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Rapid evolution and behavioral plasticity following introduction to an environment with reduced predation risk

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Abstract

Adaptive behavioral plasticity can play a beneficial role when a population becomes established in a novel environment if environmental cues allow the expression of appropriate behavior. Further, plasticity itself can evolve over time in a new environment causing changes in the way or degree to which animals respond to environmental cues. Colonization events provide an opportunity to investigate such relationships between behavioral plasticity and adaptation to new environments. Here, we investigated the evolution of behavior and behavioral plasticity during colonization of a new environment, by testing if female mate-choice behavior diverged in Trinidadian guppies 2–3 years (~6–9 generations) after being introduced to four locations with reduced predation risk. We collected wild-caught fish from the source and introduced populations, and we reared out second-generation females in the laboratory with and without predator cues to examine their plastic responses to a bright and dull male. We found introduced females were less responsive to males when reared without predator cues, but both introduced and source females were similarly responsive when reared with predator cues. Thus, the parallel evolution of behavior across multiple populations in the low-predation environment was only observed in the treatment mimicking the introduction environment. Such results are consistent with theory predicting that the evolution of plasticity is a by-product of differential selection across environments.

KEYWORDS

behavioral plasticity, guppy, mate choice, *Poecilia reticulata*, predation risk, rapid evolution

1 | INTRODUCTION

Colonization of new environments provides an opportunity to investigate adaptation on contemporary timescales (Reznick & Ghalambor, 2001). Behavioral plasticity, or the capacity of a genotype to produce different behavioral phenotypes in response to environmental conditions, is thought to be important in the process of contemporary adaptation (Foster, 2013; Ghalambor, Angeloni, & Carroll, 2010; Wcislo, 1989; West-Eberhard, 1989). Ancestral plasticity in a trait is thought to initially create non-heritable variation in the population that can later become genetically assimilated or accommodated as a heritable trait in the derived populations (Badyaev, 2005; Levis & Pfennig, 2016; Schlichting, 2004; Waddington, 1942; West-Eberhard, 2003). Selection may also result in a derived population with a different degree of phenotypic plasticity than the ancestral state through a process known as genetic accommodation (West-Eberhard, 2003). In either case, the key assumption is that there is genetic variation for plasticity in the ancestral population, such that

selection has the opportunity to drive the evolution of plasticity (Scheiner, 1993). However, new environments represent conditions where selection has not previously had an opportunity to shape patterns of plasticity, making it complicated to predict the direction of evolution in plastic responses (Foster, 2013; Ghalambor, McKay, Carroll, & Reznick, 2007).

Plasticity in a source population may be adaptive when colonizing a new environment, if past selective pressures allow individuals to respond appropriately to environmental cues (Foster, 2013; Ghalambor et al., 2010; Levis & Pfennig, 2016; West-Eberhard, 1989). Such adaptive plasticity may facilitate local adaptation by allowing for population persistence and the opportunity for directional selection to act on genetic variation (Foster, 2013; Ghalambor et al., 2007; Levis & Pfennig, 2016). On the other hand, adaptive plasticity may constrain trait evolution by shielding heritable variation and/or reducing the strength of directional selection (Foster, 2013; Ghalambor et al., 2010, 2007; Huey, Hertz, & Sinervo, 2003; West-Eberhard, 1989). Plasticity itself can also evolve in response to new environments (Foster, 2013; Ghalambor et al., 2007; Levis & Pfennig, 2016; Shaw, Scotti, & Foster, 2007). For example, a population lacking adaptive plasticity may rapidly evolve increased plasticity in a new environment that may allow it to achieve the new optimal phenotype (Lande, 2009, 2015). Empirical tests of how behavioral plasticity influences adaptation in nature are limited because few studies capture the genetic and environmental basis of behavior during the early stages of population divergence. Here, we illustrate how colonization of an environment with reduced predation risk can influence evolution and plasticity in mating behavior.

Predation risk has been shown to alter mate choice across a diversity of taxa, causing females to be less responsive to males, invest less in mate choice, and relax or reverse their preferences (Bonachea & Ryan, 2011; Demary, Michaelidis, & Lewis, 2006; Forsgren, 1992; Hedrick & Dill, 1993; Jennions & Petrie, 1997; Lima & Dill, 1990). Trinidadian guppies, *Poecilia reticulata*, are known for exhibiting a suite of behaviors that vary with predation risk (Godin & Briggs, 1996; Gong & Gibson, 1996; Houde, 1997). For example, female guppies exhibit reduced preferences for colorful males in locations with greater predation risk and in the presence of predators (Endler & Houde, 1995; Godin & Briggs, 1996; Gong & Gibson, 1996; Houde, 1997; Pocklington & Dill, 1995; Stoner & Breden, 1988), perhaps to reduce predation costs when searching for mates or mating, or to produce inconspicuous sons (Gong & Gibson, 1996; Pocklington & Dill, 1995; Schwartz & Hendry, 2007; Stoner & Breden, 1988). Thus, if adaptive plasticity facilitates adaptive behavior, female guppies that colonize environments with reduced predation risk should be more responsive to males and exhibit greater discrimination among them, with stronger preferences for colorful males.

To investigate the role of behavioral plasticity in a colonization event, we studied guppies transplanted from a high-predation environment to four low-predation sites where they had been evolving for 2–3 years (~6–9 generations; Handelsman et al., 2013; Travis et al., 2014). From these populations, second-generation (G_2) laboratory females were generated and reared with or without chemical cues of predation. We assessed the effect of this rearing environment on behavior in mate-choice trials, as a measure of behavioral plasticity. We compared the source and introduced populations under these common garden conditions to determine whether female mating behavior or behavioral plasticity evolved under reduced predation risk. If adaptive plasticity in a source population allows the expression of behavior adaptive to a new environment and shields colonizing populations from selection in a new environment, then we expected similar behavioral plasticity in source and introduced populations, and a lack of evolutionary change in behavior or its plasticity (Ghalambor et al., 2007). If a lack of adaptive plasticity in a source population prevents it from behaving adaptively in a new environment, then we expected behavior to evolve in the introduced populations, potentially through an increase in plasticity (Lande, 2015).

2 | MATERIAL AND METHODS

2.1 | Study organisms and husbandry

Females were laboratory-reared guppies descending from five populations in the Northern Range Mountains in Trinidad, West Indies, that were part of an experiment where guppies were transplanted from a high-predation site (hereafter Source) to four low-predation tributaries within the drainage (Intros1–4; Handelsman et al., 2013; Travis et al., 2014). In March 2011, 3 years post-introduction for Intro1 and Intro2 and 2 years post-introduction for Intro3 and Intro4, 40–50 juveniles collected from the source, and each introduction site were used to generate unique family lines and reared under identical conditions for two generations in a common garden environment to minimize maternal and environmental effects (following Handelsman et al., 2013).

Guppies collected from the high-predation source population and the four introduced populations were maintained at Colorado State University in 1.5 L tanks connected to a custom-made recirculating system. They were kept on a 12-hr light cycle at 27 ± 1°C and were fed flake paste made from TetraMin© Tropical Flakes in the morning and brine shrimp nauplii (*Artemia* spp.) in the evening, with food quantities adjusted for age to approximate ad libitum levels (modified from Reznick, 1982). We separated wild-caught juveniles by sex once females could be distinguished by a triangular patch of melanophores on the ventral abdomen (~after 28 days of age; Reznick, 1982). Upon reaching maturity, each wild-caught female was randomly crossed with a single wild-caught male to produce a G_1 generation, which were then separated by sex, reared to maturity, and randomly crossed with unrelated G_1 individuals to produce a G_2 generation.

To establish a split-brood experimental design, G_2 full-sibling broods from the Source, Intro3, and Intro4 were evenly split into 1.5 L tanks (2–10 siblings per tank) and randomly assigned to either a control or chemical predator cue treatment within 24 hr of birth (see below). After moving fish into their assigned treatment groups, fish were never exposed to the opposite treatment. G_2 broods from

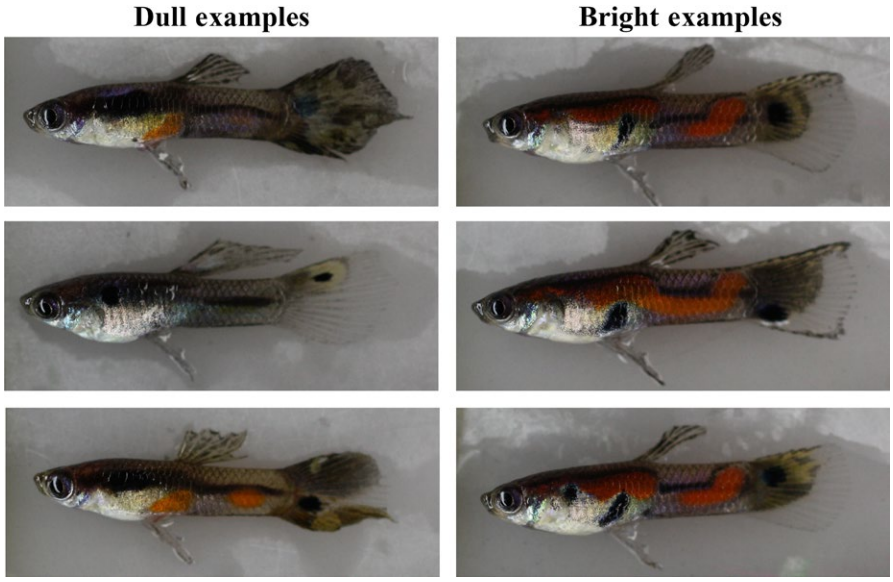

FIGURE 1 Example photographs of bright and dull males. Three "dull" males are shown on the left and three "bright" males are shown on the right; all were used in mate-choice trials [Colour figure can be viewed at wileyonlinelibrary.com]

Intro1 and Intro2 were reared only without predator cues due to space limitations. To maintain virginity, we identified females when broods reached 4 weeks old and reared females in 10 L group-tanks connected to the recirculating system separated by population and treatment. We had two group-tanks per population/treatment combination, each housing 18 females from nine families per population, with different families represented in each tank. Most families were represented by females from one brood; however, if the first brood did not produce two females, one female was used from the next brood.

Within 24 hr of birth, guppies reared with chemical predator cues were continuously maintained in recirculating systems that housed one common guppy predator, the pike cichlid (*Crenicichla frenata*), within each sump that supplied their tanks with water until the time of trial (Handelsman et al., 2013; Ruell et al., 2013). We fed pike cichlids two guppies per day so that water in the recirculating systems contained alarm pheromones released by guppy epidermal cells when consumed in addition to kairomones released by the predator. Guppies reared without predator cues were maintained in identical recirculating systems without a predator in the sump.

Male guppies used in mate-choice trials were descended from a sixth population in the Paria River in Trinidad that had been reared in the laboratory over many generations (>5 years). Using males from a single distinct population provided greater control over the range of color variation, which differs across populations, and ensured that all females were presented with males from a different population than their own. Ten mature, non-virgin males were held in individual 1.5 L tanks for 2 weeks prior to the start of experiments and through 12 weeks of behavioral experiments. The relatedness of these males was unknown. Males were categorized as "dull" (n = 6) or "bright" (n = 4) by visual assessment of the amount of orange on the body (Figure 1). The human eye is often used to assess relative coloration of male guppies (Houde, 1997; Ruell et al., 2013), and there is evidence for similarities in perception of orange coloration between humans and female guppies (Long & Houde, 1989). Differences in orange coloration between dull and bright males were confirmed in photographic images taken of the left side of each male with a Panasonic DMC FZ8 digital camera and Opteka high definition 10× macro lens under the same lighting conditions while males were anaesthetized in MS-222 (ethyl 3-aminobenzoate methane sulfonic acid salt, Sigma-Aldrich). We used the software ImageJ v1.45s to outline the body and orange elements and to calculate the proportion of the body covered by orange coloration. This analysis verified that bright males had a greater proportion of orange coloration (t_8 = 4.98, p = 0.005).

2.2 | Mate-choice trials

We conducted 148 mate-choice trials (96 reared without predator cues: 24 Source, 18 Intro1, 18 Intro2, 18 Intro3, 18 Intro4; 52 reared with predator cues: 18 Source, 18 Intro3, 16 Intro4). An arbitrarily selected dull and bright male were placed in each side of an aquarium (varying sides across trials) that contained water lacking predator cues and that was divided by an opaque barrier to prevent male–male interaction (Figure 2). A female was placed in an adjacent aquarium that contained water matching her rearing treatment (with or without predator cues) and that was blocked by an opaque barrier from the male tank. After a 10-min acclimation period, we removed the barrier between tanks to present the female with both males without contact. Behavioral observations were made through a viewing blind equipped with a full-spectrum light mounted above the aquaria. We recorded the time spent within 5 cm of each male's partition (Figure 2) for 5 min using JWatcher™. Additionally, we recorded the number of sigmoid displays performed by each male. A sigmoid display is a stereotyped courtship behavior in guppies. Males orient themselves perpendicular to a female, assume the characteristic S-shape that gives the display its name, and quiver their bodies.

We excluded trials if the female did not sample both males (i.e., swim within 5 cm of both partitions; n = 4 Intro3 females and n = 3 Intro4 females, all reared with predator cues). We quantified multiple aspects of female behavior (Archard, Partridge, & Cuthill, 2006): (a) *responsiveness*, or amount of time spent within 5 cm of either male;

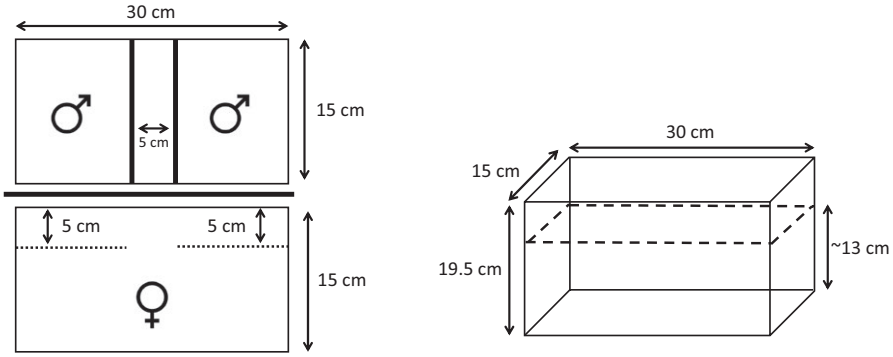

FIGURE 2 Schematic diagram of the experimental aquaria (similar to Smith et al., 2002). Bold lines indicate opaque barriers. The opaque barrier between the female and male aquaria was removed after the acclimation period. The time spent within 5 cm of each male's partition (within the dashed lines) was recorded as a measure of her interest in each male

(b) *discrimination*, or absolute difference in time spent within 5 cm of each male divided by total time spent with either male (Magurran & Seghers, 1991); and (c) *preference* for the colorful male by comparing amount of time spent with the bright versus dull male.

2.3 | Statistical analyses

To test for plasticity and the evolution of plasticity in response to predator cues in either female (1) responsiveness or (2) discrimination, we created linear mixed models that included the treatment (reared with or without predator cues), the population type (source or intro), and the interaction between treatment and population type as fixed effects (Figure 3). These models only included three populations that were reared in both conditions (Source, Intro3, and Intro4). The specific combination of males used in the trial and population ID was included as random effects. Because the number of male courtship displays may affect female behavior (Godin & Briggs, 1996), we included the difference in the number of sigmoids performed during each trial as a random effect in our models for discrimination and preference, and the total number of sigmoids performed by both males as a random effect in our models for responsiveness.

To address whether females preferred bright males, we used a linear mixed model to compare the time spent with bright and dull males. This model included color of male (bright or dull), treatment (reared with or without predator cues), population type (source or intro), and all combinations of interactions between these predictors. Again, this model only included three populations that were reared under both treatment conditions (Source, Intro3, and Intro4). Random effects included the combination of males, the trial ID, population ID, and the difference in the number of sigmoids males performed during the trial.

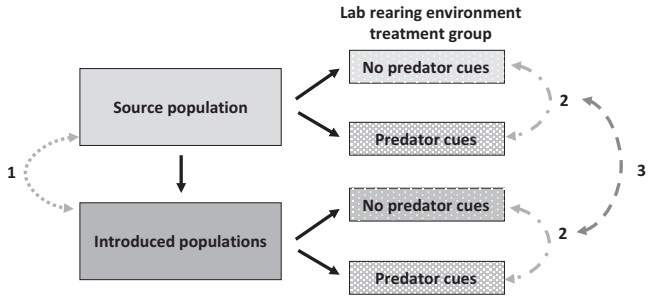

FIGURE 3 Schematic of analyses for evolution of behavior, plasticity of behavior, and the evolution of plasticity. An effect of the population type (1) would indicate evidence of evolution in the introduced populations. An effect across treatment groups (2) would indicate evidence of plasticity in the behavioral trait of interest. Evidence for the evolution of plasticity would be found by comparing the plasticity between the source population and introduced populations using an interaction term (3)

To test for additional evidence of evolution between the source and introduced populations, we repeated all three models (responsiveness, discrimination, and preference for the bright male) using all of the females reared without predator cues, removing the treatment effect (Figure 3). These models included all five populations reared without predator cues (Source, Intro1, Intro2, Intro3, and Intro4). Each of the three linear mixed models included a factor to represent the population ID as a fixed effect. Using a fixed effect for population ID allowed us to detect whether any specific introduced population differed in their evolutionary change from the source population. The combination of males used in the trial and the difference in the number of sigmoids performed were included as a random effect. In the model assessing preference for the bright male, we also included color of male (bright or dull) and the interaction between color of male and population. All proportional data were logit transformed prior to analyses because distributions were often right skewed. All analyses were conducted in R version 3.4.3 (R Core Team, 2017). Linear mixed models were run using R package "lme4" (Bates, Mächler, Bolker, & Walker, 2015).

3 | RESULTS

We tested differences between source and introduced populations in mate-choice behavior (evolution), the effects of the predator cue treatment on mate-choice behavior (plasticity), and differences in these treatment effects between the source and introduced populations (evolution of plasticity; Figure 3). For populations reared with and without predator cues (Source, Intro3, Intro4), we tested whether rearing treatment, population type (source or intro), and their interaction influenced behavior. This generated a model predicting responsiveness with a significant difference between the source and introduced populations ($t = -2.89$, $p = 0.0047$) and a marginal trend for the treatment × population type interaction ($t = 1.67$,

TABLE 1 Full results of models of the evolution of plasticity

Response variable	Variable	Estimate	*SE*	*t*-Value	*p*-Value
Responsiveness: *time spent with both males* (Figure 4)	**Intercept**	**610.17**	**58.64**	**10.41**	**<0.01**
	Treatment	27.10	89.58	0.30	0.76
	Population type	**−221.70**	**76.60**	**−2.89**	**0.0047**
	Treatment × population type	193.07	115.32	1.67	0.097
Discrimination: *absolute value of the difference in time spent with each male divided by the total time spent with either male*	**Intercept**	**−0.76**	**0.34**	**−2.23**	**0.034**
	Treatment	−0.25	0.50	−0.49	0.62
	Population type	0.014	0.44	0.032	0.98
	Treatment × population type	0.20	0.65	0.31	0.76
Preference: *time spent with each male*	Intercept	−163.81	87.46	−1.87	0.063
	Color of male	70.58	123.68	0.57	0.57
	Population type	−117.64	114.22	−1.03	0.30
	Treatment	6.81	133.59	0.051	0.96
	Color of male × treatment	−48.47	188.92	−0.26	0.80
	Color of male × population type	97.18	161.54	0.60	0.55
	Treatment × population type	116.61	171.97	0.68	0.50
	Color of male × population Type × treatment	−96.79	243.21	−0.40	0.69

Note. These linear mixed-effects models include trials from the source population, Intro3, and Intro4, across both treatment groups reared with and without predator cues. All models include random intercept terms of the male combination used in the trial, population ID, and either the difference between the number of sigmoids of each male (discrimination and preference) or the total number of sigmoids (responsiveness model). The model for preference also includes a random intercept term of trial ID. All response variables were logit transformed. The reference group for population ID was the source population, and the color of male was dull compared against bright males. For the treatment variable, females reared without predation cues were the reference group. Statistically significant ($p < 0.05$) variables are listed in bold.

$p = 0.097$; see Table 1 for full results). As shown in Figure 4, females from the introduced populations were plastic, exhibiting less responsiveness to males when reared without predator cues than with predator cues, while female responsiveness in the source population was not affected by predator cues. In other words, compared to the source population they were derived from the introduced females were less responsive to males when reared without predator cues, but did not differ when reared with predator cues. We did not detect effects of the population type, treatment, or their interaction on female discrimination of males (treatment × population type interaction: $t = 0.31$, $p = 0.76$; see Table 1 for full results). Unexpectedly, females did not spend more time with the bright male compared to the dull male ($t = 0.94$, $p = 0.35$) in an analysis pooling data across all trials. In a model comparing time spent with the bright and dull male across these populations and treatments, we did not detect population type, treatment, or interaction effects, indicating preferences did not evolve and did not exhibit plasticity with the treatment (color of male × treatment × population type interaction: $t = -0.40$, $p = 0.69$; see Table 1 for full results).

To further examine the evolution of female responsiveness to males, we compared females reared without predator cues from the source and all four introduced populations. We found a reduction in responsiveness in three of the four introduced populations compared to the source population (Intro1: $t = 0.25$, $p = 0.81$; Intro2: $t = -2.10$, $p = 0.039$; Intro3: $t = -2.01$, $p = 0.048$; Intro4: $t = -2.17$, $p = 0.032$; see Table 2 for full model; Figure 5), indicating parallel evolution in this behavior across three of the four introduced populations. Female discrimination of males did not differ among populations (Intro1: $t = -0.29$, $p = 0.77$; Intro2: $t = -1.24$, $p = 0.22$; Intro3:

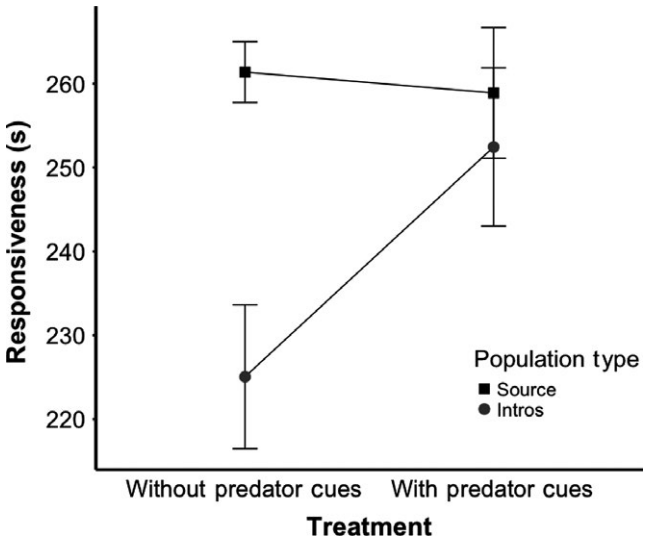

FIGURE 4 Responsiveness, or time spent with both males, of source and introduced females reared with and without predator cues. Displayed data are untransformed means ± *SE*, but data were transformed for analyses

$t = 0.046$, $p = 0.96$; Intro4: $t = 0.37$, $p = 0.71$; see Table 2 for full model), females did not prefer bright or dull males in any population (color of male: $t = 0.65$, $p = 0.52$; see Table 2 for full model), and we found no evidence that preferences evolved in the introduced populations, although there was a trend indicating Intro2 females spent relatively more time with dull males than the Source population (effects of interaction between color of male and population: Intro1: $t = 0.23$, $p = 0.82$; Intro2: $t = -1.73$, $p = 0.086$; Intro3: $t = -0.018$, $p = 0.99$; Intro4: $t = 1.22$, $p = 0.22$; see Table 2 for full model).

4 | DISCUSSION

Behavior and its plasticity are frequently cited as playing an important role in facilitating population establishment in new environments and subsequent evolutionary change (Foster, 2013; Ghalambor et al., 2010, 2007; Huey et al., 2003; Lande, 2015; Levis & Pfennig, 2016; Wcislo, 1989; West-Eberhard, 1989). However, most studies inferring the role of plasticity in adaptive evolution occur long after populations have diverged (e.g., Chapman, Galis, & Shinn, 2000; Losos et al., 2000; Magurran & Seghers, 1994), and few empirical examples exist where the ancestral and derived patterns of plasticity have been quantified during the early stages of population divergence (e.g., Handelsman et al., 2013). Here, we find that female responsiveness to males evolved after only 2–3 years (~8–12 generations) in a low-predation environment, with evidence of an increase in plasticity compared to the source population. Despite relatively small sample sizes, we found female guppies from 3 of 4 introduced populations were less responsive to males than females from the source population when reared without predator cues, but reverted back to their high-predation phenotype when reared with predator cues (Figure 3). Such parallel changes across all four introduction populations are unlikely to have arisen because of drift or founder effects. However, since females from the high-predation source population did not exhibit plasticity dependent on predator cues in their environment, the evolved changes in the introduced

TABLE 2 Full results of models of evolution across all populations

Response variable	Variable	Estimate	*SE*	*t*-Value	*p*-Value
Responsiveness: *time spent with both males* (Figure 5)	**Intercept**	**610.17**	**59.07**	**10.33**	**<0.01**
	Population ID				
	Intro1	22.07	90.12	0.25	0.81
	Intro2	**−197.85**	**94.29**	**−2.10**	**0.039**
	Intro3	**−178.97**	**89.09**	**−2.01**	**0.048**
	Intro4	**−204.92**	**94.29**	**−2.17**	**0.032**
Discrimination: *absolute value of the difference in time spent with each male divided by the total time spent with either male*	Intercept	−0.79	0.41	−1.95	0.097
	Population ID				
	Intro1	−0.14	0.48	−0.29	0.77
	Intro2	−0.62	0.50	−1.24	0.22
	Intro3	0.022	0.48	0.046	0.96
	Intro4	0.19	0.50	0.37	0.71
Preference: *time spent with each male*	**Intercept**	**−163.81**	**76.93**	**−2.13**	**0.035**
	Color of male	70.58	109.46	0.65	0.52
	Population ID				
	Intro1	−39.83	117.52	−0.34	0.74
	Intro2	72.53	117.52	0.62	0.54
	Intro3	−59.34	117.52	−0.51	0.61
	Intro4	−183.27	121.64	−1.51	0.13
	Color of male × population ID				
	Color of male × Intro1	38.81	166.20	0.23	0.82
	Color of male × Intro2	−287.05	166.20	−1.73	0.086
	Color of male × Intro3	−2.96	166.20	−0.018	0.99
	Color of male × Intro4	209.83	172.03	1.22	0.22

Note. These linear mixed-effects models include trials from the source population, Intro1, Intro2, Intro3, and Intro4, all reared without predator cues. All models include a random intercept term for the male combination used in the trial and either the difference between the number of sigmoids of each male (discrimination and preference) or the total number of sigmoids (responsiveness). The model for preference includes another random intercept term for trial ID. All response variables were logit transformed. The reference group for population ID was the source population, and the color of male was dull compared against bright males. Statistically significant ($p < 0.05$) variables are listed in bold.

populations were also unlikely to have been facilitated by adaptive plasticity in the source. Instead, the evolution of increased plasticity in the introduced populations appears to be a by-product of evolution for reduced responsiveness in the environment with reduced predation risk (Figure 3). These results mimic patterns observed in other morphological, life history, and physiological traits of guppies, where plasticity increases the expression of a new phenotype only in the derived low-predation environment (Fischer, Soares, Archer, Ghalambor, & Hoke, 2013; Ghalambor et al., 2015; Handelsman et al., 2013; Torres-Dowdall, Handelsman, Reznick, & Ghalambor, 2012). More broadly, these results support theories predicting that local adaptation to different environments can result in the evolution of plasticity (e.g., Czesak, Fox, & Wolf, 2006; Lande, 2015; Via & Lande, 1985).

Opposite to our prediction, we found that female responsiveness to males decreased when predation risk decreased (Gong & Gibson, 1996; Houde, 1997; Lima & Dill, 1990). One possible explanation is that a release from predation reduces investment in reproduction and the incentive to mate because of increasing residual reproductive value with greater survival (Jennions & Petrie, 1997; Real, 1990). Alternatively, females could be choosier and less likely to respond to males under reduced predation risk because of a decrease in the costs of mate choice, and therefore less likely to find either test male attractive (Jennions & Petrie, 1997; Real, 1990). However, we did not detect an increase in choosiness with our discrimination metric. A third possibility is that our measure of responsiveness actually reflects an interest in associating with conspecifics for predator defense (i.e., shoaling, Candolin & Heuschele, 2008). Guppies exhibit greater defensive shoaling in high-predation environments (Magurran & Seghers, 1991). Thus, the rapid evolution of reduced responsiveness we observed in the introduced populations may represent a decrease in antipredator behavior rather than a decrease in mating behavior with lower predation risk. It is important to note that we assessed each female's mating behavior under the same conditions as her rearing treatment (with or without predator cues), which mimics the continuity of predation risk experienced by guppies in nature and allows for effects of predator cues over both the long and the short term. Future studies could explore whether the behavioral plasticity we observed is dependent on long-term predation risk in the rearing environment, immediate risk during a mating event, or a combination of both.

We did not detect a difference in female discrimination of males between populations or the evolution of preferences for bright males in the introduced populations, supporting findings that preferences evolve slowly (Easty, Schwartz, Gordon, & Hendry, 2011). Unexpectedly, females did not prefer bright males in this study, perhaps because they found all males attractive. Though our bright males had more orange coloration than our dull males, they could have been similarly attractive to female guppies if females choose males surpassing an absolute threshold of coloration rather than evaluating relative differences in coloration (similar to Houde, 1987; Schwartz & Hendry, 2007). Further, because our females were virgins, they may have been particularly interested in mating, diminishing their preferences for one male over the other. Thus, we may have detected a preference for orange coloration and greater discrimination if we had used males that varied more dramatically in color, or if we had used non-virgin females. On the other hand, our findings may correctly reflect a lack of female preference for orange coloration in these populations, as other studies have found female preferences for orange coloration are not universal across guppy populations (e.g., Easty et al., 2011; Endler & Houde, 1995; Houde & Endler, 1990; Hughes, Houde, Price, & Rodd, 2013; Schwartz & Hendry, 2007).

Adaptive behavioral plasticity is thought to play a beneficial role in new environments, but as demonstrated here, not all behavioral traits are plastic. Indeed, colonization and adaptation to novel environments can cause a rapid initial increase in plasticity as a consequence of selection favoring a new optimal phenotype, which may be followed by a gradual reduction in plasticity of the new phenotype (Lande, 2015). Establishing general patterns for the long-term fate of behavioral plasticity in new environments will require studies comparing ancestral and derived populations over time post-colonization.

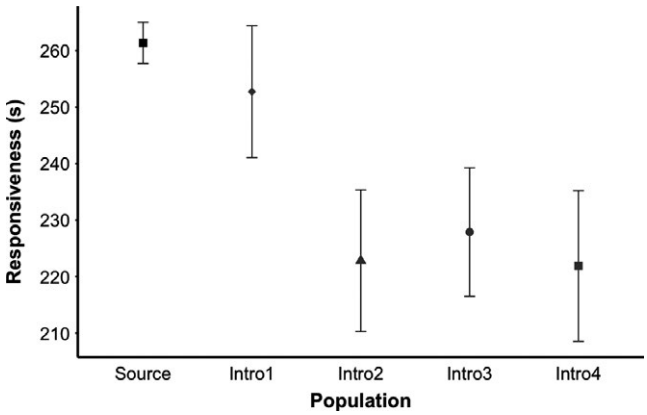

FIGURE 5 Responsiveness of females from each population reared without predator cues. Displayed data are untransformed means ± *SE*, but data were transformed for analyses

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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