



Original Article

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

Felipe Dargent,^{a,*} Lisa Chen,^a Gregor F. Fussmann,^a Cameron K. Ghalambor,^b and Andrew P. Hendry^{a,c}

^aDepartment of Biology, McGill University, 1205 Dr. Penfield Av., Montreal H3A 1B1, QC, Canada,

^bDepartment of Biology, Colorado State University at Fort Collins, 1878 Campus Delivery, Fort Collins CO 80523, USA, and ^cRedpath Museum, McGill University, 859 Sherbrooke St. West, Montreal H3A 0C4, Canada

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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 (at a minimum: parasitism, predation, and 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 male 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 (Hendry et al. 2007; Nosil 2012). However, many instances of local adaptation to divergent environments seem not to generate substantial progress toward reproductive isolation (e.g., Hendry 2009; Nosil et al. 2009; Rundell and Price 2009; Svensson 2012; Hendry 2017). 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 (Nosil et al. 2009; Chevin et al. 2014). Such shifts are hypothesized to increase the total strength of divergent selection and the number of independent trait dimensions that show divergence (i.e., multifarious selection). The consequence is thought to be greater and more rapid progress toward local adaptation and ecological speciation (Nosil et al. 2009; Nosil 2012). 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

Address correspondence to F. Dargent, who is now at Department of Biology, University of Ottawa, 30 Marie Curie Private, Ottawa, K1N 6N5, ON, Canada. E-mail: felipe.dargent@mail.mcgill.ca.

hybrids can occur as a result of their maladaptation to local parasites (MacColl and Chapman 2010; Ricklefs 2010), predators (Stoks et al. 2005; Nosil and Crespi 2006; Langerhans et al. 2007), or available diets (Rundle et al. 2000; Boughman et al. 2005; 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 resistance 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 traits 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 all 3 populations 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 unfeasible 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* sp.) 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 (Zandonà 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 Tetraamin 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 Nyltex 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_n), a proxy of overall health, following Le Cren (1951): $K_n = (BM)/a(SL)^b$, 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 1985; 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 male 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_n . 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 \pm 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: $F_{2,118} = 4.17$, $P = 0.018$; K_n : $F_{2,118} = 8.24$, $P < 0.001$). 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: $X^2 = 9.26$, $P = 0.01$; K_n : $X^2 = 10.91$, $P = 0.004$). 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

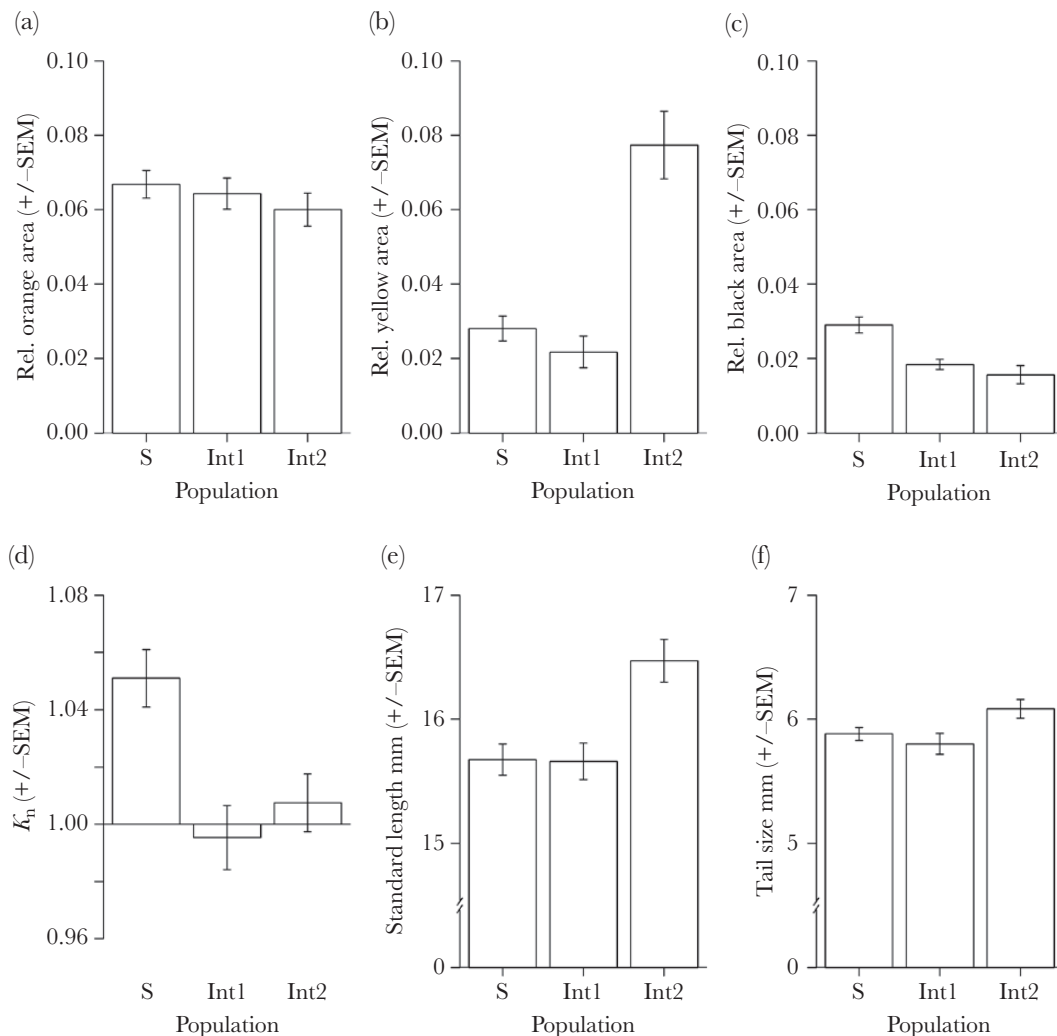


Figure 1

Male signaling trait divergence. Mean (a) relative orange area, (b) relative yellow area, (c) relative black area, (d) body condition (relative condition index - K_n), (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.

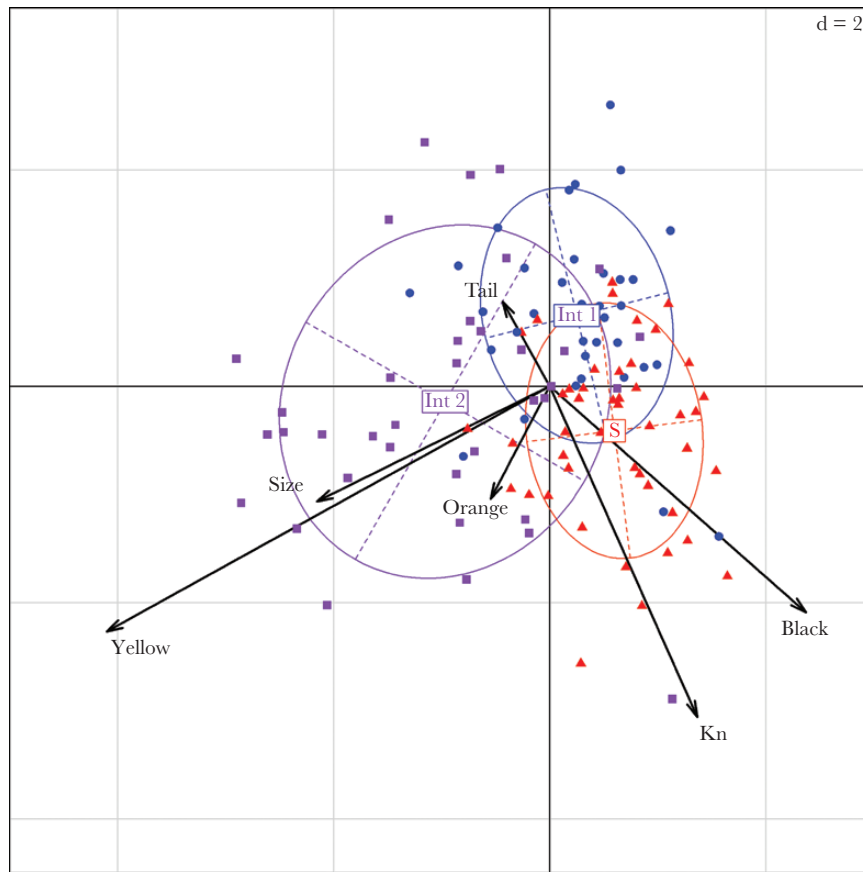


Figure 2

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).

Table 1

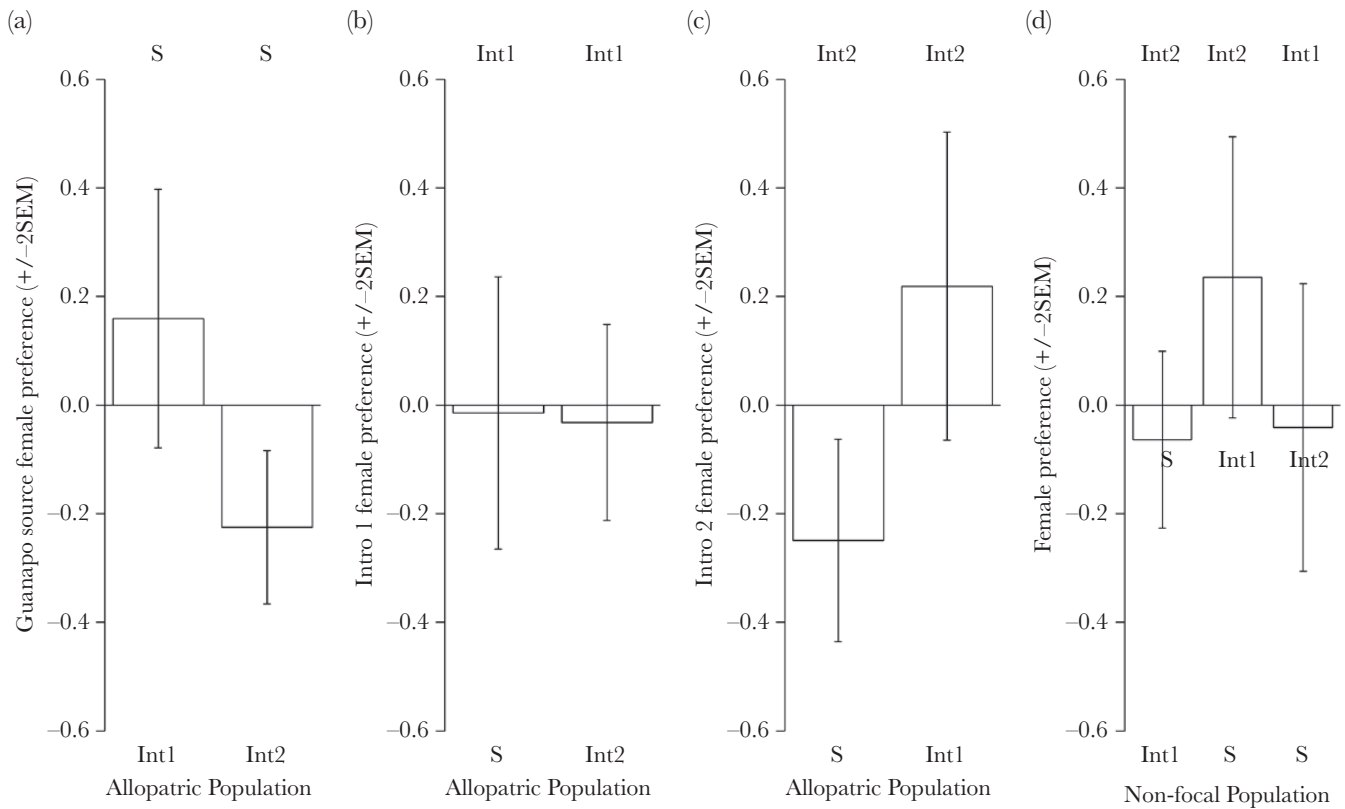
Female preference

		Female population	Male population-pair	<i>t</i> -value	df	<i>P</i> -value
A	Sympatric vs. Allopatric	Guanapo source	S-Int1	1.34	8	0.217
			S-Int2	-3.19	10	0.01
		Introduction 1	Int1-S	-0.12	9	0.91
			Int1-Int2	-0.36	9	0.729
		Introduction 2	Int2-S	-2.68	9	0.025
			Int2-Int1	1.54	9	0.157
B	Allopatric vs. Allopatric	Guanapo source	Int2-Int1	-0.78	9	0.455
		Introduction 1	Int2-S	1.82	9	0.102
		Introduction 2	Int1-S	-0.31	9	0.764

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).

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).

**Figure 3**

Female preference between alternative male choices. Female preference measured as time spent with a sympatric male minus time spent with an allopatric male divided by the total time spent with both males (relative preference for focal male) for females from the Guanapo source (a), Introduction 1 (b), and Introduction 2 (c) populations. Positive values represent preference for the sympatric male and negative values preference for allopatric males. (d) Preference for males of 2 allopatric populations; preference for males of the (arbitrary) focal population denoted by positive values and female population names immediately below bars. Abbreviations for population names: Guanapo source (S), and Introduction 1 (Int1) and Introduction 2 (Int2) populations. Letters on top of panels a-c coincide with female population (i.e., sympatric males).

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.

Do male traits influence female preference for sympatric versus allopatric males?

Female preference was also influenced by male signaling traits (Table 3). The first canonical variate (relative black and yellow areas, and size – Figure 2), which was the main axis of divergence between the Guanapo source and the Introduction 2 populations, explained some variation in female preference among sympatric vs. allopatric males (Table 3), whereas the second canonical variate had no significant effect. Nonetheless, inclusion of male signaling traits did not change our results regarding variation in female preferences for sympatric males in relation to MPP. Including the identity and family “id” of the sympatric male as random factors did not qualitatively change

Table 2

Planned comparisons of male pair differences within female population

Female population	Male paired populations 1	Male paired populations 2	<i>t</i> -value	df	<i>P</i> -value
Guanapo source	S-Int1	S-Int2	2.9	18	0.01
Introduction 1	Int1-Int2	Int1-S	0.11	18	0.91
Introduction 2	Int2-S	Int2-Int1	-2.76	18	0.013

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) population, Introduction 1 (Int1 -Lower Lalaja), and Introduction 2 (Int2 -Taylor).

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

	df	ss	F	P
LD1	1	0.51	4.39	0.041
LD2	1	0.15	1.27	0.266
Male pair by female population	5	1.43	2.46	0.045
Residuals	52	6.06		

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 replicate nature of our introductions. Second, we found no progress toward assortative mate preference despite strong multifarious shifts in selection and attendant trait divergence. Below, we discuss these results in more detail.

Rapid evolution of male traits

Consistent with previous studies on experimentally translocated guppies (e.g., Endler 1980; Kemp et al. 2009), our results show rapid evolution of male signaling traits, albeit at a faster rate than was suggested by those previous experiments. Furthermore, consistent with mate choice studies (see review by Easty et al. 2011) indicating female preference for larger males and higher carotenoid-based (i.e., orange and yellow) and melanin-based colors (i.e., black), we found that female preference in our study was linked to the first canonical variate of our signaling traits, the axis accounting for most variation in male size, and relative yellow and black area, but not orange. Recently, Gordon et al. (2015) directly assessed selection on and the evolution of male secondary sexual traits (i.e., size, and carotenoid-based and melanin-based colors), in the Guanapo source population and 2 introduced populations (Lower Lalaja, here called Introduction 1, and Upper Lalaja) during the first year postintroduction. Consistent with our results, Gordon et al. (2015) found that wild guppies experienced a decrease in melanin-based colors relative to the ancestral stock; nonetheless, their laboratory assessment of second-generation males grown in common garden showed no genetic differences in melanin-based coloration between the Guanapo source and Introduction 1 populations. Our finding of rapid evolution toward a decreased proportion of black area in the introduced populations is not explained by the

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 guppy mate choice studies (e.g., Kodric-Brown, 1985; Godin and Dugatkin 1995; Godin et al. 2005; Auld et al. 2016). We also controlled for potentially confounding factors unrelated to trait preference such as mate copying (Godin et al. 2005), low mate receptivity during pregnancy (Houde 1997), or avoidance of infected conspecifics (Croft et al. 2011). It is conceivable that females could have been associating with males for reasons other than mating. We think this is unlikely. First, female association in guppies has been shown to correlate with mating probability (Dugatkin and Godin 1992; Kodric-Brown 1993; Brooks and Endler 2001). Second, we used virgin females which were sexually mature and receptive, unlike pregnant females (Houde 1997).

Importantly, the combination of male trait and female preference divergence in our study system facilitated *negative* assortative preference (choosy females preferred novel allopatric males) as opposed to the *positive* assortative preference typically assumed under local adaptation (Rundle et al. 2000; Schluter 2000; Nosil 2012). We suggest that female preferences for novel males, commonly expressed when females choose among males from their own population (details below), were conserved in our experiment and thus led to negative assortative mating when average male trait differences among populations were large enough to form a basis for female discrimination. These differences in the patterns of male trait divergence between our 2 introduced populations have the potential to lead to differences in patterns of progress toward

(or away from) reproductive isolation, as they can influence the female's ability to discriminate local from foreign.

Why no progress toward reproductive barriers?

The general lack of progress toward reproductive isolation in guppies has been long ruminated on (Magurran 1998; Herdegen et al. 2014), including in relation to divergent selection and, hence, ecological speciation (Schwartz and Hendry 2006; Schwartz et al. 2010). The conundrum is that guppies rapidly and repeatedly evolve dramatic adaptive divergence between environments (Endler 1995; Magurran 2005), which should promote the evolution of reproductive barriers (Schluter 2000; Rundle and Nosil 2005; Nosil 2012); yet many guppy populations show only minimal, if any, reproductive isolation related to ecological differences (Magurran 2005). Our study reinforces this perspective by finding that multidimensional ecological shifts (certainly parasites, predators, and diet/resources—but probably other ecological variables too), which presumably generated strong and multifarious selection, did not generate the contemporary evolution of positive assortative mating. Perhaps, the simplest explanation might be that not enough time has yet passed for a substantial response to selection. Yet, many previous studies of other taxa have shown that strong partial barriers to gene flow can evolve on short time scales when populations colonize different environments (Hendry et al. 2007; Svensson and Gosden 2007). Indeed, we consider the “lack of time” explanation unlikely for our study given that male signaling traits had enough time to evolve (this study, Gordon et al. 2015), that assortative preference (albeit negative) did actually arise, and the above-described precedent of weak or no positive assortative mating in several populations of this system. Although studies at future time-points will provide important evidence against (or in support of) the “lack of time” argument, we see no a priori reason to expect behavioral traits to evolve at a slower rate than morphological ones. Thus, the most original question to address here is: Why does environmental change along multiple axes lead to the contemporary evolution of male signaling traits that causes *disassortative* preferences?

As mentioned earlier, a number of studies in both natural and 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 (Servodio 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; Svensson 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.

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REFERENCES

- Arendt JD, Reznick DN, López-Sepulcre A. 2014. Replicated origin of female-biased adult sex ratio in introduced populations of the Trinidadian guppy (*Poecilia reticulata*). *Evolution*. 68:2343–2356.
- Auld HL, Pusiak RJP, Godin J. 2016. Independent mating preference for male body size and coloration in female Trinidadian guppies. *Ethology*. 122:597–608.
- Badyaev AV, Young RL, Oh KP, Addison C. 2008. Evolution on a local scale: developmental, functional, and genetic bases of divergence in bill form and associated changes in song structure between adjacent habitats. *Evolution*. 62:1951–1964.
- Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *J Stat Softw*. 2015. 67:48.
- Becher SA, Magurran AE. 2004. Multiple mating and reproductive skew in Trinidadian guppies. *Proc Biol Sci*. 271:1009–1014.
- Blumstein DT, Evans CS, Daniel JC. 2006. JWATChER v. 1.0. www.jwatcher.ucla.edu (Accessed 4 January 2019).
- Boughman JW, Rundle HD, Schluter D. 2005. Parallel evolution of sexual isolation in sticklebacks. *Evolution*. 59:361–373.
- Brooks R, Endler JA. 2001. Female guppies agree to differ: phenotypic and genetic variation in mate-choice behavior and the consequences for sexual selection. *Evolution*. 55:1644–1655.
- Chevin L-M, Decorzent G, Lenormand T. 2014. Niche dimensionality and the genetics of ecological speciation. *Evolution*. 68:1244–1256.
- Crispo E, Bentzen P, Reznick DN, Kinnison MT, Hendry AP. 2006. The relative influence of natural selection and geography on gene flow in guppies. *Mol Ecol*. 15:49–62.
- Croft D, Edenbrow M, Darden S, Ramnarine I, van Oosterhout C, Cable J. 2011. Effect of gyrodactylid ectoparasites on host behaviour and social network structure in guppies *Poecilia reticulata*. *Behav Ecol Sociobiol*. 65:2219–2227.
- Dargent F, Chen L, Fussmann GF, Ghalambor CK, Hendry AP. 2018. Data from: female preference for novel males constrains the contemporary evolution of assortative mating in guppies. Dryad Digital Repository. <http://dx.doi.org/10.5061/dryad.44hh4pj>
- Dargent F, Rolshausen G, Hendry AP, Scott ME, Fussmann GF. 2016. Parting ways: parasite release in nature leads to sex-specific evolution of defence. *J Evol Biol*. 29:23–34.
- Dargent F, Scott ME, Hendry AP, Fussmann GF. 2013. Experimental elimination of parasites in nature leads to the evolution of increased resistance in hosts. *Proc Biol Sci*. 280:20132371.
- Dargent F, Scott ME, Hendry AP, Fussmann GF. 2014. Experimental evolution of parasite resistance in wild guppies: natural and multifarious selection. *Proc Biol Sci*. 281:20141820.
- Dray S, Dufour A-B. 2007. The ade4 package: implementing the duality diagram for ecologists. *J Stat Softw*. 22:1–20.
- Dugatkin LA, Godin JG. 1992. Reversal of female mate choice by copying in the guppy (*Poecilia reticulata*). *Proc Biol Sci*. 249:179–184.
- Easty LK, Schwartz AK, Gordon SP, Hendry AP. 2011. Does sexual selection evolve following introduction to new environments? *Anim Behav*. 82:1085–1095.
- Eizaguirre C, Lenz TL, Traulsen A, Milinski M. 2009. Speciation accelerated and stabilized by pleiotropic major histocompatibility complex immunogenes. *Ecol Lett*. 12:5–12.
- Eizaguirre C, Lenz TL. 2010. Major histocompatibility complex polymorphism: dynamics and consequences of parasite-mediated local adaptation in fishes. *J Fish Biol*. 77:2023–2047.

- El-Sabaawi RW, Zandonà E, Kohler TJ, Marshall MC, Moslemi JM, Travis J, López-Sepulcre A, Ferrière R, Pringle CM, Thomas SA, Reznick DN, Flecker AS. 2012. Widespread intraspecific organismal stoichiometry among populations of the Trinidadian guppy. *Funct Ecol*. 26:666–676.
- Endler JA. 1980. Natural selection on color patterns in *Poecilia reticulata*. *Evolution*. 34:76–91.
- Endler JA. 1987. Predation, light intensity and courtship behavior in *Poecilia reticulata* (Pisces, Poeciliidae). *Anim Behav*. 35:1376–1385.
- Endler JA. 1995. Multiple-trait coevolution and environmental gradients in guppies. *Trends Ecol Evol*. 10:22–29.
- Endler JA, Houde AE. 1995. Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution*. 49:456–468.
- Fitzpatrick SW, Torres-Dowdall J, Reznick DN, Ghalambor CK, Funk WC. 2014. Parallelism isn't perfect: could disease and flooding drive a life-history anomaly in Trinidadian guppies? *Am Nat*. 183:290–300.
- Fraser BA, Neff BD. 2009. MHC class IIB additive and non-additive effects on fitness measures in the guppy *Poecilia reticulata*. *J Fish Biol*. 75:2299–2312.
- Fraser BA, Ramnarine IW, Neff BD. 2010. Temporal variation at the MHC class IIB in wild populations of the guppy (*Poecilia reticulata*). *Evolution*. 64:2086–2096.
- Gamble S, Lindholm AK, Endler JA, Brooks R. 2003. Environmental variation and the maintenance of polymorphism: the effect of ambient light spectrum on mating behaviour and sexual selection in guppies. *Ecol Lett*. 6:463–472.
- Garant D, Forde SE, Hendry AP. 2007. The multifarious effects of dispersal and gene flow on contemporary adaptation. *Funct Ecol*. 21:434–443.
- Ghalambor CK, Hoke KL, Ruell EW, Fischer EK, Reznick DN, Hughes KA. 2015. Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature*. 525:372–375.
- Gilliam JF, Fraser DF, Alkinkoo M. 1993. Structure of a tropical stream fish community - a role for biotic interactions. *Ecology*. 74:1856–1870.
- Godin J-GJ, Dugatkin LA. 1995. Variability and repeatability of female mating preference in the guppy. *Anim Behav*. 49:1427–1433.
- Godin J-GJ, Herdman EJE, Dugatkin LA. 2005. Social influences on female mate choice in the guppy, *Poecilia reticulata*: generalized and repeatable trait-copying behaviour. *Anim Behav*. 69:999–1005.
- Gordon SP, Reznick D, Arendt JD, Roughton A, Ontiveros Hernandez MN, Bentzen P, López-Sepulcre A. 2015. Selection analysis on the rapid evolution of a secondary sexual trait. *Proc Biol Sci*. 282:20151244.
- Gotanda KM, Delaire LC, Raeymaekers JA, Pérez-Jvostov F, Dargent F, Bentzen P, Scott ME, Fussmann GF, Hendry AP. 2013. Adding parasites to the guppy-predation story: insights from field surveys. *Oecologia*. 172:155–166.
- Gotanda KM, Hendry AP. 2014. Using adaptive traits to consider potential consequences of temporal variation in selection: male guppy colour through time and space. *Biol J Linn Soc*. 112:108–122.
- Grether GF, Millie DF, Bryant MJ, Reznick DN, Mayea W. 2001. Rain forest canopy cover, resource availability, and life history evolution in guppies. *Ecology*. 82:1546–1559.
- Hampton KJ, Hughes KA, Houde AE. 2009. The Allure of the distinctive: reduced sexual responsiveness of female guppies to 'redundant' male colour patterns. *Ethology*. 115:475–481.
- Handelsman CA, Broder ED, Dalton CM, Ruell EW, Myrick CA, Reznick DN, Ghalambor CK. 2013. Predator-induced phenotypic plasticity in metabolism and rate of growth: rapid adaptation to a novel environment. *Int Comp Biol*. 53:975–988.
- Harris PD, Lyles AM. 1992. Infections of *Gyrodactylus bullatarudis* and *Gyrodactylus turnbulli* on guppies (*Poecilia reticulata*) in Trinidad. *J Parasitol*. 78:912–914.
- Hendry AP. 2009. Ecological speciation! Or the lack thereof? *Can J Fish Aqua Sci*. 66:1383–1398.
- Hendry AP. 2017. *Eco-evolutionary dynamics*. Princeton (NJ): Princeton University Press.
- Hendry AP, Huber SK, De León LF, Herrel A, Podos J. 2009. Disruptive selection in a bimodal population of Darwin's finches. *Proc Biol Sci*. 276:753–759.
- Hendry AP, Nosil P, Rieseberg LH. 2007. The speed of ecological speciation. *Funct Ecol*. 21:455–464.
- Herdegen M, Alexander HJ, Babik W, Mavárez J, Breden F, Radwan J. 2014. Population structure of guppies in north-eastern Venezuela, the area of putative incipient speciation. *BMC Evol Biol*. 14:28.
- Houde AE, Endler JA. 1990. Correlated evolution of female mating preferences and male color patterns in the guppy *Poecilia reticulata*. *Science*. 248:1405–1408.
- Houde AE, Torio AJ. 1992. Effect of parasitic infection on male color pattern and female choice in guppies. *Behav Ecol*. 3:346–351.
- Houde AE. 1997. *Sex, color and mate choice in guppies*. Princeton: Princeton University Press.
- Huber SK, De León LF, Hendry AP, Bermingham E, Podos J. 2007. Reproductive isolation of sympatric morphs in a population of Darwin's finches. *Proc Biol Sci*. 274:1709–1714.
- Hughes KA, Du L, Rodd FH, Reznick DN. 1999. Familiarity leads to female mate preference for novel males in the guppy, *Poecilia reticulata*. *Anim Behav*. 58:907–916.
- Hughes KA, Houde AE, Price AC, Rodd FH. 2013. Mating advantage for rare males in wild guppy populations. *Nature*. 503:108–110.
- Johnson PT, Rohr JR, Hoverman JT, Kellermanns E, Bowerman J, Lunde KB. 2012. Living fast and dying of infection: host life history drives interspecific variation in infection and disease risk. *Ecol Lett*. 15:235–242.
- Karim N, Gordon SP, Schwartz AK, Hendry AP. 2007. This is not déjà vu all over again: male guppy colour in a new experimental introduction. *J Evol Biol*. 20:1339–1350.
- Kemp DJ, Batistic FK, Reznick DN. 2018. Predictable adaptive trajectories of sexual coloration in the wild: evidence from replicate experimental guppy populations. *Evolution*. 72:2462–2477.
- Kemp DJ, Reznick DN, Grether GF, Endler JA. 2009. Predicting the direction of ornament evolution in Trinidadian guppies (*Poecilia reticulata*). *Proc Biol Sci*. 276:4335–4343.
- Kennedy CEJ, Endler JA, Poynton SL, McMinn H. 1987. Parasite load predicts mate choice in guppies. *Behav Ecol Sociobiol*. 21:291–295.
- Kodric-Brown A. 1985. Female preference and sexual selection for male coloration in the guppy (*Poecilia reticulata*). *Behav Ecol Sociobiol*. 17:199–205.
- Kodric-Brown A. 1993. Female choice of multiple male criteria in guppies: interacting effects of dominance, coloration and courtship. *Behav Ecol Sociobiol*. 32:415–420.
- Kohler TJ, Heatherly TN, El-Sabaawi RW, Zandonà E, Marshall MC, Flecker AS, Pringle CM, Reznick DN, Thomas SA. 2012. Flow, nutrients, and light availability influence neotropical epilithon biomass and stoichiometry. *Freshwater Sci*. 31:1019–1034.
- Kolluru GR, Grether GF, Dunlop E, South SH. 2009. Food availability and parasite infection influence mating tactics in guppies (*Poecilia reticulata*). *Behav Ecol*. 20:131–137.
- Labonne J, Hendry AP. 2010. Natural and sexual selection giveth and taketh away reproductive barriers: models of population divergence in guppies. *Am Nat*. 176:26–39.
- Langerhans RB, Gifford ME, Joseph EO. 2007. Ecological speciation in *Gambusia* fishes. *Evolution*. 61:2056–2074.
- Le Cren ED. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *J Anim Ecol*. 20:201–219.
- Lenormand T. 2002. Gene flow and the limits to natural selection. *Trends Ecol Evol*. 17:183–189.
- Lopez S. 1998. Acquired resistance affects male sexual display and female choice in guppies. *Proc Biol Sci*. 265:717–723.
- López-Sepulcre A, Gordon SP, Paterson IG, Bentzen P, Reznick DN. 2013. Beyond lifetime reproductive success: the posthumous reproductive dynamics of male Trinidadian guppies. *Proc Biol Sci*. 280:20131116.
- Macario A, Croft DP, Endler JA, Darden SK. 2017. Early social experience shapes female mate choice in guppies. *Behav Ecol*. 28:833–843.
- MacColl ADC, Chapman SM. 2010. Parasites can cause selection against migrants following dispersal between environments. *Funct Ecol*. 24:847–856.
- Magurran AE. 1998. Population differentiation without speciation. *Philos Trans Roy Soc*. 353:275–286.
- Magurran AE. 2005. *Evolutionary ecology: the Trinidadian guppy*. New York: Oxford University Press.
- Magurran AE, Seghers BH, Shaw PW, Carvalho GR. 1994. Schooling preferences for familiar fish in the guppy, *Poecilia reticulata*. *J Fish Biol*. 45:401–406.
- Matthews B, Harmon LJ, M'Gonigle L, Marchinko KB, Schaschl H. 2010. Sympatric and allopatric divergence of MHC genes in threespine stickleback. *PLoS One*. 5:e10948.
- Milinski M. 2006. The major histocompatibility complex, sexual selection, and mate choice. *Annu Rev Ecol Evol Syst*. 37:159–186.

- Millar N, Hendry A. 2012. Population divergence of private and non-private signals in wild guppies. *Environ Biol Fishes*. 94:513–525.
- Nosil P. 2012. *Ecological speciation*. New York: Oxford University Press.
- Nosil P, Crespi BJ. 2006. Experimental evidence that predation promotes divergence in adaptive radiation. *Proc Natl Acad Sci USA*. 103:9090–9095.
- Nosil P, Crespi BJ, Sandoval CP. 2003. Reproductive isolation driven by the combined effects of ecological adaptation and reinforcement. *Proc Biol Sci*. 270:1911–1918.
- Nosil P, Harmon LJ, Seehausen O. 2009. Ecological explanations for (incomplete) speciation. *Trends Ecol Evol*. 24:145–156.
- Ojanguren AF, Magurran AE. 2004. Uncoupling the links between male mating tactics and female attractiveness. *Proc Biol Sci*. 271(Suppl 6):S427–S429.
- Olendorf R, Rodd FH, Punzalan D, Houde AE, Hurt C, Reznick DN, Hughes KA. 2006. Frequency-dependent survival in natural guppy populations. *Nature*. 441:633–636.
- van Oosterhout C, Mohammed RS, Hansen H, Archard GA, McMullan M, Weese DJ, Cable J. 2007. Selection by parasites in spate conditions in wild Trinidadian guppies (*Poecilia reticulata*). *Int J Parasitol*. 37:805–812.
- Pérez-Jvostov F, Hendry AP, Fussmann GF, Scott ME. 2012. Are host-parasite interactions influenced by adaptation to predators? A test with guppies and *Gyrodactylus* in experimental stream channels. *Oecologia*. 170:77–88.
- Pitcher TE, Neff BD, Rodd FH, Rowe L. 2003. Multiple mating and sequential mate choice in guppies: females trade up. *Proc Biol Sci*. 270:1623–1629.
- R Core Team. 2014. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing.
- Reznick DA, Bryga H, Endler JA. 1990. Experimentally induced life-history evolution in a natural population. *Nature*. 346:357–359.
- Reznick D, Nunney L, Tessier A. 2000. Big houses, big cars, superfleas and the costs of reproduction. *Trends Ecol Evol*. 15:421–425.
- Ricklefs RE. 2010. Host-pathogen coevolution, secondary sympatry and species diversification. *Philos Trans Roy Soc*. 365:1139–1147.
- Rodríguez RL, Boughman JW, Gray DA, Hebets EA, Höbel G, Symes LB. 2013. Diversification under sexual selection: the relative roles of mate preference strength and the degree of divergence in mate preferences. *Ecol Lett*. 16:964–974.
- Ruell EW, Handelsman CA, Hawkins CL, Sofaer HR, Ghalambor CK, Angeloni L. 2013. Fear, food and sexual ornamentation: plasticity of colour development in Trinidadian guppies. *Proc Biol Sci*. 280:20122019.
- Rundell RJ, Price TD. 2009. Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends Ecol Evol*. 24:394–399.
- Rundle HD, Nagel L, Wenrick Boughman J, Schluter D. 2000. Natural selection and parallel speciation in sympatric sticklebacks. *Science*. 287:306–308.
- Rundle HD, Nosil P. 2005. Ecological speciation. *Ecol Lett* 8:336–352.
- Schluter D. 2000. *The ecology of adaptive radiation*. Oxford: Oxford University Press.
- Schwartz AK, Hendry AP. 2006. Sexual selection and the detection of ecological speciation. *Evol Ecol Res* 8:399–413.
- Schwartz AK, Hendry AP. 2007. A test for the parallel co-evolution of male colour and female preference in Trinidadian guppies (*Poecilia reticulata*). *Evol Ecol Res*. 9:71–90.
- Schwartz AK, Weese DJ, Bentzen P, Kinnison MT, Hendry AP. 2010. Both geography and ecology contribute to mating isolation in guppies. *PLoS One*. 5:e15659.
- Servedio MR, Doorn GSV, Kopp M, Frame AM, Nosil P. 2011. Magic traits in speciation: ‘magic’ but not rare? *Trends Ecol Evol*. 26:389–397.
- Servedio MR, Noor MAF. 2003. The role of reinforcement in speciation: theory and data. *Annu Rev Ecol Evol Syst*. 34:339–364.
- Shohet AJ, Watt PJ. 2009. Female guppies *Poecilia reticulata* prefer males that can learn fast. *J Fish Biol*. 75:1323–1330.
- Skarstein F, Folstad I, Rønning HP. 2005. Spawning colouration, parasites and habitat selection in *Salvelinus alpinus*: initiating speciation by sexual selection? *J Fish Biol*. 67:969–980.
- Stoks R, Nystrom JL, May ML, McPeck MA. 2005. Parallel evolution in ecological and reproductive traits to produce cryptic damselfly species across the holarctic. *Evolution*. 59:1976–1988.
- Svensson EI. 2012. Non-ecological speciation, niche conservatism and thermal adaptation: how are they connected? *Organisms Div Evol*. 12:229–240.
- Svensson EI, Gosden TP. 2007. Contemporary evolution of secondary sexual traits in the wild. *Funct Ecol*. 21:422–433.
- Torres Dowdall J, Handelsman CA, Ruell EW, Auer SK, Reznick DN, Ghalambor CK. 2012. Fine-scale local adaptation in life histories along a continuous environmental gradient in Trinidadian guppies. *Funct Ecol*. 26:616–627.
- Travis J, Reznick D, Bassar RD, López-Sepulcre A, Ferriere R, Coulson T. 2014. Do eco-evo feedbacks help us understand nature? Answers from studies of the Trinidadian guppy. In: Jordi Moya-Laraño JR, Guy W, editors. *Adv Ecol Res*. Oxford: Academic Press. p. 1–40.
- Weese DJ, Gordon SP, Hendry AP, Kinnison MT. 2010. Spatiotemporal variation in linear natural selection on body color in wild guppies (*Poecilia reticulata*). *Evolution*. 64:1802–1815.
- Weese DJ, Schwartz AK, Bentzen P, Hendry AP, Kinnison MT. 2011. Eco-evolutionary effects on population recovery following catastrophic disturbance. *Evol Appl*. 4:354–366.
- Zajitschek SR, Brooks RC. 2008. Distinguishing the effects of familiarity, relatedness, and color pattern rarity on attractiveness and measuring their effects on sexual selection in guppies (*Poecilia reticulata*). *Am Nat*. 172:843–854.
- Zandonà E, Auer SK, Kilham SS, Howard JL, López-Sepulcre A, O’Connor MP, Bassar RD, Osorio A, Pringle CM, Reznick DN. 2011. Diet quality and prey selectivity correlate with life histories and predation regime in Trinidadian guppies. *Funct Ecol*. 25:964–973.