

## Original Article

## Does male behavioral type correlate with species recognition and stress?

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The persistence of gynogenetic organisms is an evolutionary paradox. An ideal system for examining the persistence of gynogens is the unisexual–bisexual mating complex of the unisexual Amazon molly (*Poecilia formosa*), and the bisexual, parent species, the sailfin molly (*Poecilia latipinna*) and the Atlantic molly (*Poecilia mexicana*). Insight into the maintenance of this mating complex might be enhanced by taking a more holistic view of male and female behavior through a behavioral syndrome framework. In this study, we examined whether male mate choice is part of a behavioral syndrome. We quantified behaviors related to activity, boldness, exploration, and sociability in male sailfin mollies, as well as their mate preference for conspecific females or the all-female species of Amazon mollies. In addition, we explored the relationship between behavioral type and cortisol (a fish stress hormone) production in male sailfin mollies. We found evidence for behavioral correlations in male sailfin mollies, but individual behavioral type was not correlated with their mate preference or stress hormone production. However, we did find differences in preexperience cortisol production related to male boldness behaviors. The lack of correlation between behavioral types, mate preference, and stress hormone production emphasizes that the nature of behavioral–hormonal interactions is complex. In summary, neither individual traits nor the behavioral types found here are adequate to explain the maintenance of this unisexual–bisexual mating system.

**Key words:** Amazon molly, behavioral syndrome, cortisol, gynogenesis.

## INTRODUCTION

A holistic view of male and female behavior, incorporating a behavioral syndrome framework, provides a model to explain observations of limited plasticity in behavioral traits, termed a “behavioral carryover,” which may result in the expression and maintenance of seemingly “suboptimal” and/or maladaptive behaviors (Sih, Bell, and Johnson 2004; Sih, Bell, Johnson, and Ziemba 2004; Bell 2007). Individuals may be constrained in their ability to tailor behavior to their current environment (Sih, Bell, and Johnson 2004; Sih, Bell, Johnson, and Ziemba 2004; Dingemanse and Réale 2005). These constraints can be attributed to factors such as correlational selection on multiple behaviors, which could result in indirect selection on nontarget behavioral traits (Lande and Arnold 1983; Bell 2007; Bell and Sih 2007). For example, female fishing spiders (*Dolomedes triton*) are more likely to engage in precopulatory sexual cannibalism if they foraged aggressively as juveniles (Johnson and Sih 2005). The behavioral carryover in this case has resulted in the maintenance of a seemingly maladaptive trait, precopulatory sexual cannibalism, via correlational selection for juvenile aggressiveness. Limited behavioral plasticity may also arise due

to physiological constraints on organisms (Bell 2007), such as pleiotropy and/or hormonal regulation of behaviors (Ketterson and Nolan 1999; Bell 2007).

Correlation between behaviors may be a result of hormones mediating the expression of suites of correlated traits (McGlothlin and Ketterson 2008). Several studies demonstrate a relationship between correlated traits and glucocorticoid (stress hormones: corticosteroid in birds, reptiles, and amphibians and cortisol in fish) levels. Higher levels of circulating corticosterone in zebra finches (*Taeniopygia guttata*) following a mild stressor result in greater exploratory behavior (Martins et al. 2007). Similarly, there is a positive relationship between cortisol production and measures of exploratory tendencies and activity, in the livebearing fish *Brachyrhaphis episcopi* (Archard et al. 2012). Likewise, there is a positive correlation between both aggressiveness and boldness and preexperience cortisol levels in mangrove rivulus, *Kryptolebias marmoratus* (Chang et al. 2012). In addition, selection experiments in rainbow trout (*Oncorhynchus mykiss*) have led to 2 lines that vary in their behaviors and responses to stress. The HR (high responsiveness) lines show high cortisol responsiveness that is associated with increased swimming activity and exhibited more “anxiety-like erratic behavior,” whereas the LR (low responsiveness) lines show a behavioral strategy of remaining passive, and ignoring intruders (in low-quality territories) (Overli et al. 2005). However, Thomson et al. (2011)

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found that there is no relationship between boldness (a behavioral type) and stress hormones in rainbow trout. The complex nature of hormonal-behavioral interactions and behavioral syndromes are highlighted in Conrad et al. (2011) and suggest that selection may have different effects on how proximate mechanisms mediate behaviors of interest.

Individual behavioral types also can influence mate choice. For example, highly exploratory female zebra finches show lower selectivity in mate choice (David and Cezilly 2011), and highly exploratory female zebra finches prefer highly exploratory males, suggesting that females may select mates on either (or both) genetic and behavioral compatibility (Schuett et al. 2011). Mate choice consists not only of selecting high-quality mates (which may be a relative trait) but also of selecting a mate of the same species (species recognition). Mate-quality recognition and species recognition are overlapping processes that share similar neuroendocrine bases (Adkins-Regan 2011).

One system in which species recognition and mate choice has been extensively studied from multiple behavioral and physiological perspectives is the sailfin molly (*Poecilia latipinna*) and Amazon molly (*Poecilia formosa*) mating system. Amazon mollies are a gynogenetic, all female lineage of hybrid origin (Hubbs and Hubbs 1932; Dawley 1989). Sperm from one of the parent species (*P. latipinna* or *Poecilia mexicana*) is required by the Amazon molly to initiate embryogenesis (Hubbs and Hubbs 1932), but the male genome is not incorporated into the diploid gamete of the Amazon molly. Males are therefore considered sexually parasitized by the Amazon molly and gain no direct fitness benefits (but see Schlupp et al. (1994) and Heubel et al. (2008) for indirect benefits accruing from mate choice copying). Although there is geographic variation in male mate choice, as well as population polymorphisms in male mate choice (Gabor and Aspbury 2008), on average, males prefer to mate with conspecifics over Amazon mollies (Ryan et al. 1996; Gabor and Ryan 2001; Gabor and Aspbury 2008; Robinson et al. 2008; Aspbury et al. 2010; Gabor and Grober 2010), and male sailfin mollies prime more sperm for conspecifics over Amazon mollies (Aspbury and Gabor 2004). The mechanisms underlying the variation in male mate preferences for conspecifics versus Amazon mollies are not fully understood.

Behavioral carryovers from different behavioral types could help explain the maintenance of polymorphic male mate choice by male sailfin molly (i.e., the persistence of some males that are more likely to fail in species recognition). Here, we test the hypothesis that male mate choice for conspecifics over heterospecifics is part of a behavioral type in male sailfin mollies. In addition, we predicted that a stress hormone (cortisol) is correlated with any behavioral types observed in male sailfin mollies.

## MATERIALS AND METHODS

### Fish maintenance

Male and female sailfin mollies, as well as Amazon mollies, were collected from the headwaters of San Marcos River, Spring Lake, San Marcos, Hays County, TX (29°89'N, 97°82'W) from April to September 2011. Fishes were maintained in the laboratory for at least 2 weeks prior to experimentation. Males were maintained in mixed-sex aquaria (38L). All fish were on a 14:10h light:dark cycle and were fed a combination of brine shrimp (*Artemia* spp.; Bio-Marine; [www.aquafauna.com](http://www.aquafauna.com)) and Spirulina Flakes mixed with Aquamax® Fry Starter 200 (PMI Nutrition International) once daily between 1630 and 1730 hours to control for any hormonal changes due to feeding.

### Identification of behavioral types

Individual males ( $N = 50$ ) were exposed to each of 4 assays to examine behavioral types: 1) activity/boldness/exploration, 2) sociability, 3) male mate preference, and 4) repeatability of activity/boldness/exploration. All tests occurred between 0800 and 1700 hours, and assays 3 and 4 occurred on the days following assays 1 and 2.

We obtained preexperience cortisol release rates (via water-borne hormone samples) 24h before assay 1. Hormone samples were taken between 0800 and 1300 hours to control for circadian variation in hormone levels (Lorenzi et al. 2008). Water-borne hormone collection methods followed Gabor and Grober (2010). Briefly, individual males were placed into a 250-mL beaker filled with 100 mL of conditioned water and then removed after 1 h. Although placing a fish in a beaker could cause stress, this procedure is less invasive than bleeding a fish to quantify blood plasma levels of circulating stress hormones. Moreover, in sailfin mollies, we found no change in cortisol across 4 days of sampling, indicating minimal stress across repeated sampling events (Gabor and Contreras 2012). Water samples were stored at  $-20^{\circ}\text{C}$  until hormone assays were completed at a later date (Ellis et al. 2004). All subsequent hormone assay procedures followed the methods of Gabor and Grober (2010). To assay cortisol levels, we used commercially available enzyme immunoassay (EIA) kits (Cayman Chemical). Gabor and Contreras (2012) validated the use of water-borne hormones to measure cortisol on EIA plates, as well as verified the correlation between water-borne release rates and plasma cortisol production for sailfin mollies. All samples were run in duplicate on 96-well plates and read by a fluorescent plate reader (BioTek Powerwave XS).

### Assay 1: Activity/boldness/exploration and cortisol release rates

Assay 1 quantified activity, boldness, and exploration in a novel environment using methods adapted from Cote et al. (2010) and Smith and Blumstein (2010). First, we placed individual males into an opaque, acclimation chamber (15 cm in diameter) for 5 min within an experimental arena ( $70 \times 40 \times 10$  cm), which was marked with a grid of 36 total squares (each  $7.5 \times 8.5$  cm). After acclimation, we remotely opened a door located on the exterior of the chamber and then recorded the time to emerge for up to 10 min. We quantified *boldness* as (maximum time to exit) – (individual time to exit), such that individuals that exited more quickly had higher scores than those that exited more slowly. We also measured time spent swimming (s) (*activity 1a*) in the experimental arena and the number of grids entered (*exploration*) for 5 min after emergence using video following the trial.

### Assay 2: Sociability

This assay examined individual sociability using methods adapted from Cote et al. (2010) and Smith and Blumstein (2010) and took place in a 75-L dichotomous-choice tank, which was divided into a large, center compartment (60 cm; *no-choice zone*) and 2 smaller compartments (each 10 cm) with sealed, plexiglass dividers. The *preference zone* was defined as the 10 cm in front of each small side compartment. One side compartment housed a mixed-species shoal during testing, whereas the other (control) contained only water. Shoals contained 2 conspecific and 2 heterospecific females, and we randomized shoal placement (i.e., right vs. left) for each focal individual. We established 2 stimulus shoals, which were housed together in 38-L aquaria prior to testing. Finally, we randomized which one of the 2 shoals was presented to the males. First, we acclimated the focal male for 5 min under an opaque, acclimation

chamber (16 cm in diameter) in the center of the no-choice zone. After acclimation, we removed the chamber and recorded the time spent in the preference zone (*sociability*) and total time swimming (*activity 2a*) for 10 min.

### Assay 3: Male mate preference

This assay examined the mate preference of male sailfin mollies. Because males show preferences for female body size (Gabor 1999; Gabor and Aspbury 2008), and prefer larger females, regardless of species (Gumm and Gabor 2005), we used size-matched females to isolate the preference for species identity. One male sailfin molly was housed with a size-matched female pair, consisting of 1 female sailfin molly and 1 Amazon molly (female standard length [SL] within  $\pm 2$  mm of each other and within  $\pm 9$  mm male SL). Groups of fish were housed for 18–24 h in individual aquaria (19 L), with a transparent divider separating the sexes. Females were isolated from males either for 30 days or, if housed individually, were used approximately 1 week after parturition of fry.

On the day following assays 1 and 2, males were given access to the female pair. The central divider was removed, and the time until the first gonopodial thrust (i.e., mating attempts) toward a female was recorded. Following the first thrust, the total number of thrusts by the male at each female was recorded for 10 min. At the end of the trial, females were removed from the aquaria. If no mating behavior was observed, the trial ended after 30 min. We measured strength of preference (SOP) as the number of thrusts toward conspecifics over the total number of thrusts.

### Assay 4: Repeatability of behavior

This assay examined the repeatability of behaviors measured in assay 1. Repeatability is the intraclass correlation coefficient based on variance components obtained from a one-way analysis of variance and can represent the upper limits on the heritability of a trait (Lessels and Boag 1987). Measures of repeatability are necessary in studies of behavioral syndromes to examine individual changes in trait expression across time relative to changes at the population level (Lessels and Boag 1987; Bell et al. 2009; Cote et al. 2010). The behavioral syndrome framework predicts that individuals should maintain a consistent pattern of behavior over time; however, most studies find relatively low levels of repeatability (Bell et al. 2009; Cote et al. 2010). Additionally, we examined the correlations among behaviors by conducting a Spearman's rank correlation to compare the change in individual ranks for each behavioral metric over a 2-day time span.

## Statistical analyses

To identify behavioral syndromes, we first examined whether there were significant, pairwise correlations among the behavioral metrics with Spearman's rank correlation (Table 1). Because the behavioral metrics were all significantly correlated (except for sociability–exploration; Table 1), we used a principal component (PC) analysis to identify multiple correlations among behaviors (i.e., personality trait dimensions; Cote et al. 2010). We identified important PCs as those with eigenvalues  $>1.0$  (Kaiser's rule; Kaiser 1960). This allowed us to extract individual component scores from the first 2 PCs (Table 2). We also assessed repeatability ( $r$ ) at the population level following the method outlined in Lessels and Boag (1987), which is commonly used in similar studies (reviewed by Bell et al. 2009).

We used Spearman's rank correlation to examine relationships between the individual behavioral metrics and SOP, as well as to examine the relationship between preexperience cortisol levels and the individual behavioral metrics. We used a Wilcoxon signed-ranks test to compare differences in number of gonopodial thrusts and latency to thrust at conspecific versus heterospecific females. Additionally, we used a Mann–Whitney  $U$  test to examine differences in preexperience cortisol levels between males that exited the acclimation chamber in assay 1 and those that did not.

## RESULTS

### Behavioral correlations, repeatability, and mate preferences

There were significant, pairwise correlations among all behavioral metrics (except for sociability–exploration; Table 1). Activity (1a; from assay 1), boldness, and exploration loaded positively onto PC1, which explained 59% of the variance, whereas activity (2a; from assay 2) and sociability loaded positively onto PC2, which explained 23.6% of the variance (Table 2). Individual males were highly consistent (Spearman rank correlations [ $\rho$ ] range = 0.49–0.59) and repeatable (range in  $r = 0.4$ –0.5) in their boldness, exploratory, and activity behavior (Table 3). Males preferred to mate with conspecific versus heterospecific females (Wilcoxon signed-ranks test:  $z = 3.95$ ,  $P < 0.0001$ ; Figure 1). Male SOP for conspecifics was not significantly correlated with any of the individual, behavioral metrics (Table 1).

**Table 1**

**Spearman's rank correlations among the 5 behavioral categories that were measured, also including SL and male SOP for conspecifics**

	Activity 1a	Activity 2a	Boldness	Exploration	SL	Sociability
Activity 2a	0.434**	—	—	—	—	—
Boldness	0.690***	0.385**	—	—	—	—
Exploration	0.932***	0.407**	0.653***	—	—	—
SL	−0.052	−0.099	0.0002	−0.030	—	—
Sociability	0.294*	0.486**	0.362**	0.250 <sup>a</sup>	−0.031	—
SOP	−0.078	−0.070	0.090	−0.069	−0.012	−0.012
Preexperience cortisol (pg SL <sup>−1</sup> h <sup>−1</sup> )	0.215		0.3246 <sup>b</sup>	0.2706		

1a indicates data from assay 1 and 2a indicates data from assay 2.

<sup>a</sup> $P = 0.08$ .

<sup>b</sup> $P = 0.06$ .

\* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.0001$ .

**Table 2**  
Component loadings of behaviors onto 2 orthogonal PCs and the variance explained by each

Behavior	PC1	PC2
Activity 1a	<b>0.492</b>	−0.367
Activity 2a	0.413	<b>0.561</b>
Boldness	<b>0.416</b>	−0.268
Exploration	<b>0.508</b>	−0.348
Sociability	0.395	<b>0.599</b>
Variance explained (%)	59.001	23.585
Cumulative variance explained (%)	59.001	82.585

Values in bold represent the behaviors with the strongest impacts on that component.

**Table 3**  
Individual consistency (Spearman's rank correlation;  $\rho$ ) and repeatability (intraclass correlation coefficient;  $r$ ) over 2 days of the 3 behaviors measured in assay 1

Behavioral context	Rank correlation	Repeatability
Activity	$\rho = 0.559$ , $P < 0.0001$	$r = 0.470 \pm 0.157$ , $F_{49,99} = 2.77$ , $P < 0.0001$
Boldness	$\rho = 0.491$ , $P = 0.0003$	$r = 0.402 \pm 0.169$ , $F_{49,99} = 2.35$ , $P = 0.002$
Exploration	$\rho = 0.589$ , $P < 0.0001$	$r = 0.505 \pm 0.150$ , $F_{49,99} = 3.04$ , $P < 0.0001$

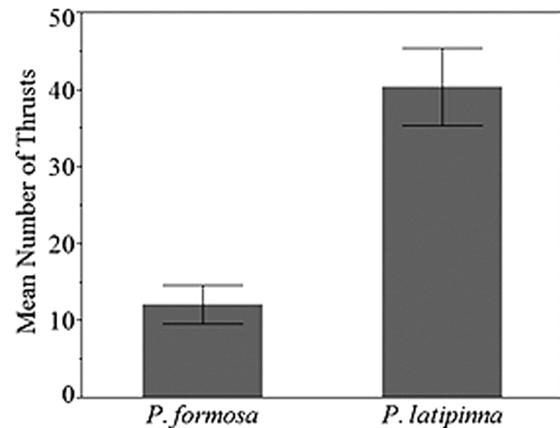
### Correlations between behaviors and stress hormones

There was no significant correlation between preexperience cortisol release rates and the behaviors examined in assay 1 (Table 1). In this experiment, 15 of the 50 males did not exit the acclimation chamber, and there was a significant difference in preexperience cortisol release rates between those individuals that did and those that did not exit the acclimation chamber (Mann–Whitney:  $U = -2.30$ ,  $df = 3$ ,  $P = 0.02$ ). Males that did exit the acclimation chamber had significantly higher preexperience release rates of cortisol than males that did not exit (Figure 2).

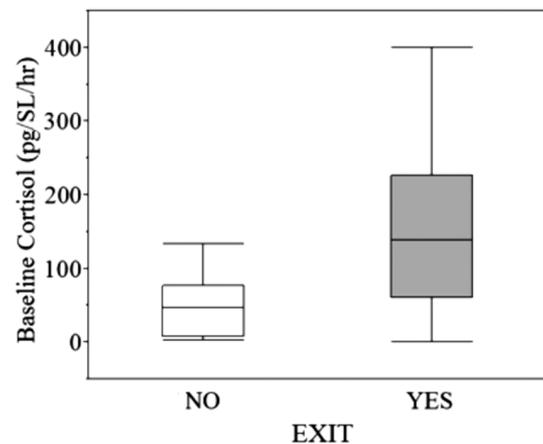
## DISCUSSION

Similar to prior work on behavioral syndromes (Cote et al. 2010; Smith and Blumstein 2010), we found that boldness, exploration, activity, and sociability are correlated behaviors in male sailfin mollies. We found significant, pairwise correlations among most of the behavioral metrics (Table 1). Additionally, individual males were highly consistent and repeatable in their behavior over 2 days (Table 3). We also confirmed prior conclusions regarding mate choice in this system (Ryan et al. 1996; Gabor and Ryan 2001; Gabor and Aspbury 2008; Robinson et al. 2008; Aspbury et al. 2010; Gabor and Grober 2010); male sailfin mollies from this population show, on average, a preference to mate with conspecific females, over Amazon mollies. However, we did not detect a relationship between individual behavioral types and male mate preference, indicating that behavioral type does not influence species discrimination abilities.

Contrary to our results, there is increasing evidence in other taxa that individual behavioral types can influence mate preferences. For example, Schuett et al. (2011) found that more exploratory female zebra finches prefer highly exploratory males. In addition,



**Figure 1**  
Number of gonopodial thrusts ( $\pm$ SE) by male sailfin mollies ( $N = 50$ ) to heterospecific (*Poecilia formosa*) and conspecific (*Poecilia latipinna*) females.



**Figure 2**  
Box plot representing preexperience cortisol release rates ( $\text{pg SL}^{-1} \text{h}^{-1}$ ; median  $\pm$  1st and 3rd quartiles) in males that did (YES; gray) or did not (NO; white) exit the acclimation chamber in assay 1.

individual behavioral types also explain variation in female mate choice and the consistency of choice, such that females that are more exploratory show lower preference scores and are more consistent in their preference (David and Cezilly 2011). In our study, we predicted that males with a more active/bold/exploratory/social behavioral type would have a higher SOP for conspecifics, potentially as a result of increased inspection time with females. In another study that tested male sailfin mollies from a population allopatric to Amazon mollies, Seda et al. (2012) found a relationship between courtship and boldness traits and determined that certain combinations of behaviors within the behavioral syndrome were size dependent, whereas others were not associated with male size. Our results differ from those of Seda et al. (2012), in that we did not find a significant correlation between conspecific mate choice and boldness, nor did we find significant correlations between male size and the measured behaviors (Table 1). One methodological consideration that could have obscured correlations is that we were unable to randomize the order of the behavioral assays (as we did not want to confound the third assay on mate choice with previous experience). Apart from this consideration, we hypothesize that in the population of sailfin mollies that we used, there may not be enough variation in male size to detect size-based behavioral

correlations. Sailfin mollies are one of several species of live-bearing fish that exhibit alternative reproductive strategies in males, and these strategies are associated with male size (reviewed in Farr 1989). Smaller males are more likely to attempt sneak copulations with females because larger males are known to act aggressively toward smaller males and thereby limit the access of small males to females (Travis and Woodard 1989; Travis et al. 1990). Therefore, it would be useful to explore species discrimination in populations with greater variation in male size distributions that may exhibit different behavioral types associated with male size.

Assortative mating may arise when individuals with similar behavior types preferentially mate with similar individuals. For example, Kralj-Fišer et al. (2013) show that more aggressive male bridge spiders (*Larinioides sclopetarius*) are more likely to mate with aggressive females than with nonaggressive females. In the sailfin molly/Amazon molly population that we used in this study, female sailfin mollies are more exploratory than Amazon mollies (Muraco JM, Gabor CR and Aspbury AS, unpublished data). This difference in behavior between females of the 2 species also informed our prediction that more exploratory male sailfin mollies would prefer conspecific females over Amazon mollies. Contrary to the observed differences between female sailfin mollies and Amazon mollies in our studied population, Scharnweber et al. (2011) found that the expression of boldness traits was similar between female sailfin mollies and Amazon mollies. However, they were unable to detect an overall behavioral syndrome due to lack of correlations between the measured behavioral traits, possibly due to small sample sizes. Further assessment based on individual behavior types in this system is warranted.

### Behavioral correlations and hormones

In this study, we also examined whether individual, male behavioral type was correlated with preexperience stress hormone (cortisol) release rates. We did not find significant correlations between preexperience cortisol release rates and activity, boldness, or exploratory behaviors (Table 1). These results are similar to results from rainbow trout, in which there is no relationship between cortisol production and boldness (Thomson et al. 2011). However, our results are in contrast to Archard et al. (2012) as they found a positive relationship between cortisol production and known correlates to boldness in another live-bearing fish. Similarly, other nonfish systems have found a relationship between exploratory behavior and stress hormones (Martins et al. 2007; Thaker et al. 2009).

Although overall there were no correlations with behaviors and cortisol, we do demonstrate that preexperience cortisol release rates are higher in individuals that exited the acclimation chamber in assay 1 compared with those that did not (~30% of tested males; Figure 2). In this situation, males that did not exit are considered shy, whereas males that did exit are considered bold. This result suggests that individuals with a bold/active/exploratory behavioral type will have higher baseline (i.e., preexperience) cortisol levels compared with individuals with a shy/inactive/nonexploratory/asocial behavioral type. High cortisol levels may be associated with the behavioral type in which individuals cope with stressful events more effectively. For example, elevated levels of glucocorticoids can result in increased survival of individual tree lizards (*Urosaurus ornatus*) via enhanced antipredator responsiveness (Thaker et al. 2009).

Boldness traits are linked with other traits such as exploration tendencies and activity in several taxa (Cote et al. 2010, 2011; Smith and Blumstein 2010), and these traits may be linked with organismal stress responses (De Boer et al. 1990; Koolhaas et al. 1999). Although we found that preexperience cortisol release rates are higher in

individuals that exited the acclimation chamber (bold males) compared with those that did not (shy males), we did not find a relationship across all males between any of the correlated behavioral traits and cortisol release rates in our system. This result contrasts with results found in another live-bearing fish, *B. episcopi*, where the cortisol response is apparent after individuals from sites differing in population pressure were subjected to a mild stressor. In this case, differential selective pressures across populations arising from variation in predation can have effects on the physiology and behavior of individuals (Archard et al. 2012). Future studies in the sailfin molly–Amazon molly system should examine the configuration of correlated behavioral traits and their relationship with stress hormone production after a mild stressor in different populations of sailfin mollies, specifically focusing on populations that are either allopatric to or sympatric with the unisexual Amazon molly. Differential selection from being sexually parasitized or not across populations could influence not only male species recognition (Gabor et al. 2013) but also the relationships between correlated traits and stress hormones. Alternatively, differences between populations could be a result of other environmental correlates of allopatric or sympatric populations, such as differential predation pressures.

Production of glucocorticoids can suppress activity of the hypothalamus–pituitary–gonadal axis, resulting in decreased secretion of gonadal hormones, thereby decreasing the expression of androgen-mediated mating behavior (reviewed by Adkins-Regan 2005; Milla et al. 2009). Gabor and Grober (2010) show that the teleost steroid hormone 11-ketotestosterone (KT) plays a role in species recognition in male sailfin mollies, but there is no correlation between preexperience cortisol release rates and preexperience KT release rates for male sailfin mollies, and cortisol release rates are not significantly correlated with the number of times males attempted to mate (Gabor and Contreras 2012). These results indicate that preexperience cortisol release rates are not suppressing male mating behaviors in this system.

### CONCLUSIONS

Taken together, the results of this study suggest that there is no predictable relationship between boldness (and its correlates) and male sailfin molly mating preference in this unisexual–bisexual system. Although suites of correlated traits may explain the maintenance of maladaptive behaviors in some situations, they do not do so predictably in the context of this unique mating system. Additionally, we have provided evidence of a relationship between boldness and stress hormones, though this relationship is neither the driving force behind the correlated traits nor the expression of mating activity of males in this system.

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