Female preference for novel males constrains the contemporary evolution of assortative mating in guppies

Felipe Dargent, Lisa Chen, Gregor F. Fussmann, Cameron K. Ghalambor, and Andrew P. Hendry

Department of Biology, McGill University, 1205 Dr. Penfield Av., Montreal H3A 1B1, QC, Canada, Department of Biology, Colorado State University at Fort Collins, 1878 Campus Delivery, Fort Collins CO 80523, USA, and Redpath Museum, McGill University, 859 Sherbrooke St. West, Montreal H3A 0C4, Canada

Received 9 August 2018; revised 17 December 2018; editorial decision 18 December 2018; accepted 24 December 2018.

Progress toward local adaptation is expected to be enhanced when divergent selection is multidimensional, because many simultaneous sources of selection can increase the total strength of selection and enhance the number of independent traits under selection. Yet, whether local adaptation ensues from multidimensional selection also depends on its potential to cause the build-up of reproductive barriers such as sexual signals and preference for these signals. We used replicate experimental introductions of guppies (Poecilia reticulata) in nature to test whether an abrupt and dramatic shift in multiple important ecological dimensions (parasitism, predation, diet/resources) promoted the contemporary evolution of assortative mating. After 8–12 postintroduction guppy generations in the wild, we bred descendants of each population in a common-garden laboratory environment for 2 generations, after which we recorded the preferences of females from each population for males from all populations. We found contemporary evolution of mate traits (size, body condition, color) that should influence mate choice, but no evidence for the occurrence of positive assortative preferences. That is, females in a given evolving population did not prefer males from that population over males from other populations. Instead, females tended to prefer novel males (i.e., disassortative mating), which likely acts as a mechanism preventing the evolution of reproductive isolation. Preferences for novelty may explain why many cases of local adaptation do not lead to the evolution of reproductive barriers and ecological speciation.

Key words: ecological speciation, experimental evolution, mate choice, Poecilia reticulata, reproductive isolation.

INTRODUCTION

Local adaptation resulting from divergent natural selection among environments is expected to promote the build-up of reproductive barriers among the diverging populations, either as a pleiotropic by-product of trait divergence or owing to selection against maladaptive hybridization. However, many instances of local adaptation to divergent environments seem not to generate substantial progress toward reproductive isolation. Current research efforts thus increasingly focus on determining the factors that promote and constrain progress toward the evolution of reproductive isolation. One proposed promoter is the increased dimensionality of divergence among environments, i.e., environmental change along multiple ecological axes. Such shifts are hypothesized to increase the total strength of divergent selection and the number of independent trait dimensions that show divergence. The consequence is thought to be greater and more rapid progress toward local adaptation and ecological speciation. Here, we explore whether a dramatic shift along multiple ecological axes of selection could generate the contemporary evolution of reproductive barriers in natural populations of guppies. We experimentally introduced guppies into 2 replicated sites that differed from the source location in parasitism, predation, diet/food availability, and presumably other unmeasured factors. Divergent selection from each of these 3 sources has the potential to shape local adaptation and reproductive isolation in several ways. First, direct natural selection against migrants and
hybrids can occur as a result of their maladaptation to local parasites (MacColl and Chapman 2010; Ricklefs 2010), predators (Stoks et al. 2003; Nosil and Crespi 2006; Langerhans et al. 2007), or available diets (Rundle et al. 2000; Boughman et al. 2003; Badyaev et al. 2008; Hendry et al. 2009). Second, mating isolation between populations can occur, either because of selection that reduces maladaptive between-type mating (e.g., “reinforcement”) or because the traits influencing differential success against enemies or competitors also pleiotropically influence mate choice. For example, divergence in parasite communities can lead to divergent selection on the immune system (i.e., MHC) which pleiotropically generates preferences for local mates (Eizaguirre et al. 2009; Matthews et al. 2010); traits influencing differential predation can also pleiotropically influence mate choice (Nosil et al. 2003; Servedio and Noor 2003; Servedio et al. 2011); and feeding morphology associated to specialized food items or ecology can lead to emergent assortative mating (Rundle et al. 2000; Huber et al. 2007). Parasite-mediated divergent sexual selection (i.e., mate choice based on divergent traits that better communicate fitness in each environment) can also generate positive assortative mating (Skarstein et al. 2005). Finally, differences in overall levels of food availability (independent of the composition of the diet) may result in divergent selection for resource-use efficiency, leading to morphs that are more competitive under high or low resource availability (Reznick et al. 2000).

For guppies, in particular, each of these agents has the potential to drive reproductive isolation. Infection with the common (Harris and Lyles 1992) monogenean ectoparasite Gyrodactylus spp. has detrimental consequences for guppy fitness (van Oosterhout et al. 2007; Pérez-Jvostov et al. 2012; Dargent et al. 2013), and influences MHC evolution (Fraser and Neff 2009; Fraser et al. 2010), association preferences, mate choice, and male signaling traits (Kennedy et al. 1987; Houde and Torio 1992; Lopez 1998; Kolluru et al. 2009). Individuals moving from low-predation to high-predation environments are strongly selected against (Weese et al. 2010) and under divergent selection from predators also influence mate choice (Endler and Houde 1995). Life-history traits and feeding behavior of different guppy populations correlate with different diets (Zandonà et al. 2011) and levels of food availability (Grether et al. 2001). Furthermore, divergence in predation and dietary resources among guppy populations are key drivers of adaptive divergence in morphological, life-history, and behavioral traits that may influence mating success (Endler 1995; Magurran 2005).

Divergent selection owing to infection, predation and food resources can sometimes occur simultaneously, as they do in our experimental field sites. In particular, the source guppies of our study originated from a site characterized by high predation (from piscivorous fishes; Gilliam et al. 1993), high parasitism (especially from the monogenean ectoparasites Gyrodactylus spp.; Gotanda et al. 2013; Dargent et al. 2014), high invertebrate-consumption relative to detritus and periphyton-consumption (Zandonà et al. 2011), and high food availability (due to an open forest canopy that allows high light availability for periphyton and phytoplankton growth and results in lower intraspecific competition; Kohler et al. 2012; Travis et al. 2014). By contrast (same references as above), the introduction sites were characterized by low predation (no major piscivorous fishes), low parasitism (no Gyrodactylus), high algae- and detritus-consumption diets, and low food availability (owing to more closed canopies). Fitting these multifarious environmental shifts, the introduced populations in this study have shown rapid evolution of resistance to Gyrodactylus (Dargent et al. 2013; Dargent et al. 2016), brain gene expression in relation to predator cues (Ghalambor et al. 2015), metabolic rate and growth rate (Handelsman et al. 2013), and male carotenoid and melanin-based coloration (Gordon et al. 2015; Kemp et al. 2018). Yet, missing from this multifaceted picture of divergent evolution is whether mate preferences for local males have also evolved. Specifically, given that male color and female preference can rapidly evolve as a set of correlated traits (Houde and Endler 1990), we ask if assortative mate preferences evolve on the same timescale as this would facilitate local adaptation. Alternatively, if no evidence for assortative mating is observed it may reflect that it evolves more slowly or never evolves because of preference for novelty (see papers by Hughes et al. 1999; Zajitschek and Brooks 2008; Hampton et al. 2009; Hughes et al. 2013; Macario et al. 2017).

Previous work has reported some (often weak) positive assortative mate choice between (nonintroduced) guppies in high-predation versus low-predation sites (Endler and Houde 1995; Schwartz et al. 2010), which probably also differed in parasitism, diets, and resources (e.g., Grether et al. 2001; Zandonà et al. 2011; El-Sabaawi et al. 2012; Gotanda et al. 2013). Yet the evolution of strong and repeatable assortative mating in such populations—and in our experiment—is not a given owing to constraints imposed by several aspects of female preference in guppies (Becher and Magurran 2004; Labonne and Hendry 2010). For instance, females sometimes show preferences that are conserved across populations (Schwartz and Hendry 2007), females sometimes prefer “novel” males (Hughes et al. 1999), female preferences can be frequency dependent (Hughes et al. 2013), and males can use “sneaky” copulations to circumvent female choice (Ojanguren and Magurran 2004). Furthermore, the one previous introduction experiment that tested for assortative mating did not find it (Easty et al. 2011). Yet, the introduction used by Easty et al. (2011) experiment did not include replicate populations, did not consider parasites, and used methodologies that might not have been optimal with respect revealing the contemporary evolution of mate choice (Easty et al. 2011).

Our experiment introduced guppies from a single ancestral (source) population into 2 previously guppy-free new (introduced) environments, and then allowed them to evolve in situ for 8–12 generations. Guppies were then collected from each population and reared for 2 generations in a common-garden laboratory environment to assess genetically-based changes in a suite of traits, including female mate preference. These laboratory guppies were then used to address 3 specific questions. First, to what extent have male signaling traits that might influence mate choice diverged among populations? This is a necessary prerequisite for the evolution of assortative mating. Second, do females prefer sympatric (from the same population) over allopatric (from a different population) males: i.e., positive assortative mating? Third, is female preference for sympatric (or allopatric) males influenced by specific male signaling traits?

METHODS
Ethical note
All field collections were approved by the Trinidad and Tobago Ministry of Fisheries. All field and laboratory procedures were approved by McGill University’s Animal Care Committee in accordance with the Canadian Council on Animal Care in Sciences guidelines (AUP #5759).
Experimental introductions

We compared guppies from an ancestral population to guppies from 2 descendant populations established through a large scale translocation experiment (Dargent et al. 2013; Handelsman et al. 2013; López-Sepulcre et al. 2013; Ruell et al. 2013; Arendt et al. 2014; Travis et al. 2014; Ghalambor et al. 2015). Briefly, juvenile guppies were collected in 2008 from the main channel of the Guanapo River in Trinidad (“Guanapo source”; 10° 38’ 23”N, 61°14’54”W and 10° 39’ 14”N, 61°15’ 18”W). The collected guppies were quarantined and treated with medications for a wide spectrum of pathogens, and—once mature—were introduced (37 males and 37 females) into a tributary stream of the Guanapo River (Lower Lalaja: Introduction 1). To increase genetic diversity, the introduced females had earlier been mated in groups of 5 with groups of 5 nonintroduced males from the source population. In 2009, this process was repeated, with the guppies being introduced into another tributary of the Guanapo River (Taylor – 64 guppies of each sex: Introduction 2). The Introduction 1 and Introduction 2 environments are mostly similar to each other with the exception that the canopy of the Introduction 2 environment was experimentally trimmed by 28%, thus increasing its productivity (Kohler et al. 2012), as part of a larger experimental evolution assay beyond the scope of our paper (see Travis et al. 2014). No reintroductions were performed in the source population because stream morphology made feasible the initial removal of all individuals present.

This source site (Guanapo) has 4 key features relevant to our study: 1) the monogenean ectoparasite Gyrodactylus spp. is present, 2) large predatory fishes (including Crenicichla spp.) are present, 3) productivity is high, and 4) invertebrate availability is high. At the introduction sites, by contrast, Gyrodactylus spp. are absent (Dargent et al. 2014), large piscivorous predators are absent, productivity is lower, and invertebrate availability is lower (Zanodonà et al. 2011; Kohler et al. 2012; Dargent et al. 2013; Arendt et al. 2014). Furthermore, other unmeasured factors differ between the introduction sites and the source population, such as stream morphology, as well as population age and fish size distributions, which influence intraspecific competition, and ultimately influence trait divergence and local adaptation (Torres Dowdall et al. 2012). Thus, we expect that the source and introduction populations experienced strong divergent selection along multiple ecological axes. Following introduction, the guppies were allowed to experience this selection for 3 (Introduction 1) and 2 (Introduction 2) years, which are equivalent to approximately 12 and 8 guppy generations (Ruell et al. 2013).

In 2011, guppies were collected from the source and introduction sites, transported to laboratory facilities, and raised for 2 generations in a common-garden environment (for details, see Dargent et al. 2013). For this reason, any observed difference among the populations in suites of traits, including female mate preference, likely reflects genetic effects rather than plasticity or maternal effects, while also limiting the potential effects of selection under laboratory conditions for more generations (Reznick et al. 1990). Field-collected guppies and F1 laboratory-reared guppies were raised and bred by C. Ghalambor at Colorado State University (Fort Collins, Colorado). The F1 guppies were then transferred to McGill University (Montreal, Quebec) to breed the F2 laboratory-reared generation and to perform the mate choice trials (see below). As the F2 guppies grew, males and females were separated before reaching sexual maturity and before males had begun to express color patterns. This allowed females to remain virgin, and thus receptive to males (Houde 1997); and without familiarity-induced preferences (Pitcher 2003), and thus without past experience biases; until the start of the experiments described below. Families—individuals born from the same mother—were also kept separate from each other. All guppies were fed paste made from water and Tetramin Tropical Flakes (Tetra, Melle, Germany) and were housed in an aquatic housing system (Aquaneering Inc., San Diego, CA) that standardized water conditions.

Mate choice trials

Mate choice trials were conducted in 5 gallon glass tanks (40.6 × 20.3 × 25.4 cm) filled with municipal water that was carbon filtered and treated with Freshwater Biozyme (Mardel, Oklahoma city, USA) and Prime (Seachem Laboratories, Madison, USA)—the same water used to fill the aquatic housing systems. The tanks were divided into 3 compartments (Supplementary Material S1) by means of transparent acrylic sheets (0.080 inch Clear Acrylic Sheet -Plaskolite, Columbus, USA) bordered by 1.5 cm of 700 μm Nytex net (Safar Inc., Buffalo, USA) to allow for the flow of any chemical cues. At both ends of the central compartment, a 5 cm wide (approximately 2 female body lengths) preference zone was delineated on the exterior of the tank to quantify female association with males in the different compartments. Similar tank designs are commonly used in studies of mate preferences in fish, including guppies (Kodric-Brown 1985; Godin and Dugatkin 1995; Shohet and Watt 2009), and association strongly correlates with probability of mating (Dugatkin and Godin 1992; Kodric-Brown 1993; Brooks and Endler 2001) which we expect to be higher in receptive (virgin) females (Houde 1997).

Prior to a trial, a female guppy was placed into the central compartment to habituate overnight (20 h). On the day of the trial, the experimental tank was illuminated from overhead by an 18” full-spectrum fluorescent light (Aqueon Products, Franklin, USA). To prevent female–male interactions prior to the onset of a trial, the female was isolated in a 12 cm diameter opaque cylinder in the center of the tank, while males from 2 different populations were added into the side compartments and allowed to habituate for 10 min. After habituation, the opaque cylinder was gently removed to release the female and a thin layer of cheesecloth was placed on top of the experimental tank to simulate dusk/dawn conditions when courtship is highest in nature (Endler 1987; Gamble et al. 2003). After starting the video recording equipment (Canon PowerShot SD1000 - Canon, Melville, USA) the experimenter left the room and allowed the trial to run for 20 min.

Following each trial, the guppies were anesthetized in 0.02% Tricaine Methanesulfonate (MS-222 -Argent Chemical Laboratories, Redmond, USA) buffered to a neutral pH with NaCO3. Each guppy was then weighed (nearest 0.0001 g) and photographed on its left side with a Nikon D90 camera (Nikon, Mississauga, Canada), with an attached Speedlight Commander Kit R1C1 flash (Nikon, Mississauga, Canada). Additional illumination was provided with 2 full-spectrum fluorescent bulbs (Aqueon Products, Franklin, USA), and a scale and a X-Rite color checker card (X-Rite, Grand Rapids, USA) were visible in each image. To reduce and thoroughly mix any residual odors after each trial, two thirds of the water in the tank was replaced with fresh-prepared water and allowed to sit for a day before a new trial was performed.

Three types of “male population pairings” (MPPs: Guanapo source vs. Introduction 1; Guanapo source vs. Introduction 2;
Introduction 1 vs. Introduction 2) were generated and tested with females of each population, which led to 9 possible “female population by male population pairings” (FMPPs). Each FMPP was replicated 10 times, generating a total of 90 trials conducted between December 2012 and February 2013. Each female was used in only one trial and each male was used in a maximum of 2 trials. If a male was used twice, it could not be tested for at least 10 days after the first trial, and had to be tested against a female from a different population than the one used in the first trial. We defined family as a set of individuals which were maternal siblings. The specific guppies used in any given trial were selected at random with the following criteria: sympatric (from the same population) males and females could not be from the same family, if more than one female from a given family was used they had to be tested with males from different families than those used to test their sisters, and no male-male family pairs could be repeated. That is, all female–male and male–male family pairs were unique to minimize potential family and individual identity effects. We had an average of 2.72 females from 11 families in the Guanapo source population, 2.14 females from 14 families in the Introduction 1 population, and 3 females from 10 families in the Introduction 2 population. For males, we had on average 3.83 individuals from 12 families in the Guanapo source, 2.85 individuals from 13 families the Introduction 1, and 2.92 individuals from 13 families in the Introduction 2 population.

**Phenotypic traits and female preferences**

For each male guppy, we used ImageJ 1.46r (National Institutes of Health, Bethesda, USA) to measure (nearest 0.001 mm) standard length (SL — tip of lower jaw to end of caudal peduncle), tail length and body area. SL and body mass (BM) were used to calculate individual condition (relative condition index, $K_c$), a proxy of overall health, following Le Cren (1951): $K_c = (BM)/a\times SL^b$, where $a$ and $b$ are the intercept and slope of a least-squares regression of log-BM on log-SL across all male guppies. We also measured the area of the body covered by 3 colors that influence mate choice in guppies: orange (including red), yellow, and black (Kodric-Brown 1983; Houde and Endler 1990; Schwartz and Hendry 2007). Following standard procedures (e.g., Gotanda et al. 2013), the colors were first visually identified and categorized. The area of each color spot was then measured in ImageJ and summed across all spots of that color to obtain the total area of each color on a guppy. Color areas were then expressed as a relative proportion of guppy area: i.e., color area divided by guppy area. All measurements were performed by the same person (LC) and were highly repeatable: orange area ($R^2 = 0.86$), yellow area ($R^2 = 0.85$), black area ($R^2 = 0.83$), total area ($R^2 = 0.99$), SL ($R^2 = 0.94$), and tail size ($R^2 = 0.94$).

We used JWatcher Version 1.0 (Blumstein et al. 2006) to analyze the video recordings for female preference (time spent in each preference zone). Each video was analyzed for 20 min (i.e., full trial) by the same person (LC), starting 30 s after the experimenter had removed the opaque container to allow the female to see both males. To facilitate the analysis of the behavioral trials, we introduced the distinction between “focal males” and “nonfocal males.” In sympatric-allopatric FMPP combinations (female from the same population as one of the males), the focal male was the sympatric male. In allopatric-allopatric FMPP combinations (female from a different population than both males), Introduction 2 males were (arbitrarily) considered focal for Guanapo source and Introduction 1 females, whereas Introduction 1 males were (arbitrarily) considered focal for Introduction 2 females. Two measures of female preference were quantified: 1) time with the focal male (details below) minus time with the nonfocal male divided by time spent with both males (i.e., relative preference for focal male), and 2) time with focal male minus time with the nonfocal male divided by the trial duration (i.e., absolute preference for focal male). In principle, these 2 measures could differ since they show the preference for a given male relative to the total time the female was observed (absolute preference), versus the total time a female was actively making a choice (relative preference, a measure which could inflate the perceived strength of female choice). Nonetheless, these 2 measures yielded qualitatively similar results (Supplementary Materials S2, S3, and S4), and so, we here present results based only on relative preference.

**Statistical analysis**

To assess how males differed in signaling traits among populations, a key prerequisite for assortative mating, we used a multivariate analysis of variance (MANOVA), where the predictor variable was population and the response variables were the male signaling traits: proportion of orange area, proportion of yellow area, proportion of black area, SL, tail size, and $K_c$. We also analyzed each trait individually in univariate analysis of variance (ANOVA) followed with Tukey HSD tests. Given that males used in the experiment did not come from an equally diverse array of families (average number of individuals per family ± SEM: Guanapo source =3.83 ± 0.53, Introduction 1 = 2.85 ± 0.46 and Introduction 2 = 2.92 ± 0.33), we also performed mixed effect models for each signaling trait as a response variable, male population as a fixed factor and the family from which the males originated as a random factor. We used the lme4 (Bates et al. 2015) and MASS packages in R (R Development Core Team 2014). In addition, we used the ade4 package (Dray and Dufour 2007) to perform a linear discriminant analysis with male population as the categorical grouping variable. For males that were used in 2 trials, we analyzed signaling trait data from the first trial only—although similar results were obtained using data for both trials (Supplementary Material S5). Also, including SL as a covariate instead of as a response variable did not change qualitatively the results, with the exception of tail size (results not shown).

To assess variation in female preference, we first tested for whether or not preferences diverged from zero (i.e., no preference for either focal or nonfocal male) within each FMPP, and then for how preferences differed between sympatric-allopatric FMPPs. For the first inference, we used one-sample $t$-tests. For the second inference, we performed an ANOVA with female preference as a response variable and FMPP as a fixed factor. Finally, to test whether female preferences for (or against) sympatric males were context dependent (i.e., depended on the specific allopatric male population in the pairing and its ecological context), we performed planned-comparison $t$-tests that compared the 2 sympatric-allopatric male pairs within each female population.

To assess how male signaling traits influenced female preferences for (or against) sympatric males, we modeled female preference (relative preference for or against sympatric males) using GLMs with a normal distribution and identity link function. The model included FMPP as a fixed factor and the difference between the sympatric and allopatric males’ canonical scores for the first and second canonical variates. All analyses were conducted in R (R Development Core Team 2014).
RESULTS
Did male signaling traits diverge?
Males differed in signaling traits among populations in MANOVA (Pillai’s trace = 0.651, F_{2,118} = 9.171, P < 0.001, Figure 1) and in individual ANOVAs. In fact, with the exception of relative orange area (F_{2,118} = 0.72, P = 0.49), all measured traits differed among populations (relative black area: F_{2,118} = 11.92, P < 0.001; relative yellow area: F_{2,118} = 25.68, P < 0.001; SL: F_{2,118} = 9.62, P < 0.001; tail size: F_{2,118} = 4.17, P = 0.018; Kn: F_{2,118} = 8.24, P < 0.001). Specifically, 1) Guanapo source males had more relative black area and were in higher condition than did Introduction 1 males (Lower Lalaja), 2) Guanapo source males had more relative black area and higher condition but less relative yellow area and smaller SL and tails than did Introduction 2 males (Taylor), and 3) Introduction 2 males had more relative yellow area, larger SL and larger tails than did Introduction 1 males. Including the family from which the males originated as a random factor in the analysis did not change the results qualitatively. That is, all measured traits, with the exception of relative orange area (GLMM, X^2 = 0.92, P = 0.63) and tail size (GLMM, X^2 = 2.52, P = 0.28), differed among populations (GLMM, relative black area: X^2 = 13.6, P = 0.001; relative yellow area: X^2 = 21.39, P < 0.001; SL: X^2 = 9.26, P = 0.01; Kn: X^2 = 10.91, P = 0.004). Although males from the 3 populations showed some trait overlap, they were discriminated from each other in the linear discriminant analysis (% misclassified: Guanapo source = 21.7%, Introduction 1 =35.1%, and Introduction 2 = 39.5%; Figure 2). In general, Introduction 1 males were often intermediate between Guanapo source and Introduction 2 males (Figures 1 and 2) which would explain the higher rate of misclassification. Indeed, classification improved considerably when Introduction 1 males were excluded from the discriminant analysis (percent misclassified: Guanapo source = 17.3% and Introduction 2 = 9.4%).

Do females prefer sympatric vs. allopatric males?
The t-tests showed significant female preferences for a particular male population in some FMPPs but not others (Table 1, Figure 3). Females from the Introduction 1 population did not show any significant preferences (Table 1, A; Figure 3B), whereas females from the other populations showed preferences that

![Figure 1](https://academic.oup.com/beheco/advance-article-abstract-redirect/doi/10.1093/beheco/ary202/5288456)

**Figure 1**
Male signaling trait divergence. Mean (a) relative orange area, (b) relative yellow area, (c) relative black area, (d) body condition (relative condition index - Kn), (e) standard length (SL in mm), and (f) tail size (in mm) values for males from the Guanapo source population (S), Introduction 1 (Int1), and Introduction 2 (Int2) populations.
depended on their particular male population pairing (Table 1, A; Figure 3A, C): When given the choice between a sympatric and an allopatric male, they preferred allopatric males as long as they were not from the Introduction 1 population. Specifically, Guanapo source females preferred Introduction 2 males over their own sympatric Guanapo males (Table 1, A; Figure 3C), and Introduction 2 females preferred Guanapo males over their own sympatric Introduction 2 males (Table 1, A; Figure 3C). That is, Guanapo source and Introduction 2 females preferred males from the other population, whereas Introduction 1 females showed no particular preference for males of any population. Furthermore, females from each population showed no difference in their preference when given a choice between 2 allopatric males (Table 1, B; Figure 3D).

**Table 1**

<table>
<thead>
<tr>
<th>Female population</th>
<th>Male population-pair</th>
<th>t-value</th>
<th>df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Sympatric vs. Allopatric</td>
<td>Guanapo source</td>
<td>S-Int1</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S-Int2</td>
<td>−3.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Introduction 1</td>
<td>Int1-S</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Int1-Int2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Introduction 2</td>
<td>Int2-S</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Int2-Int1</td>
</tr>
<tr>
<td>B</td>
<td>Allopatric vs. Allopatric</td>
<td>Guanapo source</td>
<td>Int2-Int1</td>
<td>−0.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Introduction 1</td>
<td>Int2-S</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Int1-S</td>
</tr>
</tbody>
</table>

*t*-test for female preference (relative preference for focal male) between alternative males derived from different populations by female population and male pair combination. A significant difference represents female preference for males of a given population more than would be expected by chance. Positive *t*-values indicate preference for the population of the first male in the male population-pair column. Abbreviations for population names: Guanapo source (S) population, Introduction 1 (Int1 -Lower Lalaja), and Introduction 2 (Int2 -Taylor).

**Figure 2**

Discriminant analysis plot of male guppies differentiated by their signaling traits. Divergence between male guppies from the Guanapo source (S, triangles), Introduction 1 (Int1, circles), and Introduction 2 (Int2, squares) populations in the first (X) and second (Y) discriminant axes, differentiated by their signaling traits (relative black area, relative orange area, relative yellow area, standard length, relative condition index, and tail size). Loading plot of signaling trait weights in the first (X) and second (Y) discriminant axes in black (arrows).
When exploring female preference from the perspective of differences in the strength of preference for sympatric males between alternative sympatric-allopatric male pairings, we find that female preferences for sympatric males varied among FMPPs (ANOVA: $F_{5,54} = 3.16$, $P = 0.014$). In particular, Guanapo source and Introduction 2 females showed differences in the degree of preference for sympatric males between MPPs (Table 2). That is, preference for sympatric males, relative to allopatric ones, was contingent on the particular population used as the allopatric contrast, such that both Guanapo source and Introduction 2 females showed stronger preference when the allopatric males were from the Guanapo source or Introduction 2 populations instead of the Introduction 1 population.

**Table 2**

Planned comparisons of male pair differences within female population

<table>
<thead>
<tr>
<th>Female population</th>
<th>Male paired populations 1</th>
<th>Male paired populations 2</th>
<th>$t$-value</th>
<th>df</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guanapo source</td>
<td>S-Int1</td>
<td>S-Int2</td>
<td>2.9</td>
<td>18</td>
<td>0.01</td>
</tr>
<tr>
<td>Introduction 1</td>
<td>Int1-Int2</td>
<td>Int1-S</td>
<td>0.11</td>
<td>18</td>
<td>0.91</td>
</tr>
<tr>
<td>Introduction 2</td>
<td>Int2-S</td>
<td>Int2-Int1</td>
<td>-2.76</td>
<td>18</td>
<td>0.013</td>
</tr>
</tbody>
</table>

$T$-test for differences in the strength of female preference for sympatric males between alternative sympatric-allopatric male pairings. A significant difference indicates that female preference for sympatric males varies depending on the allopatric male population. Abbreviations for population names: Guanapo source (S), and Introduction 1 (Int1 -Lower Lalaja), and Introduction 2 (Int2 -Taylor).

Table 2, our results (Supplementary Material S6), as would be expected given that all male identities and family “id”s in each FMPP were unique and the numbers of families were relatively high.

**DISCUSSION**

After experimentally establishing populations in new environments that differed along multiple environmental axes (parasites,
Table 3  
Female preference explained by male difference in canonical variates

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>ss</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD1</td>
<td>1</td>
<td>0.51</td>
<td>4.39</td>
<td>0.041</td>
</tr>
<tr>
<td>LD2</td>
<td>1</td>
<td>0.15</td>
<td>1.27</td>
<td>0.266</td>
</tr>
<tr>
<td>Male pair by female population</td>
<td>5</td>
<td>1.43</td>
<td>2.46</td>
<td>0.045</td>
</tr>
<tr>
<td>Residuals</td>
<td>52</td>
<td>6.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Generalized linear model with a normal distribution of the errors and identity link of female preference for sympatric males (relative preference for focal male) as response variable, male pair combination by female population (MFPP) as factor and the difference in canonical scores between the sympatric and allopatric males for the first (LD1) and second (LD2) discriminant axes as covariates.

predators, diets/food availability, and presumably other factors) compared to their source environment, we assessed the degree to which male signaling traits, female preferences, and assortative mate choice evolved after 8–12 generations. We found that male signaling traits in the introduced populations had diverged, albeit to different degrees, relative to males from the source population, and therefore fulfill a key prerequisite for the evolution of assortative mating. Furthermore, we also found that differences in female preference among populations were partially explained by this male trait divergence (Table 3). However, when females showed a clear preference between male types (which was not always the case), they actually preferred novel (allopatric) males over their local (sympatric) males (Table 1, Figure 3), despite those allopatric males being from a divergent ecological regime and thus potentially maladapted. Our findings highlight 2 general issues that we address below. First, we found a degree of divergence despite the seemingly rapid evolution of male traits from the source population. Specifically, the Introduction 1 population (3 years in the novel environment) showed less evolution of male signaling traits and these males were favored by females from the source population. By contrast, the Introduction 2 population (3 years in the novel environment) showed no genetic differences in melanin-based coloration a direct comparison among studies is precluded. Additionally, Gordon et al. (2015) found the rapid evolution of carotenoid-based colors in Introduction 1 males, whereas we only report such increase for Introduction 2 (albeit from an increase in yellow coloration rather than orange). An increase in carotenoid-based coloration is consistent with previous experimental introductions (e.g., Endler 1980) and common to upper reaches of streams where predators are scarce (Endler 1995), yet Kemp et al. (2018) found that at a later time point the introduced populations had experience no change (Introduction 1) or a loss of orange (Introduction 2) relative to the source population, and argue that such initial increase followed by a decrease could be caused by sex-linked preferences and color patterns which may take several generations for recombination to unlink. With the data available to us we cannot currently evaluate whether the above fluctuations in color evolution are associated to fluctuations in the strength of mate choice, yet it seems that these changes will only be relevant if they allow females to identify males from allopatric populations as novel (see below).

Our 2 introduced populations showed different patterns of male trait divergence from the source population. Specifically, the Introduction 2 population (2 year in the novel environment) showed the evolution of multiple male signaling traits and these males were favored by females from the source population. By contrast, the Introduction 1 population (3 years in the novel environment) showed less evolution of male signaling traits and these males were neither favored nor disfavored by females. This contrast between 2 independent introductions from the same source indicates a substantial variation in guppy trait evolution, which is consistent with recent findings from other guppy studies (Karim et al. 2007; Kemp et al. 2009; Weese et al. 2010; Millar and Hendry 2012; Fitzpatrick et al. 2014; Gotanda and Hendry 2014). These differences could arise due to different time scales (the 2 populations had different lengths of time for evolution), different genetic backgrounds (perhaps the groups of fish used for the 2 introductions were—by chance—genetically different), or environmental differences (perhaps the 2 introduction sites were less similar than they appear).

The first 2 of these explanations for differences between the introduced populations seem unlikely in our case. For time scales, the population (Introduction 1) showing less male trait evolution was actually the population that had been introduced earlier and so had had more time to evolve. Although we cannot discount possible genetic differences, the 2 introductions each used a moderate numbers of females (Introduction 1 = 37; Introduction 2 = 64) and males (Introduction 1 = 37; Introduction 2 = 64), and females had had the opportunity to mate with an additional 37 and 64 males (Introduction 1 and Introduction 2, respectively) before being introduced. Genetic variation therefore should have been representative and similar to the source population for the 2 introductions. By contrast, the third explanation seems more likely given that the 2 introduction sites did manifest some environmental differences.

fact that we sampled the populations at a later time point (2 and 3 years postintroduction instead of 1 year). A later study by Kemp et al. (2018) has reported no change in the evolution black area relative to the source population 6 years postintroduction for males from Introduction 1 and a decrease in black area for Introduction 2 males 5 years postintroduction (consistent with our result). In aggregate, these studies (Gordon et al. 2015; Kemp et al. 2018, and this study) suggest that the direction of melanin-based color evolution may fluctuate through time; yet since we measured only black spot areas and did not include fuzzy-black areas (i.e., facultatively expressed melanin coloration) a direct comparison among studies is precluded. Additionally, Gordon et al. (2015) found the rapid evolution of carotenoid-based colors in Introduction 1 males, whereas we only report such increase for Introduction 2 (albeit from an increase in yellow coloration rather than orange). An increase in carotenoid-based coloration is consistent with previous experimental introductions (e.g., Endler 1980) and common to upper reaches of streams where predators are scarce (Endler 1995), yet Kemp et al. (2018) found that at a later time point the introduced populations had experience no change (Introduction 1) or a loss of orange (Introduction 2) relative to the source population, and argue that such initial increase followed by a decrease could be caused by sex-linked preferences and color patterns which may take several generations for recombination to unlink. With the data available to us we cannot currently evaluate whether the above fluctuations in color evolution are associated to fluctuations in the strength of mate choice, yet it seems that these changes will only be relevant if they allow females to identify males from allopatric populations as novel (see below).

Our 2 introduced populations showed different patterns of male trait divergence from the source population. Specifically, the Introduction 2 population (2 year in the novel environment) showed the evolution of multiple male signaling traits and these males were favored by females from the source population. By contrast, the Introduction 1 population (3 years in the novel environment) showed less evolution of male signaling traits and these males were neither favored nor disfavored by females. This contrast between 2 independent introductions from the same source indicates a substantial variation in guppy trait evolution, which is consistent with recent findings from other guppy studies (Karim et al. 2007; Kemp et al. 2009; Weese et al. 2010; Millar and Hendry 2012; Fitzpatrick et al. 2014; Gotanda and Hendry 2014). These differences could arise due to different time scales (the 2 populations had different lengths of time for evolution), different genetic backgrounds (perhaps the groups of fish used for the 2 introductions were—by chance—genetically different), or environmental differences (perhaps the 2 introduction sites were less similar than they appear).

The first 2 of these explanations for differences between the introduced populations seem unlikely in our case. For time scales, the population (Introduction 1) showing less male trait evolution was actually the population that had been introduced earlier and so had had more time to evolve. Although we cannot discount possible genetic differences, the 2 introductions each used a moderate numbers of females (Introduction 1 = 37; Introduction 2 = 64) and males (Introduction 1 = 37; Introduction 2 = 64), and females had had the opportunity to mate with an additional 37 and 64 males (Introduction 1 and Introduction 2, respectively) before being introduced. Genetic variation therefore should have been representative and similar to the source population for the 2 introductions. By contrast, the third explanation seems more likely given that the 2 introduction sites did manifest some environmental differences.
For instance, productivity was lower in the Introduction 1 site than in the Introduction 2 site owing to a reduced canopy cover at the second site which allowed higher light penetration (Kohler et al. 2012). Notably, higher light intensities have been associated to higher rates of color evolution (Kemp et al. 2018). However, given the multidimensional nature of differences between the source and introduction environments, and the limited number of replicated populations ($n = 2$), we cannot at present attribute this divergence to specific environmental differences.

**Trait evolution and (non-)assortative preferences**

Differences between the 2 introduced populations in male signaling trait evolution can explain some of the patterns of female preference we report. For instance, the lesser divergence of Introduction 1 males, and their resulting intermediate phenotype between the other 2 populations (Figures 1 and 2), could explain why females did not distinguish between males from the Introduction 1 versus males from either of the other 2 populations (Figure 3). In addition, Introduction 1 females showed the least discrimination between their sympatric males versus males from the other populations (Figure 3B, Tables 1 and 2). Further, Guanapo source and Introduction 2 females showed no discrimination between their own males versus Introduction 1 males. This general association between divergence in male signaling traits and divergence in female preferences has been found to varying degrees in other studies of guppies (Schwartz and Hendry 2006) and other taxa (Rodríguez et al. 2013).

Our mate choice design measured under a common-garden setting female associational preferences expected to have evolved in the wild, but did not directly assess mating success since males were separated from females by a transparent partition. Laboratory common-garden assays cannot integrate potentially complex environment by genetic interactions that may play a role in mate choice under field conditions. But our design isolates potential genetic variation in preference and is directly comparable to several standard laboratory contexts have shown that female guppies often prefer rare (novel) phenotypes within their own population (Hughes et al. 1999; Zajitschek and Brooks 2008; Macario et al. 2017; Hampton et al. 2009; Hughes et al. 2013). Suggested reasons include inbreeding avoidance (Johnson et al. 2012), reduced probability of remating with the same male (Hampton et al. 2009), and a survival advantage for rare individuals (Olendorf et al. 2006). Another possibility is that mating with rare males increases MHC diversity (Milinski 2006), which could enhance resistance to parasites (Eizaguirre and Lenz 2010). We therefore suggest that selection favoring mating with rare males within populations could lead to disassortative mating between populations as a coincidental by-product. That is, disassortative preference in our populations seems to arise not through the evolution of a novel preference, but instead through the evolution of traits that facilitate the expression of a pre-existing preference. Although this by-product could be maladaptive and thus be selected against, this situation would only take place in the presence of reasonably high dispersal between populations (Servedio and Noor 2003). Indeed, Schwartz et al. (2010) showed that high-predation females generally preferred low-predation males (disassortative mating as in our study), except when the low-predation males were from the population immediately upstream (suggesting reinforcement). Similarly, Magurran et al. (1994) reported that female
guppies were unable to discriminate between unfamiliar individuals from their local or foreign populations. In our study populations, gene flow (following the introduction itself) would be rare or absent between the source and introduction sites owing to physical barriers to upstream movement and the large distances between source and introduction populations. Thus, we would not expect direct selection against between-population mating, which means that if rare-male preferences previously evolved due to within-population selection they would not be opposed by between-population selection.

Overall, our findings are congruent with recent suggestions that reproductive isolation following divergent selection is not so easily accomplished (Hendry 2009; Nosil et al. 2009; Rundell and Price 2009; Swenson 2012). That is, divergent selection might be present but progress toward the formation of reproductive barriers might be strongly constrained—for several reasons. First, high gene flow might prevent local adaptation despite divergent selection (Lenormand 2002; Garant et al. 2007). Second, adaptive divergence might occur but make only modest contributions to reproductive isolation. In guppies, for instance, migrants between predation environments can suffer low fitness, which can impose a reproductive barrier (Weese et al. 2011); however, at the same time, conserved patterns of sexual selection can lead to disassortative mating between those populations (Schwartz and Hendry 2006; Labonne and Hendry 2010; present study). In short, guppies provide a good example of how divergent natural selection can simultaneously generate reproductive barriers (e.g., selection against migrants) and reproductive enhancers (disassortative mating) that potentially lead to no net effect on gene flow (Crispo et al. 2006; Labonne and Hendry 2010; Schwartz and Hendry 2010) and, hence, little or no progress toward reproductive isolation.

Implications

We detected the contemporary evolution of guppy signaling traits after experimental introductions into a novel environment, but that male trait evolution was not coupled to female preference evolution. The combination of these 2 outcomes was the facilitation of disassortative mating between populations. Our study highlights the likely importance of conserved sexual selection—here for novel males—in sometimes counteracting the isolating influence of even strong and multifarious divergent natural selection. Theoretical and empirical studies of local adaptation should pay more attention to the various influences of mating system variation in enhancing or constraining progress toward adaptation and speciation. Just as ecological speciation requires both divergent selection and assortative mating, positive assortment by mate choice requires a mating system that couples divergent trait evolution to female preferences in specific ways.

SUPPLEMENTARY MATERIAL

Supplementary data are available at Behavioral Ecology online.

FUNDING

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) through a Special Research Opportunity grant (GFF and APH - # 356373-07), Research Tools and Instruments grant to APH, and a Vanier Canada Graduate Scholarship to F.D. ED. was also supported by a Centre for Host-Parasite Interactions bridge fund, a NSERC Banting Postdoctoral Fellowship and an NSERC Discovery Accelerator Supplement to T. Sherratt. Research at the Ghalambor lab was supported by a National Science Foundation’s Faculty Early Career grant to CKG (DEB-0846175).

We thank D. Reznick, E. Ruell, D. Fraser, and the FIBR team for supplying us with guppies derived from the FIBR sites in Trinidad, for their support, and for their advice. We thank S. Porter and S. Reader for advice on data analysis, A. Howard for laboratory assistance, H. Auld, S. Potter, R. Pusiak, and D. Reznick for comments on an earlier version of this manuscript, and K. Gotanda for training in color assessment. Four anonymous reviewers and M. Wegner from Axios Review provided helpful advice.

Data accessibility: Analyses reported in this article can be reproduced using the data provided by Dargent et al. (2018).

Handling editor: Ulrika Candolin

REFERENCES


