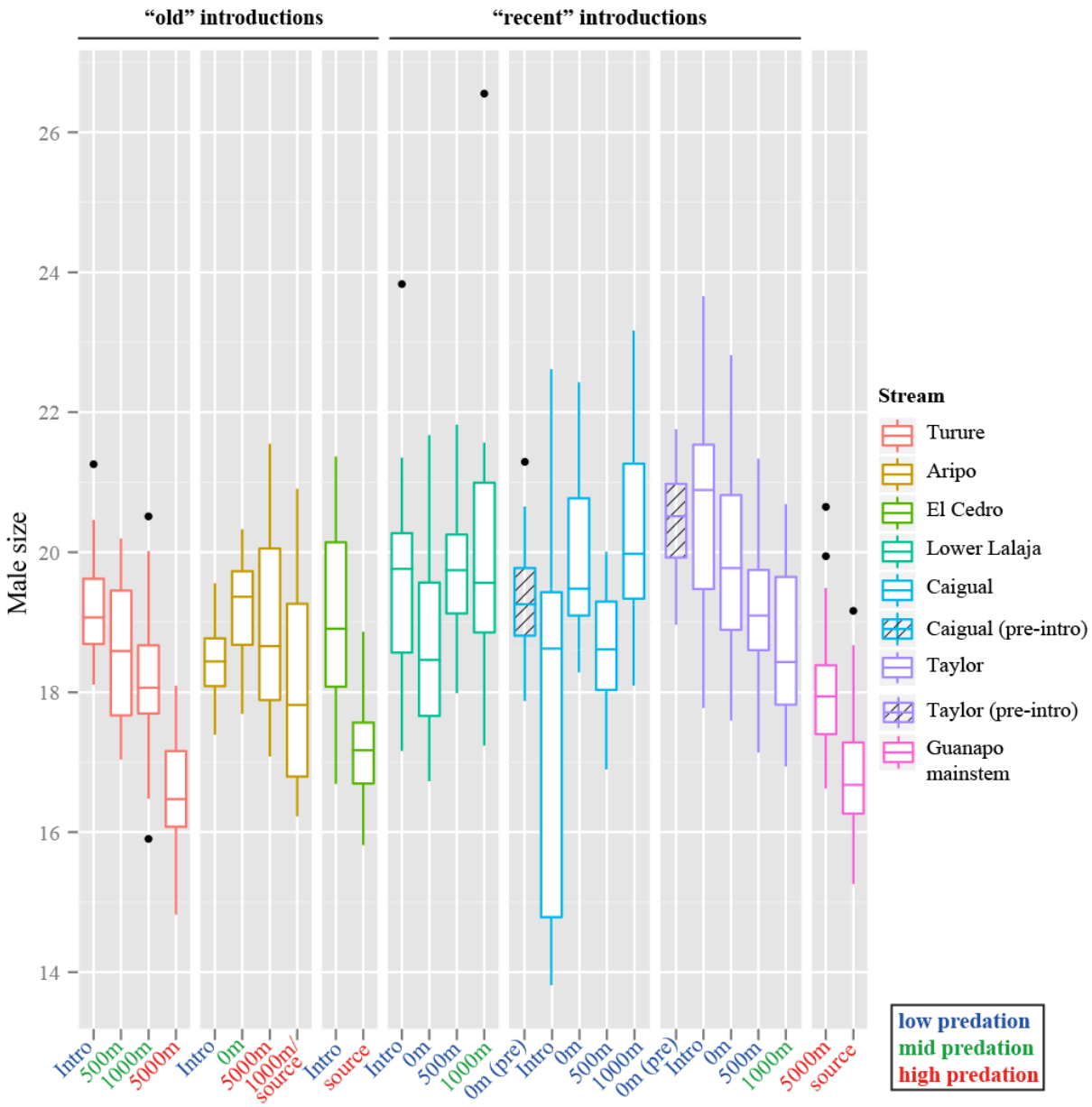
"old" introductions
"recent" introductions
Male size
26
24
22
20
18
16
14
Intro
500m
1000m
5000m
Intro
0m
5000m
1000m/
source
Intro
source
Intro
0m
500m
1000m
0m (pre)
Intro
0m
500m
1000m
0m (pre)
Intro
0m
500m
1000m
5000m
source
Stream
Turure
Aripo
El Cedro
Lower Lalaja
Caigual
Caigual (pre-intro)
Taylor
Taylor (pre-intro)
Guanapo mainstem
low predation
mid predation
high predation

Fig S3.3c

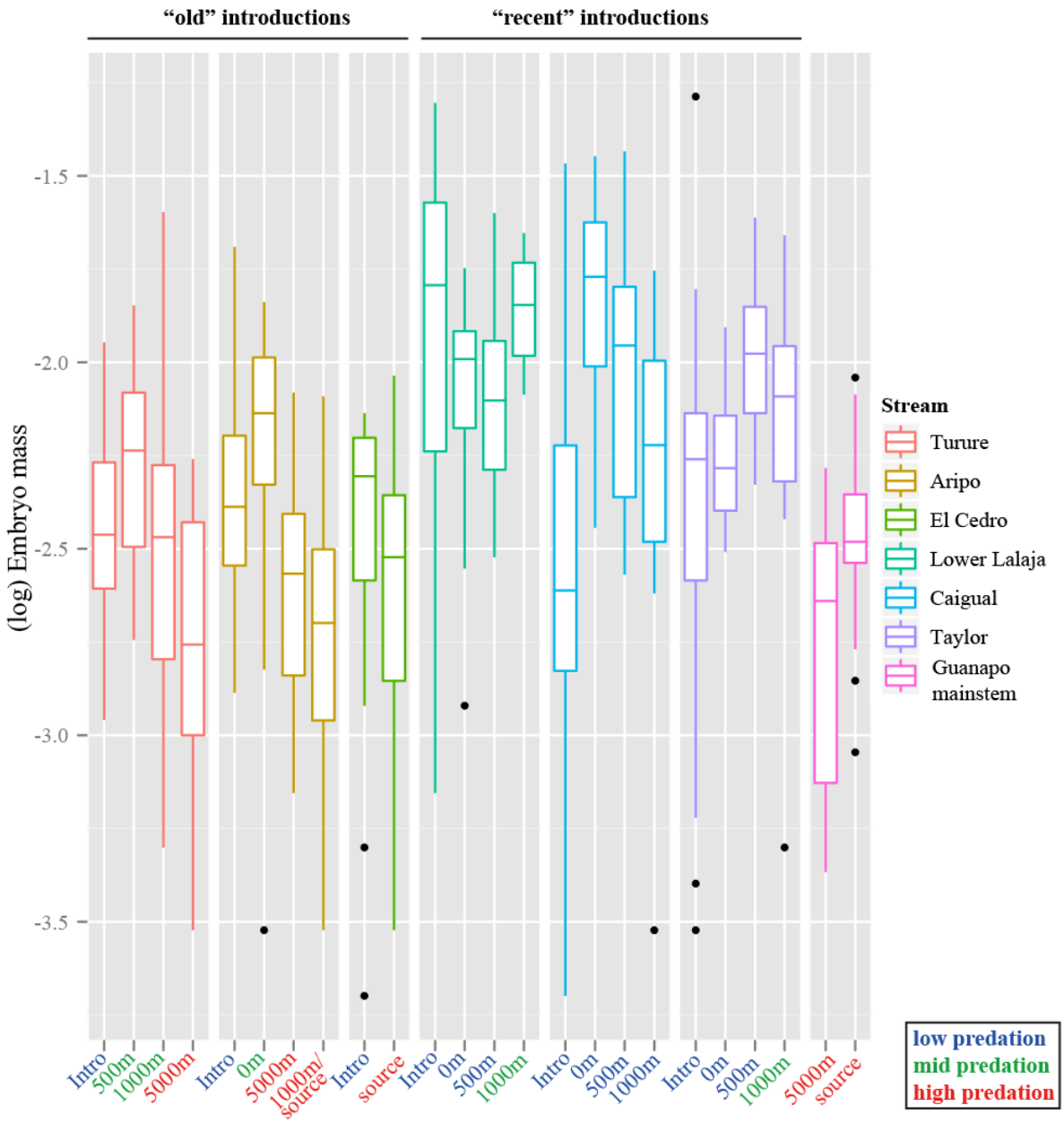
"old" introductions
"recent" introductions
(log) Embryo mass
-1.5
-2.0
-2.5
-3.0
-3.5
Intro
500m
1000m
5000m
Intro
0m
5000m
1000m/
source
Intro
source
Intro
0m
500m
1000m
Intro
0m
500m
1000m
Intro
0m
500m
1000m
5000m
source
Stream
Turure
Aripo
El Cedro
Lower Lalaja
Caigual
Taylor
Guanapo
mainstem
low predation
mid predation
high predation

Fig S3.3d

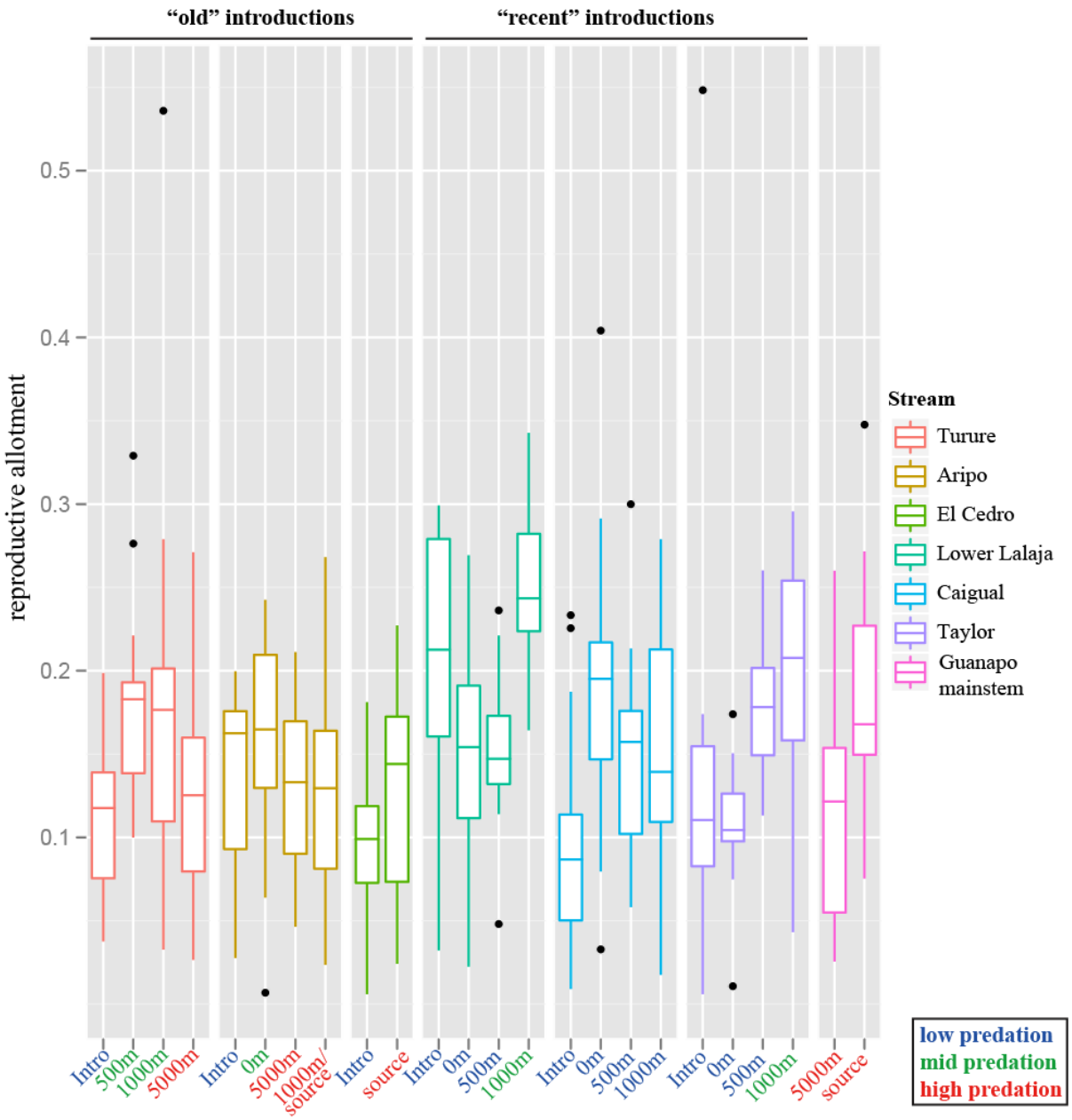
"old" introductions
"recent" introductions
reproductive allotment
0.5
0.4
0.3
0.2
0.1
Intro
500m
1000m
5000m
Intro
0m
5000m
1000m/
source
Intro
source
Intro
0m
500m
1000m
Intro
0m
500m
1000m
Intro
0m
500m
1000m
5000m
source
Stream
Turure
Aripo
El Cedro
Lower Lalaja
Caigual
Taylor
Guanapo
mainstem
low predation
mid predation
high predation

Fig S3.3e

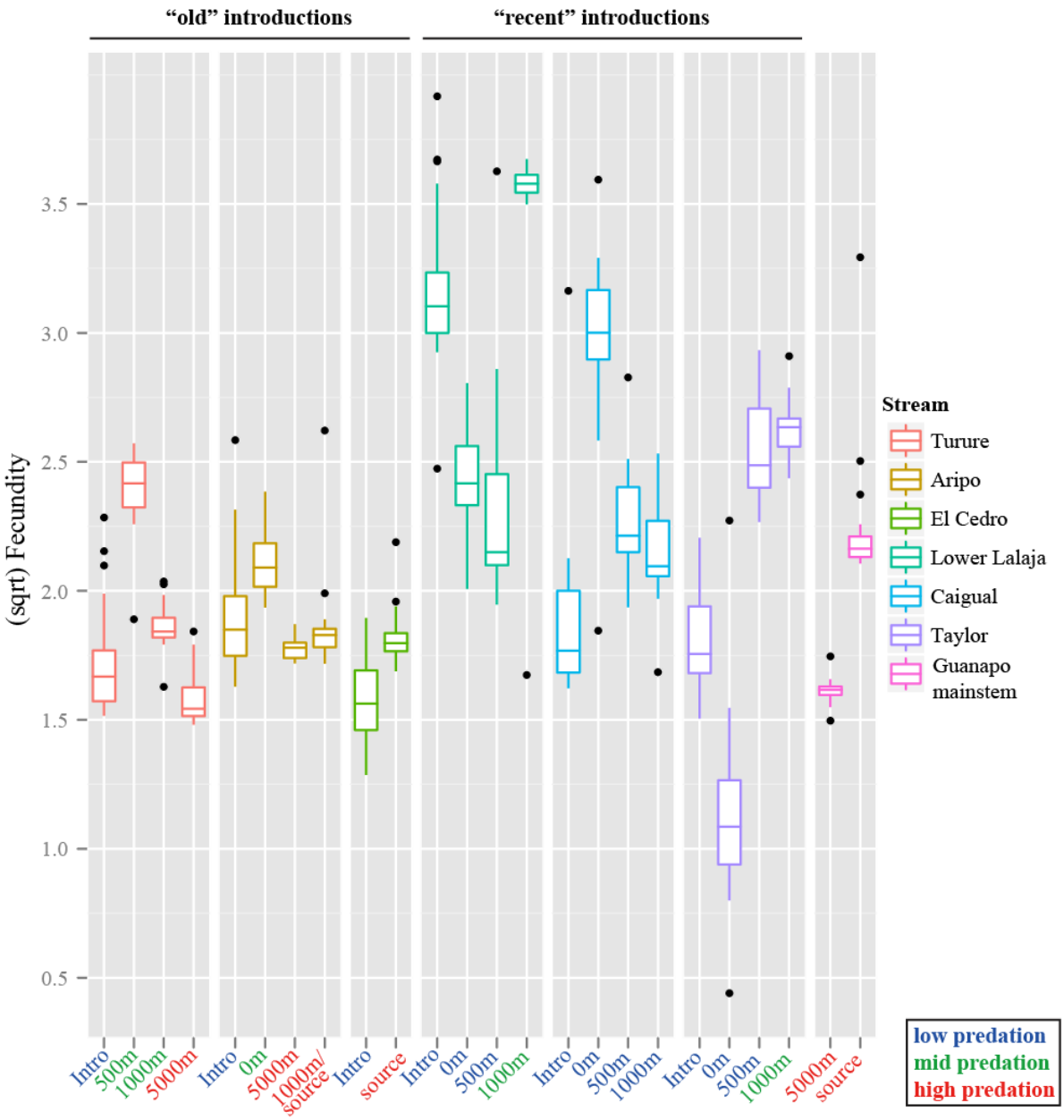
"old" introductions
"recent" introductions
(sqrt) Fecundity
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Intro
500m
1000m
5000m
Intro
0m
5000m
1000m/
source
Intro
source
Intro
0m
500m
1000m
Intro
0m
500m
1000m
Intro
0m
500m
1000m
5000m
source
Stream
Turure
Aripo
El Cedro
Lower Lalaja
Caigual
Taylor
Guanapo
mainstem
low predation
mid predation
high predation

Fig S3.3f

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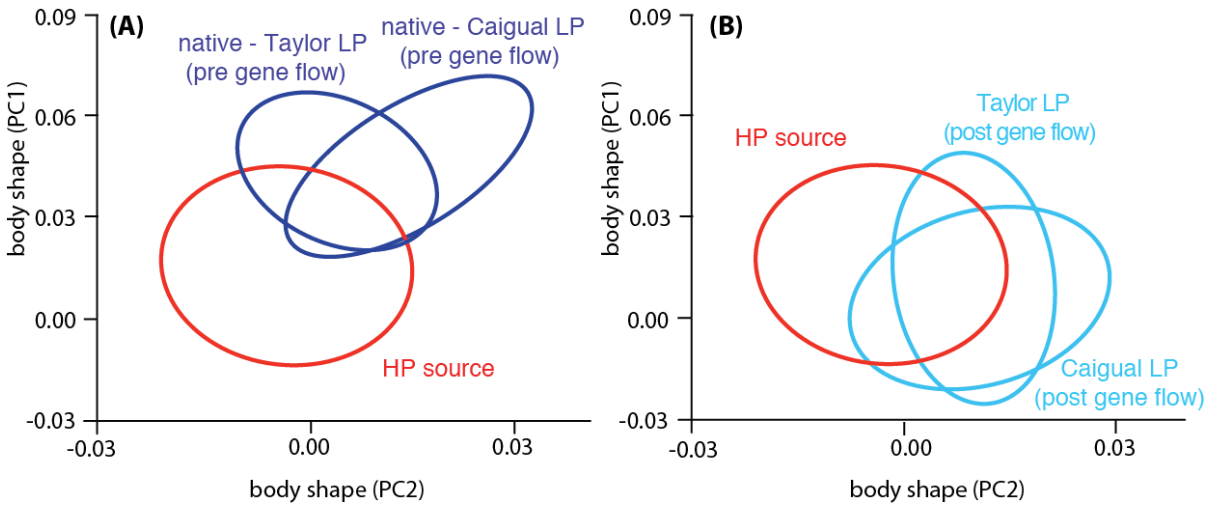

Figure S4.1 Bivariate plots of correlations between common garden measured male body shape principal component axes PC1 and PC2 in the high predation source population (red) and native low predation populations (purple) **(A)** and the high predation source and same low predation populations sampled approximately 10 generations after gene flow (light blue). **(B)** Gene flow from the source population led to changes in the structure of genetically based phenotypic correlations (i.e., the shape and size of the ellipses). Ellipses represent 1.5 standard deviations from the population mean.

APPENDIX 5.1

Development and characterization of 12 microsatellite loci for the Trinidadian guppy

I used independent, neutral, and variable microsatellite loci to identify unmarked fish as recruits from the native Caigual and Taylor populations, new HP immigrants, or hybrids and to reconstruct the wild pedigree. I first screened 80 of 126 microsatellite loci that had been developed for this species prior to our work (Paterson et al., 2005; Shen et al., 2007; Watanabe et al., 2003, 2004). I did not find adequate polymorphism using these loci. For example, 42 out of 58 loci that amplified in both native populations were homozygous and fixed for the same allele. Due to the lack of genetic variation found in pre-existing loci, I developed a new microsatellite library for my study using Illumina sequencing in collaboration with the Evolutionary Genomics Core Facility at Cornell University.

Genomic DNA was purified from muscle tissue of five native Caigual and five native Taylor guppies using Qiagen DNeasy Blood and Tissue Kits. DNA was eluted with 100 µl AE buffer and concentration was determined on a Qubit 2.0 fluorometer. Each DNA sample was given one of two barcodes based on population (Caigual or Taylor) in order to filter loci for those with allelic variants in both populations. The following steps were thus completed using two sets of pooled DNA from five individuals per population. Genomic DNA (50-100 ng) was digested with the restriction enzymes *AluI*, *RsaI*, and *Hpy166II*, in three separate reactions. After heat inactivation of the restriction enzymes equal amounts of the three digests were combined in a single tube and the blunt ends were adenylated (+A) with Klenow (exo-) and dATP. After heat inactivation of the Klenow (exo-), the reactions were supplemented with ATP to 1 mM and an Illumina Y-adaptor was ligated with T4 DNA ligase. Fragments were enriched for microsatellites

by hybridization to 3'-biotinylated repeat probes (representing two unique dimers, five unique trimers, four unique tetramers and two unique pentamers). Enriched genomic fragments were captured by streptavidin-coated magnetic beads, and fragments were amplified with Platinum Taq polymerase and a pair of Illumina primers (one universal, one index). PCR products were analyzed on an agarose gel and quantified with a Qubit 2.0 fluorometer. Equal amounts of each library were pooled and fragments 300-600 basepairs (bp) were recovered with Ampure beads. Libraries were submitted to the Sequencing and Genotyping Facility at the Cornell Life sciences Core Laboratory Center (CLC) for 2 x 250 paired end sequencing on an Illumina MiSeq.

Barcode-sorted reads were trimmed of adapter sequences and assembled with SeqMan NGen v4.1.0.147. Consensus files and singleton reads were exported as fasta files and simple repeats and associated genotyping primers were summarized with msatcommander v1.0.3. For primer design I chose a product size range of 150-450 bp, primer minimum, optimum, and maximum sizes of 22,23, and 24 bases respectively. Minimum, optimum, and maximum annealing temperatures were set respectively to 58, 60, and 62 °C.

A total of 116 loci were discovered after filtering the total set to include only tetramers found in both Caigual and Taylor populations and had variable repeat lengths in at least one population. I conducted an initial screening for variability on 36 loci using a "universal tag" approach (Schuelke, 2000). PCR amplifications were carried out in 12.5 µl reactions containing 8.4 µl H_2O, 1.6 µl 10x ABI buffer I with added $MgCl_2$, 0.25 µl dNTPs, 0.1 µl BSA, 0.28 µl reverse primer (10 µM), 0.15 µl forward primer (10 µM), 0.15 µl dye-labeled M13 primer (10 µM), 0.06 µl AmpliTaq DNA polymerase, and 1.5 µl DNA. All reactions were performed using thermocycling conditions of: 95 °C for 3 min; 40 cycles at 95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s; 8 cycles at 94 °C for 30 s, 53 °C for 30 s, 72 °C for 45 s; and a final extension at 72 °C

for 10 min. PCR products were mixed with HiDi formamide and LIZ ladder (500 GeneScan) and read on an ABI 3730 genetic analyzer (Life Sciences Core Laboratories at Cornell University). Fragment sizes were manually confirmed using GENEMARKER® v1.91 (SoftGenetics, LLC, State College, PA, USA).

Sixteen out of 36 loci amplified and were polymorphic in seven individuals (three Caigual, four Taylor). I next tested these 16 loci at 20 additional individuals from each of Caigual and Taylor native populations using the same PCR protocol described above. Conformity of genotype proportions to Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) was tested using GENEPOP v4.2 (Raymond and Rousset, 1995). Microsatellite loci were examined for evidence of null alleles and scoring error due to stutter or large allele dropout using MICROCHECKER v2.2.3 (van Oosterhout et al., 2006).

I recovered a final set of 12 loci that fit HWE expectations, did not show evidence for LD or null alleles, and were variable in native Caigual and Taylor populations. I divided these 12 loci into three panels of four loci each for multiplexing PCR reactions. Dye-labeled forward primers were ordered using 6-FAM from Integrated DNA Technologies and the Applied Biosystems G5 dye set (PET, VIC, NED). I performed multiplexed PCR reactions on the remainder of individuals using the QIAGEN Type-it Microsatellite PCR kits. These reactions contained 4 µl of H_2O, 6.25 µl of Type-it Master Mix, 0.1 µl of BSA, 1.25 µl of the primer mix (each primer at 2 µM), and 1 µl of DNA. All reactions were performed using thermocycling conditions of: 95 °C for 10 min; 35 cycles at 95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s; and a final extension at 60 °C for 30 min. Fragment analysis was performed using the same protocol described above. I confirmed that peaks obtained from multiplex reactions corresponded to those from single-locus PCRs.

Simulations to optimize genetic class assignments in NEWHYBRIDS

I assessed the power of NEWHYBRIDS to correctly assign individuals to genotypic classes by analyzing a set of simulated (i.e., genotypes of known genetic ancestry). To generate simulated data, I used twenty known pure native Caigual individuals sampled prior to gene flow, and twenty individuals known to originate from the introduction site. From these pure "parental" genotypes, I generated 100 genotypes in HYBRIDLAB 1.0 (Nielsen et al., 2006) for each of the following genotypic classes: Native, Immigrant, F1, F2, F1xNative, F1xImmigrant, for a total of 600 individuals. I then used this simulated dataset in NEWHYBRIDS with default settings for 100,000 MCMC iterations and discarding the first 10,000 as burn-in. I repeated this process using twenty pure Taylor individuals as one of the parental populations.

NEWHYBRIDS returns posterior probability values that represent each individual's probability of belonging to one of the six genotypic classes. To optimize the posterior probability threshold value for my dataset, I calculated efficiency and accuracy scores and obtained an "overall performance score" across all simulated genotypes, using a range of threshold values (0.5-0.95), as recommended by (Vähä and Primmer, 2006). An optimized performance score should maximize the number of identified members of a genotypic class while maintaining high accuracy. Using NEWHYBRIDS results from simulated data, the posterior probability threshold that optimized overall performance score (averaged across each genotypic class) and had the lowest standard deviation was 0.50 (Figure S5.1). I used this threshold for classifying individuals into genetic groups as described in the main text.

Modeling detection probability with capture-mark-recapture data

I estimated detection probability (p) by fitting Cormack-Jolly-Seber (CJS) model in Program MARK v8.0 to the full 29 months of individual capture histories. I expected detection probability

to vary by stream due to differences in pool structure and flow and by month due to seasonal differences in flow. I did not have a priori reasons to expect differences in detection probability between sexes or among genetic classification groups, and thus did not include them as factors. All models included the most general structure for survival (ϕ); a three-way interaction among sex, stream, and month. I compared the most complex model for *p,* which included an interaction between stream and month, to all possible model simplifications including an additive interaction, single factors, and the constant model. Model fitting was done by Maximum Likelihood and models were compared using Akaike's Information Criterion adjusted for sampled size AICc and AICc weights.

The top-ranking model, with 100% of the weight of evidence, supported the most general model structure with an interaction between stream and month (Table S5.2). Overall, detection probability was high. Temporal variation in capture probability was consistent with seasonal changes in water level and flow (Figure S5.2).

Table S5.1 Characteristics of 12 microsatellite loci in *Poecilia reticulata*.

						All	native Caigual			native Taylor		
Locus	**Panel**	**Dye**	**Repeat motif**	**Forward primer**	**Size range (bp)**	N_A	N_A	H_O	H_E	N_A	H_O	H_E
Prgf006	2	FAM	AGAT	**F:**AAGAAACAAAGCCAGTCCAACAC **R:** TGCCTCTGGTTGGATTTATTGAC	161-269	20	5	0.48	0.45	4	0.41	0.52
Prgf008	1	PET	AGAT	**F:**CATGAGGGTCTGTTCTTTCCATG **R:** TCTCTTACGCCAGATAGATCGATC	193-353	17	5	0.43	0.37	4	0.41	0.46
Prgf021	1	VIC	AGAT	**F:**CAGGTTGCTGTCTTGTTGCTTC **R:** TGTCGATGTTGTCTACTGCAAAG	208-284	18	7	0.66	0.79	6	0.63	0.76
Prgf025	3	VIC	AAAG	**F:**TCGCTAAGCAACGTATGAAACAC **R:** ACTAATACGAGGGAAGTGGAAGG	228-344	20	7	0.60	0.73	5	0.82	0.74
Prgf027	1	NED	AGAT	**F:**GTGGATGCAGTGTCTCTATCATG **R:** TTGTCACTGTTTAAGCATCTGGG	188-260	18	11	0.71	0.83	3	0.30	0.31
Prgf034	1	FAM	AAAG	**F:**CCCATTCACCCTATTTCCCAAAG **R:** GCCCACTCCCTTTCCGTAATATC	253-341	20	4	0.07	0.12	3	0.19	0.24
Prgf038	2	PET	AGAT	**F:**GGTCACGTGGTTTGGAAATGTC **R:** AAAGCATCCCGACAGTATGATTC	174-298	17	5	0.59	0.63	4	0.26	0.24
Prgf039	3	NED	AAAC	**F:**TCCCTTTCCTTGCTGAAGTTTAAG **R:** ACAAAGGTCTGCATAATTGTGATG	208-282	10	2	0.19	0.23	2	0.11	0.11
Prgf040	2	NED	AGAT	**F:**AGCATTGTTAGCATCACAGACAG **R:** ACAGCCACCAATTAAGAAACCAG	175-235	15	2	0.26	0.32	4	0.19	0.21
Prgf042	2	VIC	AGAT	**F:**ACATAACATTCCTTTAGTGCACG **R:** AGGAGCAATAAGAAGAAGGGTTC	170-230	10	3	0.20	0.19	2	0.37	0.35
Prgf043	3	PET	ATCC	**F:**CCTTTCCCTGTGGTGAATATTGG **R:** AGTCTTTGCCTCCCTACTTAGAC	194-280	17	3	0.31	0.27	2	0.22	0.31
Prgf053	3	FAM	ATCC	**F:**CTGTACTTTGAAGCCACCCATC **R:** GTTCATCTGCGTTCCAAGGATC	114-244	12	3	0.36	0.37	5	0.56	0.56

Table S5.2. Model selection results for detection probability (p). Model structures were ranked using Akaike Information Criteria corrected for sample size (AIC_c). Relative AIC_c, Akaike weight (w), number of parameters (K), and deviance are reported. All reported model structures were run with the most general model structure in the survival parameter: ϕ(stream × sex × month).

***p* model structure**	**AICc**	**ΔAICc**	**w**	**K**	**Deviance**
Stream × month	38313	0	1	142	6634
Stream + month	38397	84	0	120	6763
Month	38404	91	0	119	6772
Stream	38520	208	0	98	6930
.	38527	214	0	97	6939

Table S5.3 Model selection results for survival (ϕ) and lamda (λ) using full capture-mark-recapture dataset with 29 capture occasions. Model structures were ranked using Akaike Information Criteria corrected for sample size (AIC_c). Relative AIC_c, Akaike weight (w), number of parameters (K), and deviance are reported. All reported model structures were run with the best supported model structure for detection probability: p(stream × month).

ϕ model structure	AICc	ΔAICc	w	K	Deviance
Stream × sex × month	38313	0	1	142	6634
Stream × month	38975	662	0	94	7393
Sex × month	39090	777	0	95	7506
Stream × sex	39248	935	0	52	7750
Sex	39638	1325	0	50	8145
Month	39780	1467	0	71	8245
Stream	39882	1569	0	50	8389
.	40298	1985	0	49	8807

λ model structure	AICc	ΔAICc	w	K	Deviance
Stream × sex × month	96492	0	1	238	6634
Stream × month	96606	113	0	190	6845
Sex × month	96752	260	0	193	6985
Month	96865	372	0	169	7147
Stream × sex	97705	1213	0	150	8025
Stream	97712	1220	0	148	8036
Sex	97755	1263	0	148	8079
.	97759	1267	0	147	8085

Table S5.4 Model selection results for survival (ϕ) and lamda (λ) using the genotyped subset of capture-mark-recapture data with 17 capture occasions. Model structures were ranked using Akaike Information Criteria corrected for sample size (AIC_c). Relative AIC_c, Akaike weight (w), number of parameters (K), and deviance are reported. All reported model structures were run with the best supported model structure for detection probability: p(stream × month). Survival (ϕ) was modeled with the Cormack-Jolly-Seber model. Lamda (λ) was modeled using the Pradel model. All model structures for λ were run with ϕ(sex × stream × month).

ϕ model structure	**AICc**	**ΔAICc**	**w**	**K**	**Deviance**
Gen × sex × stream	14672	0	1	48	4387
Sex × stream	14693	20	0	36	4432
Gen × stream	14768	96	0	40	4499
Stream	14796	124	0	34	4539
Month	14885	213	0	47	4602
Gen × sex	15037	365	0	40	4768
Sex	15103	431	0	34	4847
Gen	15139	467	0	36	4878
.	15230	558	0	33	4975
λ model structure	**AICc**	**ΔAICc**	**w**	**K**	**Deviance**
Gen × sex × stream	29292	0	1	114	6473
Gen × stream	29324	32	0	106	6521
Gen × sex	29472	180	0	106	6669
Gen	29513	221	0	102	6719
Stream	30775	1483	0	100	7984
Sex × stream	30775	1483	0	102	7981
.	30783	1491	0	99	7995
Sex	30784	1493	0	100	7994

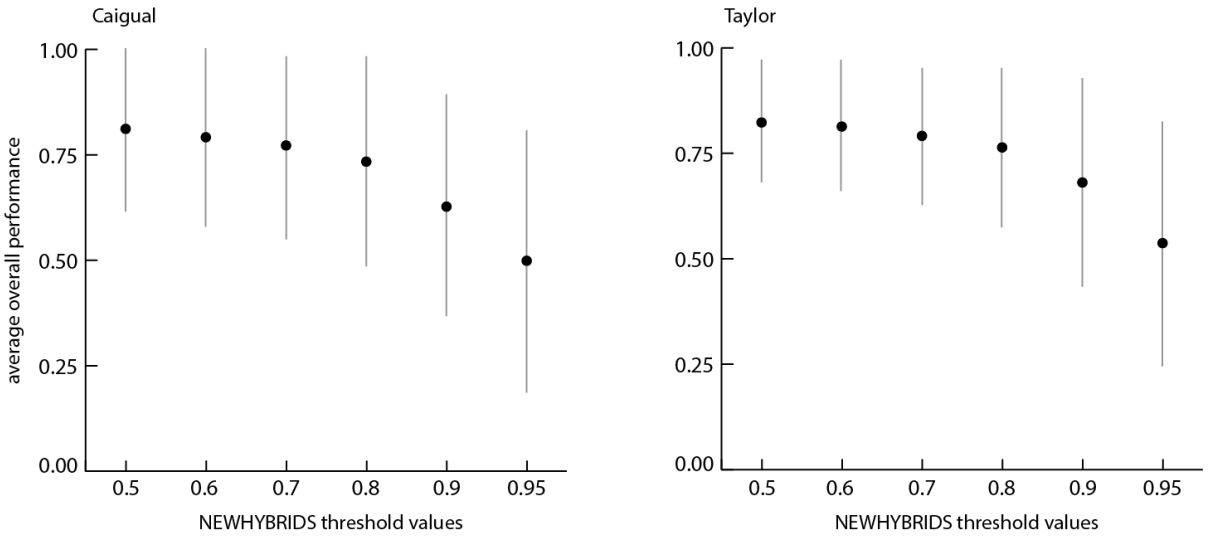

Figure S5.1 Distribution of average overall performance scores (Vähä and Primmer, 2006) as a function of the threshold value used to assign individuals to genotypic classes in NEWHYBRIDS. We determined that a threshold of 0.5 was most appropriate based on the overall performance score and distribution of the data around the mean.

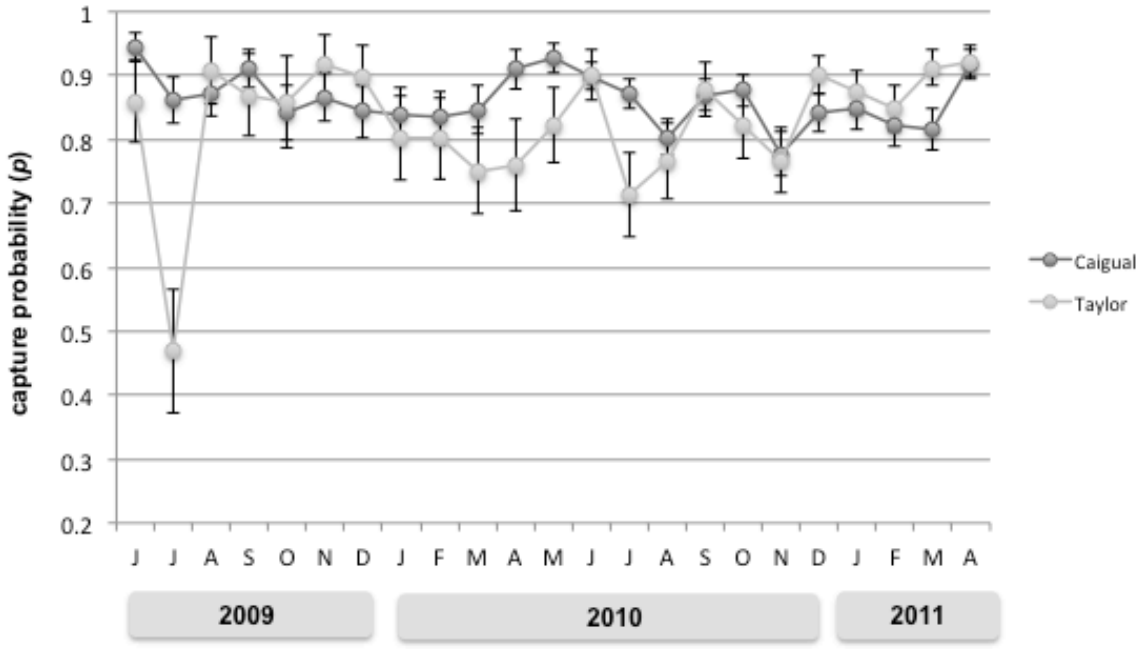

Figure S5.2 Monthly estimates of detection probability (*p*)

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