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Experience and mate choice in sailfin mollies (*Poecilia latipinna*)

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Experience and mate choice in sailfin mollies (*Poecilia latipinna*)

by

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Thesis

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Dedication

To Joshua White. A haiku: Here is my thesis. I probably should have breathed, just a little more.

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Abstract

Experience and mate choice in sailfin mollies (*Poecilia latipinna*)

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Learning and experience shape mate preferences in many species. My thesis investigates the role of experience on mating behavior of male and female sailfin mollies (*Poecilia latipinna*). In the first chapter I explore whether adult experience influences male sailfin molly mate preference for their sexual parasite, the Amazon molly (*Poecilia formosa*), and whether experience could account for reproductive character displacement (RCD) of male mate preference in this species. Sailfin males from sympatric populations show a stronger preference for conspecific females over Amazon mollies than do males from allopatric populations. I exposed males from sympatric and allopatric populations to either a sailfin female or an Amazon prior to a mating trial with an Amazon. For the allopatric population, males with recent experience with an Amazon directed fewer mating behaviors towards an Amazon during mating trials than did males with recent experience with a sailfin. Males from the sympatric population, however, performed the same amount of mating behaviors towards an Amazon regardless of experience. Thus adult experience influences mating preferences and suggests that experience may play a role in RCD in this species.

In the second chapter I investigate whether a learned sensory bias could influence female mate preferences. Sensory biases that influence mate preferences can arise through selection on the sensory system in foraging and predator detection domains. I tested whether a learned preference originating outside of the mating domain, specifically a color-based food preference, can be transferred to a color-based preference for a male trait. I trained female sailfin mollies to associate either green or blue with food and then tested their preference for animated male sailfins featuring either a blue or green spot. I found that females did not prefer the male with the same color spot to which they had been conditioned. I discuss the problem of learned preference transfer and suggest directions for future research into the role of learning in sensory bias.

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ABSTRACT

Selection on closely related species to avoid the fitness costs of hybrid mating can lead to reproductive character displacement (RCD). RCD is the pattern of increased divergence of mating traits, preferences, or both, between closely related species in areas of sympatry compared to allopatry. Increased mate discrimination against heterospecifics through experience could be a common mechanism leading to RCD. However, the extent to which learning and experience influence RCD of preferences is not clear. In areas of sympatry male sailfin mollies (*Poecilia latipinna*) act as sperm donors for the gynogentic sexual parasite, the Amazon molly (*Poecilia formosa*). Sailfin mollies show RCD of male mate preferences with sympatric populations displaying stronger conspecific preferences compared to allopatric populations. We investigated whether previous adult experience with an Amazon molly influences male sailfin molly mating behavior towards Amazons in sympatric and allopatric populations. We found that recent previous experience with an Amazon resulted in increased mate discrimination against Amazons by males from the allopatric population but not by males from the sympatric population. Whether experience can account for the pattern of RCD in sailfin male preference or whether genetic mechanisms are involved has yet to be determined.

INTRODUCTION

Choosing an appropriate, high quality mate is an important component determining an individual's fitness (Andersson 1994). Mating with an inappropriate mate, such as an individual of a different species, can result in inviable or sterile offspring. When the fitness cost of mating with a heterospecific is high, natural selection can lead to displacement of courtship signals and mate preferences in a direction that permits easier discrimination between closely related species resulting in reproductive character displacement (RCD) (Dobzhansky 1940, Higgie *et al.* 2000).

Reproductive character displacement is when traits, preferences, or both, that contribute to reproductive isolation show increased divergence between populations of two closely related species in sympatry compared to populations in allopatry (Brown and Wilson 1956, Howard 1993). RCD of traits and preferences has been documented across a wide range of taxa (frogs: Gerhardt 1994, Lemmon 2009; fish: Borland 1986, Albert and Schluter 2004, Gregorio *et al.* 2012; butterflies: Kronforst *et al.* 2006; mice: Bimova *et al.* 2011). In some cases selection on mate recognition leads to divergence of both traits and preferences. For example, populations of chorus frogs (*Pseudacris feriarium*) sympatric with a closely related species show RCD in both male courtship call characteristics and female preference such that the likelihood of hybridization is reduced (Lemmon 2009).

Even in the absence of signal divergence, RCD of mate preference is sufficient to drive reproductive isolation (Noor 1995, Peterson *et al.* 2005). Enhanced mate discrimination by populations sympatric with a close relative compared to allopatric populations has been widely documented across diverse taxa both in male preferences (birds: Ratcliffe and Grant 1983; fish: Borland 1986, Ryan *et al.* 1996, Gabor and Ryan 2001, Albert and Schluter 2004, Gregorio *et al.* 2012; insects: Kronforst *et al.* 2006) and female preferences (frogs: Marquez and Bosch 1997, Richards-Zawacki and Cummings 2011, birds: Saetre *et al.* 1997, insects: Noor 1995). Hybrid matings between *Drosophila pseudoobscura* and *D. persmilis* result in sterile male hybrid offspring. Female

Drosophila pseudoobscura demonstrate RCD of mate preferences resulting from selection against hybridization through reinforcement (Noor 1995). Male *D. pseudoobscura* mate indiscriminately with both conspecific and *D. persmilis* females. However, female *D. pseudoobscura* from sympatric populations are less likely to mate with *D. persimilis* than are females from allopatric populations (Noor 1995).

Enhanced mate discrimination displayed by populations in sympatry compared to allopatry is often attributed to genetically determined recognition mechanisms (Svensson *et al.* 2010). Genetic mechanisms responsible for RCD of mate preferences have been demonstrated in *Ficedula* flycatchers (Saether *et al.* 2007), *Drosophila pseudoobscura* (Noor 1995), Trinidadian guppies (*Poecilia reticulata*) (Magurran and Ramnarine 2005), and are assumed in many other systems. Although most models for reproductive isolation assume genetic control of mate preferences, learned preferences are also important in the development of RCD and in reproductive isolation (Servedio *et al.* 2009, Svensson *et al.* 2010). Identifying the role of learning and experience in RCD of mate recognition is necessary to understand the likelihood of reproductive isolation upon secondary contact and the potential for and rate of sympatric speciation (Servedio *et al.* 2009).

Learning to avoid heterospecifics through experience could be an important step towards developing innate discrimination abilities and RCD of mate preferences through the process of genetic assimilation (Waddington 1953, Irwin and Price 1999). In genetic assimilation a phenotype initially induced by environmental conditions, if favored by natural selection, can become genetically encoded through canalization of the trait's developmental pathway (West-Eberhard 2003). While Magurran and Ramnarine (2005) found evidence for genetically determined RCD of male mate preferences in Trinidadian guppies, they also showed that experience strongly influenced conspecific mate preferences. Trinidadian guppies sympatric with closely related swamp guppies (*Poecilia* *picta*) exhibit stronger conspecific mate preferences than those in allopatry (Magurran and Ramnarine 2004). However, given a chance to interact with both swamp guppy females and conspecific females, within one week, allopatric males show a strength of preference for conspecific over swamp guppy females indistinguishable from that shown by males from sympatric populations.

Learning and experience influence conspecific mate preferences throughout life in a variety of ways (Verzijden *et al*. 2012). Imprinting is an important and widespread mechanism by which many organisms learn conspecific mate preferences early in life. Through sexual imprinting animals learn an aspect of a parent's phenotype during a sensitive period that later serves as a template for adult mate preferences (reviewed by Irwin and Price 1999). Female cichlids in Lake Victoria, for example, learn to prefer conspecific males by imprinting their mothers, possibly based on color and olfactory cues (Verzijden and ten Cate 2007). Females of two sympatric species of three-spined sticklebacks imprint on color and odor, two traits that have diverged through ecological selection. When females are raised without fathers they show reduced mate discrimination, demonstrating the importance of imprinting on reproductive isolation in this system (Kozak 2011).

For some species the process of learning conspecific mate preferences involves experience with conspecifics while for others it involves experience with heterospecifics. Even within a single species the sexes can differ in their development of mate preferences. In two species of stickleback fish, females learn to prefer conspecific males through experience with conspecifics during rearing. Males of these species, however, learn to prefer conspecifics through experience discriminating against heterospecifics during rearing (Kozak *et al*. 2009).

In addition to learning that occurs early in life, learning and experience as an adult also shape conspecific preferences in both males and females across many taxa (reviewed by Verzijden *et al*. 2012). *Drosophila melanogaster* males reduce their courtship behavior towards closely related *D. simulans* females only after previous experience courting *D. simulans* females (Dukas 2004). Female Syrian hamsters (*Mesocricetus auratus*), although they can discriminate between conspecific and heterospecific Turkish hamster males, learn to avoid mating with Turkish hamsters only after being exposed to them as adults (delBarco-Trillo *et al*. 2010). Similarly, females of one species of damselfly (*Calopteryx splendens*) sympatric with a heterospecific learn to avoid mismating only through sexual experience with a heterospecific male (Svensson *et al*. 2010).

The unisexual-bisexual species complex of sailfin mollies and Amazon mollies is both a model system for mate recognition and an example of RCD of mate preferences. Sailfin mollies are live-bearing fish that occupy fresh, brackish, and coastal waters along the Atlantic coast from North Carolina to Florida, throughout Florida, and along the Gulf Coast from Florida to the Yucatán Peninsula in Mexico (Burgess 1980, Gabor and Ryan 2001). They have also been introduced into several locations including parts of central Texas (Hubbs *et al*. 1991). Amazon mollies occupy parts of the sailfin molly range in northern Mexico and southern Texas, and have been introduced into parts of the sailfin range in central Texas (Hubbs *et al*. 1991, Schlupp *et al*. 2002). Amazon mollies are a clonally reproducing species of gynogenetic females originating 100,000 years ago from a mating event between a sailfin and an Atlantic molly (*Poecilia mexicana*) (Schartl *et al*. 1995). As gynogens Amazon mollies require sperm from males of a parent species to complete oogenesis (Hubbs 1964).

Male sailfins show RCD of mate preferences. Sympatric males show stronger preferences for conspecific females over Amazons than do allopatric males (Ryan *et al.* 1996, Gabor and Ryan 2001). Although males obtain some reproductive benefits from mating with Amazons, such as increased conspecific matings through mate choice copying, this behavior appears maladaptive overall since matings do not result in genetic offspring for the male (Schlupp *et al.* 1994). Although mate recognition is well studied in this system, whether learning or experience influence mate recognition and RCD of mate preferences is not known.

In this study we explore the influence of prior adult experience with an Amazon molly on male mating behavior towards an Amazon to determine whether experience is important for mate recognition and RCD of male preferences in this species. Although males can discriminate between Amazons and conspecific females, typically preferring to mate with conspecifics, they show variation in preferences and will sometimes prefer an Amazon to a sailfin when it is larger or more fertile than the sailfin (Gumm and Gabor 2005, Heubel and Schlupp 2008). By testing males from populations with Amazons and populations without, we aim to identify the roles of longer-term experience and recent experience on male mate preferences for Amazon mollies.

If experience with a heterospecific is important for strengthening male conspecific mate preferences in the sailfin molly, we predict that males that have had previous experience with an Amazon molly will perform fewer mating behaviors towards an Amazon than males that have not had previous experience. We also predict that response patterns will differ between allopatric and sympatric populations. We expect to find an effect of treatment, recent experience with an Amazon or a sailfin female, on male behavior in the allopatric population but not in the sympatric population. We expect sympatric males in both treatments to show a similarly reduced mating response due to that population's history with Amazons.

METHODS

Subjects

We collected male and female sailfin mollies from two stock ponds at Brackenridge Field Laboratory in Austin, Texas. Through extensive sampling of both ponds across seasons we collected both sailfin and Amazon mollies in one pond, the "sympatric" pond, and sailfin mollies only in the second pond, the "allopatric" pond. The sailfin mollies from both ponds are from sympatric populations in Olmito, Texas but have been living in these ponds since 1988. Amazon mollies from the same location were also added to the ponds in 1988. However, they only persisted in the "sympatric" pond. Although we cannot confirm that Amazon mollies are completely absent from the second pond, we are confident that we sampled the ponds thoroughly. Apart from the difference in species composition, stock ponds comprise similar vegetation, are located next to one another, and are exposed to similar environmental conditions.

Amazon mollies used in the experience protocol and mate trials were obtained from the sympatric pond and a stock pond at The University of Oklahoma in Norman, Oklahoma. Populations of Amazon mollies originated in Olmito, Texas and Rio Purificacion, sites in southern Texas and northern Tamaulipas, approximately 80 km apart.

Male sailfins and Amazons were kept out of view of each other in the lab. Amazons were kept in group tanks containing only Amazons or Amazons and sailfin females while males were held in male-only group tanks for at least 30 days prior to testing. Testing took place in September and November 2013 and March 2014 between

0830 and 1500. Due to sampling restrictions only the allopatric population was tested in September and November and both populations were tested in March. Only females that were unfamiliar to each male were used for the experience treatment and mate trials to control for any effect of familiarity on mate preference. Sailfin females and Amazons used in the experience treatment had mean lengths of 36.09 +/- 4.40mm and 39.23 +/- 4.70mm, respectively. Test Amazons had a mean length of 40.16 +/- 4.05mm and male subjects had a mean length of 34.26 +/- 2.74mm.

Experience treatment

Males were moved to individual home tanks (40.6 X 20.3 X 25.4cm) up to three days prior to the addition of a female so that they could acclimate to their new surroundings. Forty-eight hours prior to mating trials males were given access to either a sailfin molly female or an Amazon molly in their home tank depending on treatment. Each male was randomly assigned to one of four treatment groups: 1) "*Symp formosa*", males from the sympatric population paired with an Amazon (*n*=12), 2) "*Symp latipinna*", males from the sympatric population paired with a sailfin female (*n*=12), 3) "*Allo formosa*", males from the allopatric population paired with an Amazon (*n*=10), 4) "*Allo latipinna*", males from the allopatric population paired with a sailfin female (*n*=12). After spending 24 hours with the males in their individual home tanks, females were removed and males were left alone for 24 hours prior to testing.

Mating trials

Mating trials employed an open-field design. We conducted all trials in a single test tank (40.6 X 20.3 X 25.4cm) under a combination of 500 W quartz–halogen lamp filtered following Cummings *et al*. (2003) and fluorescent overhead lighting. A male subject and an Amazon molly were placed in the test tank separated from each other by a permeable, translucent plastic barrier and allowed to acclimate for 10 minutes. After 10 minutes the barrier was removed and the female and male were free to interact for 30 minutes. After testing, we measured males and females using calipers and thoroughly rinsed the test tank. We video recorded all trials and scored them for the number of gonopodial thrusts, gonopore nips, and courtship displays performed by each male towards the Amazon. These three behaviors have been identified as behaviors that may translate to mating success in closely related guppies (*Poecilia reticulata*) (Price and Rodd 2006). Sailfin courtship displays comprise sigmoidal movements with an erected dorsal fin performed in front of a female (Travis and Woodward 1989). Males attempt to inseminate females through gonopodial thrusts that either accompany courtship displays or are performed during coercive copulation attempts. Although the function has not been confirmed, gonopore nipping is thought to allow males to assess female olfactory cues (Herdman, Kelly, & Godin, 2004).

Statistical analysis

To determine the effect of treatment and population on mating response variables we performed a series of ANOVA tests using generalized linear mixed models fitted with a Poisson distribution and Wilcoxon rank sum tests.

We ran generalized linear mixed models using gonopodial thrusts and gonopore nips as response variables. The models included the following fixed variables: treatment, population, the interaction between treatment and population, date of test, and the differences in size between the experience treatment female and the test female. We included Julian date in the model to account for any effect of seasonality on behavior because the tests spanned three months. We included the difference in size between the female used in the experience treatment and the test female to account for an effect of sequential size comparison on mating response. Test female ID was added as a random effect in the model. We performed paired comparisons between treatments within each population using Wilcoxon rank sum tests for response variables that showed significant effects of treatment. We also ran a Kruskal-Wallis rank sum test to test for difference between groups in the degree of size disparity between treatment and test females.

In addition, we ran a principal components analysis using gonopodial thrusts, gonopore nips, and courtship displays as responses to obtain a principal component score (PC1) to use as an additional response variable "mating response" in our analyses. Using PC1 as the response variable, we performed a linear mixed model using the same fixed and random variables used in the generalized linear mixed models. All analyses were performed using the programming language R (R Development Core Team 2010). Mixed models were run using the glmer and lmer functions in the lme4 package.

RESULTS

We found significant effects of treatment, exposure to either Amazon or sailfin female prior to mate test, for gonopodial thrusts, nips, and PC1 "mating response" (Table 1). Population (allopatric versus sympatric) also had an effect on the number of thrusts and nips directed towards an Amazon. The effect of population on PC1, however, was not statistically significant ($P = 0.095$). We found an interaction effect between treatment and population in number of gonopodial thrusts and nips (Table 1). Neither test date nor difference in size between the experience treatment female and the test female had a significant effect on any of the mating response variables (Table 1). However, the effect of difference in female sizes almost approached significance for number of gonopodial thrusts and nips (thrusts: $P = 0.061$, nips: $P = 0.055$). We found no difference between groups in the degree of size difference between treatment and test females using a Kruskal-Wallis rank sum test (ChiSq = 4.434, $P = 0.218$).

Response variable	**Effect**	**ChiSq**	**P-value**
Thrusts	Treatment	15.281	9.129e-05 ***
	Population	33.118	9.937e-08 ***
	Treatment:Population	49.630	1.802e-12 ***
	Female size diff.	3.418	0.061
	Test date	0.136	0.920
Nips	Treatment	4.193	0.039 *
	Population	10.663	0.001 **
	Treatment:Population	7.084	0.008 **
	Female size diff.	3.197	0.055
	Test date	1.363	0.896
"Mating response" (PC1)	Treatment	3.970	0.047 *
	Population	3.218	0.095
	Treatment:Population	1.438	0.212
	Female size diff.	0.308	0.421
	Test date	1.132	0.841

Table 1: Mixed model results showing the Chi Squared and P-values for all response variables. Significance levels: ***$P < .001$, ** $P < .01$, *$P < 0.05$.

Allopatric males differed in their mating responses towards Amazons depending on treatment. Males in the *Allo latipinna* group performed significantly more gonopodial thrusts and nips towards an Amazon than did males in the *Allo formosa* group (Table 2, Figures 1 and 2). The *Allo latipinna* group also showed a greater overall "mating response" (PC1) towards Amazons than did the *Allo formosa* group (W = 19, $P = 0.006$, Figure 3). Courtship displays were not included as a response variable in a mixed model because they were rare. All six of the males that performed courtship displays were from the allopatric population: five males from the *Allo formosa* group and one from the *Allo latipinna* group (Figure 4).

The two sympatric treatment groups (*Symp latipinna* and *Symp formosa*) did not differ from each other in any of the response variables. No males from the sympatric population performed courtship displays (Table 2, Figures 1-4).

Population	**Response variable**	**W**	**P-value**
Allopatric	Thrusts	28	0.037*
	Nips	18.5	0.007**
	PC1	19	0.006**
Sympatric	Thrusts	49	0.190
	Nips	57.5	0.417
	PC1	56.5	0.386

Table 2: Wilcoxon rank sum test results comparing treatments (Amazon vs. Sailfin) within each population (Sympatric and Allopatric). Significance levels: $**P < .01$, $*P < 0.05$.

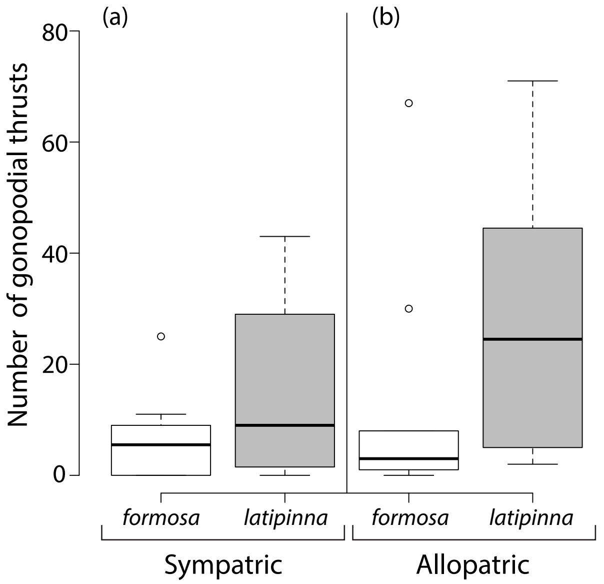

Figure 1: Boxplots showing the number of gonopodial thrusts performed towards an Amazon molly by (a) males from the sympatric population and (b) males from the allopatric population in *formosa* (white bars) and *latipinna* (grey bars) treatment groups.

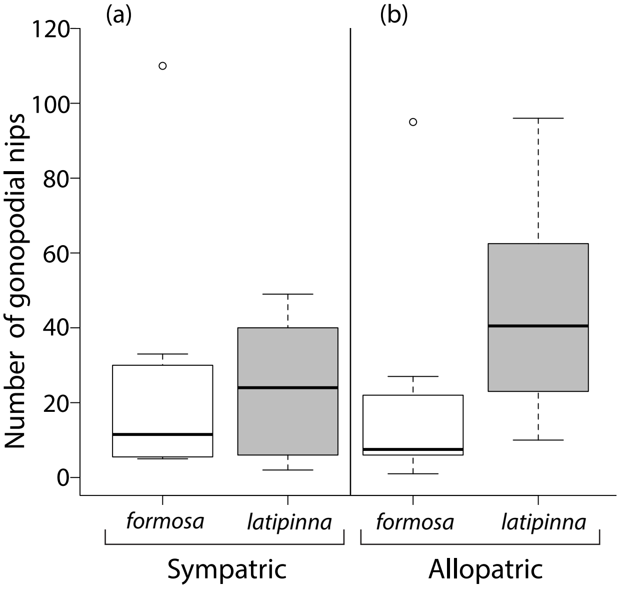

Figure 2: Boxplots showing the number of gonopore nips performed towards an Amazon molly by (a) males from the sympatric population and (b) males from the allopatric population in the *formosa* (white bars) and *latipinna* (grey bars) treatment groups.

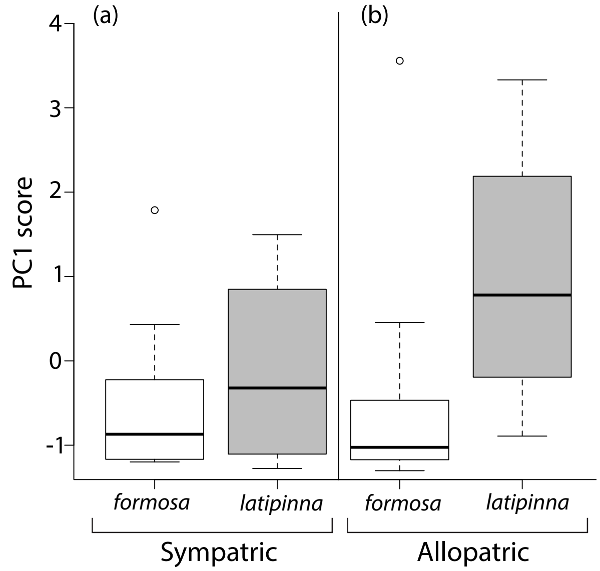

Figure 3: Boxplots showing the “Mating response,” or PC1 score, a composite of gonopodial thrusts, nips, and courtship displays performed towards an Amazon molly by (a) males from the sympatric population and (b) males from the allopatric population in the *formosa* (white bars) and *latipinna* (grey bars) treatment groups.

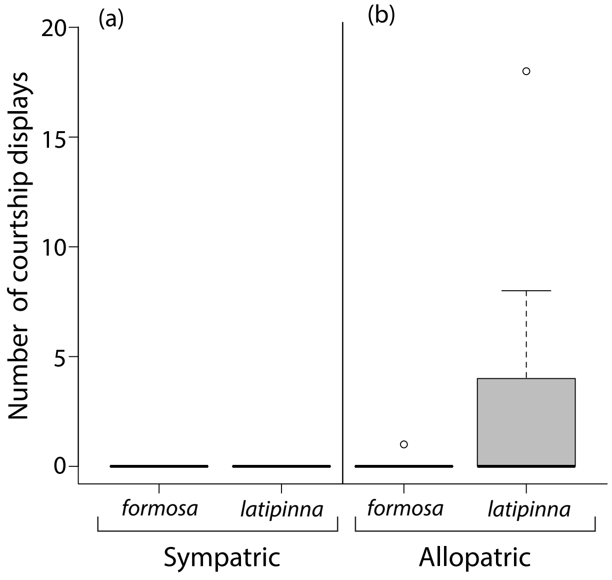

Figure 4: Boxplots showing the number of courtship displays performed towards an Amazon molly by (a) males from the sympatric population and (b) males from the allopatric population in the *formosa* (white bars) and *latipinna* (grey bars) treatment groups.

DISCUSSION

We found different effects of recent short-term experience with an Amazon on male mating responses in the allopatric population compared to the sympatric population. In the allopatric population, males exposed to an Amazon for a twenty-four hour period prior to mate trials (*Allo formosa*) directed fewer mating behaviors towards Amazons compared to males exposed to a sailfin female prior to mate trials (*Allo latipinna*). Sympatric males, however, did not differ in their mating responses towards Amazons regardless of whether they were exposed to an Amazon or a sailfin female prior to mate trials.

Other studies have shown that recent exposure to a heterospecific can result in experience-based aversion to heterospecifics of the opposite sex. Female Syrian hamsters show experience-based aversion to male heterospecific Turkish hamsters after short-term exposure to male Turkish hamsters. Interestingly, females show decreased aversion towards the heterospecific male when presented with a conspecific male prior to the heterospecific male than when presented with the heterospecific male first (delBarco-Trillo *et al*. 2010).

In our study, presentation sequence varied with treatment group. Males either encountered a heterospecific in the experience treatment followed by a heterospecific in the mate trial (*Allo formosa* and *Symp formosa*) or a conspecific in the experience trial followed by a heterospecific in the mate trial (*Allo latipinna* and *Symp latipinna*). Although the effect was not significant, the difference in size between the treatment female and the test female may have influenced the number of gonopodial thrusts and gonopore nips directed towards the test Amazon (Table 1). Collins (1995) showed that the attractiveness of a male zebra finch depends on whether that male is encountered after a more or a less attractive male. Male sailfin mollies show a preference for large body size that can result in preferences for large Amazons over smaller conspecifics (Gumm and Gabor 2005). In addition, males from allopatric populations show a stronger preference for heterospecific females when they are larger than sailfins than do males from sympatric populations (Gumm and Gabor 2005).

Differences in size between Amazons and sailfin females may have influenced mating response in males in the sailfin treatment groups (*Allo latipinna* and *Symp latipinna*) that encountered a small sailfin female in the experience treatment prior to a larger Amazon female in the mate trial. Furthermore, this effect could have been stronger in the allopatric population if those females showed a stronger preference for large females than did sympatric females. Although these effects would lead to similar results, they are likely not driving the pattern we see. Experience treatment and population had stronger effects than female size differences on all of the mating response variables used in our model. In addition, we found no significant difference in the degree of size disparity between the treatment and test females across treatment groups. Future studies should aim to tease apart the relative roles of experience with an Amazon and sequential mate choice on sailfin male mating behavior.

Although this study shows an effect of short-term adult experience on male mating response towards a heterospecific, its interpretive power is somewhat limited because we tested males from only one sympatric population and one allopatric population. The sympatric population we chose could have failed to show a significant effect of short term experience on subsequent mating response towards an Amazon for some other reason than that the population was sympatric with Amazons. Because of the similarities between the two ponds already mentioned, however, it seems more parsimonious to interpret the difference in responses between populations as an effect of long-term experience with Amazons in the sympatric population.

Hormonal feedback during and after mating may be a mechanism by which experience leads to a decrease in male mating response towards Amazons. When sailfin males attempt to mate with conspecific females, both they and the females show an increase in 11-ketotestosterone (11-KT) production that increases with mating attempts (Gabor and Grober 2010). When males mate with Amazons neither they nor the Amazons show an increase in 11-KT. Gabor and Grober (2010) suggested that this difference in androgen production that depends on the identity of the mating partner could be a mate recognition mechanism allowing sailfin males to discriminate between conspecifics and Amazons. This mechanism alone would be an imperfect discrimination mechanism requiring at least one mating attempt for complete mate discrimination to occur. Although we found that experience with Amazons led to decreased mating response towards Amazons in the allopatric population, the majority of males in the experiment, 38 of the 46 males, attempted to mate with an Amazon at least once during mating trials.

The Amazon-sailfin mating complex is a particularly interesting example of RCD due to the opposing selection pressures to avoid mating with the other species, in the case of sailfins, and to obtain matings from the other species, in the case of Amazons. Although males may be under selection to avoid mating with Amazons, as obligate sexual parasites, Amazons are under selection to get males to mate with them. If the fitness cost of mating with Amazons is great enough, this conflict of interest could lead to a cycle of sexual conflict whereby females evolve traits that are better at attracting males and males evolve better mate discrimination mechanisms to avoid mating with Amazons. Evidence suggests, however, that selection on males to avoid mating with Amazons may not be sufficiently strong. Males can decrease the cost of mating with an Amazon by decreasing sperm investment in heterospecific matings compared to conspecific matings (Schlupp and Plath 2005). Also, males can gain conspecific matings by mating with

Amazons through mate choice copying (Schlupp *et al*. 1994). More research is needed on the evolution of Amazon traits and mating tactics to understand how selection is acting on species in this mating complex.

Knowing whether learning is involved in mate recognition and RCD can influence predictions related to the probability and rate of reproductive isolation (Servidio *et al*. 2009, Svensson *et al*. 2010). Learning mate preferences through imprinting or other experience can contribute to reproductive isolation in the face of gene flow (Servidio *et al*. 2009). In the Amazon-sailfin mating complex, hybrid matings do not lead to gene flow between species even though they give rise to viable offspring. For sailfin males, however, hybrid matings lead to the evolutionary equivalent of inviable offspring since those offspring do not inherit the father's genetic material. Because Amazons have recently expanded their introduced range in central Texas, understanding the importance of learning and experience in male sailfin mating behavior can help predict how quickly sailfin populations will alter their mating responses upon contact with Amazons (Schlupp *et al*. 2002).

This study adds to the current body of research demonstrating the influence of adult experience on mate preferences and proposes a possible mechanism for RCD of male preferences in this species. To determine to what degree learned mate discrimination or innate recognition mechanisms are responsible for RCD of male sailfin mate preference, future studies should investigate the influence of genetics and rearing environment on adult male mate preferences.

Chapter 2: *No evidence for transfer of learned preferences between non-mating and mating domains*

ABSTRACT

During the process of mate choice, the female brain and sensory system dictate the attractiveness of a male trait. The sensory bias model of sexual selection proposes that preferences for male traits can arise as a consequence of pre-existing biases in the female sensory system originating outside of the mating domain that favor certain traits. Sensory bias often focuses on mate preferences resulting from tuning of the sensory system at the periphery in response to selection in non-mating domains, including foraging and predator avoidance. Processes occurring higher up in the brain, such as learning, however, may also play a role in the transfer of preferences from a non-mating domain, specifically foraging, to the mating domain. This study directly tests whether female sailfin mollies (*Poecilia latipinna*) can transfer a learned food preference to a mate preference by training females to either blue or green stimuli associated with food and then testing their preference for two male animations featuring blue or green dorsal fin spots. We found that learned food color preference had no effect on female preference for male animations. We also found an associative learning bias whereby females developed a food association with blue more easily than with green. We discuss the problem of transfer of learned preferences across domains and suggest future research directions to uncover how learning might be involved in sensory bias processes.

INTRODUCTION

Many incredible examples of morphological variation in the animal kingdom occur between the sexes within a species. Some of the most stunning traits and charismatic behaviors are those used by one sex to gain access to members of the other sex. The existence of these elaborate traits that appear to decrease survivorship initially puzzled evolutionary biologists until Charles Darwin offered an explanation with his theory of sexual selection. These elaborate traits can evolve by sexual selection if they increase reproductive success for their bearers by increasing access to mates either through direct competition with rivals or through female choice (Darwin 1871).

Females influence male trait evolution through the process of mate choice. Females can evolve preferences for traits because they gain direct benefits from the choice, their preferences are genetically correlated with the attractive male trait, which itself can be correlated with fitness benefits for offspring, yielding 'indirect' benefits, or because they have pre-existing biases for certain traits (reviewed by Ryan 1998). As opposed to direct selection and genetic correlation models of female preference in which traits and preferences evolve together, in sensory bias the preferences arise prior to the male trait and sexual selection favors traits that arise that can exploit these biases (Ryan 1998).

Preferences arise through sensory bias as pleiotropic effects of incidental, pre-existing biases in the sensory system of the receiver (reviewed by Ryan 1998). Pre-existing sensory and cognitive biases can influence trait attractiveness by dictating how well traits stimulate the female, how easy they are to detect against the background, or how easy they are to discriminate against those of other individuals (Guilford and Dawkins 1991). These biases can arise at different levels of sensory processing, from end organ tuning at the periphery to cognitive algorithms in the brain.

Certain cognitive algorithms arise during processing of sensory information in the brain that can influence attractiveness of male traits (reviewed by Ryan and Cummings 2013). Habituation is a well-known phenomenon whereby neural response decreases with repeated presentation of the same stimulus. Presenting a different stimulus causes a release from habituation and a recovery of neural response. Complex signals reduce the likelihood of habituation in the listener compared to simple signals. In some species, complex signals are favored over simple signals. Female common grackles prefer male songs with artificially increased repertoire size compared to unaltered simple songs (Searcy 1992). Biases arising through peak shift can also lead to signal elaboration through the process of learning to discriminate between two signals (ten Cate and Rowe 2007). Peak shift has been found to occur during sexual imprinting in male zebra finches leading to an adult mate preference for exaggerated beak color that is more dissimilar from the father's beak color in the direction of the mother's (ten Cate *et al*. 2006).

Cognitive algorithms also influence comparisons between signal number or magnitude. In the case of the túngara frog (*Physalaemus pustulosus*), females prefer male courtship calls with low-frequency call components, 'chucks', added to the end (Ryan and Rand 1990). Akre *et al*. (2011) demonstrated that the preference function for calls with different numbers of chucks follows Weber's Law, indicating that females perceive the ratio of chucks, not the absolute difference between chuck number. Another example of a bias that arises during sensory processing in the brain is the Ebbinghaus illusion (reviewed in Kelley and Kelley 2013). With the Ebbinghaus illusion, the perceived attractiveness of a trait changes depending on how attractive it is in relation to other traits nearby. Callander (2013) showed that female fiddler crabs (*Uca mjoebergi*) are more attracted to robotic males when they court immediately next to smaller males than when they court next to larger males.

In addition to occurring through sensory processing in the brain, sensory biases also commonly arise due to tuning of sensory end receptors. In the case of the túngara frog, females prefer male courtship calls with low-frequency chucks that match the sensitivity of an organ in the female's ear called the basilar papilla (Ryan *et al*. 1990). Ryan and Rand (1993) confirmed the sensory bias origin of this preference by showing the existence of preference prior to the trait. They found that females of related species that possess the organ but whose males do not add ‘chucks’, prefer the calls of their own males with ‘chucks’ added.

Biases at the sensory periphery can arise as pleiotropic effects of the architecture of the sensory system, as in the túngara frog, or they can arise through selection on the sensory system for prey detection and predator avoidance via sensory drive (Endler and Basolo 1998, reviewed in Ryan and Cummings 2013). Cummings (2007) demonstrated evolution of male courtship traits through sensory drive in surfperch, a group of fish that live in kelp forests. She found that species that live in different light environments have different visual pigment sensitivities driven by selection for increased prey detection ability. Male courtship traits in each species evolved to match the different visual sensitivities of their females, increasing their conspicuousness in their particular light environments.

Mate preferences arising due to tuning sensory end-organs can lead to the evolution of male traits that exploit sensory biases either by taking advantage of general sensitivities, as in the surfperch, or by mimicking the specific cue in the environment that the sensory system was tuned to detect. Trinidadian guppies show a preference for orange fruit resulting from a visual sensitivity to orange thought to have arisen through selection to detect orange fruit in the environment (Rodd *et al*. 2002). Males evolved orange spots exploiting the female sensory bias and leading to female preferences for orange spots on males (Rodd *et al*. 2002). Three-spined stickleback males also evolved red nuptial coloration through sensory exploitation by exploiting a female preference for red, carotenoid rich food (Smith *et al*. 2004).

Although sensory bias studies tend to focus on selection on sensory systems at the periphery and not at higher level brain processes, we know that higher level processes, such as learning, influence the sensory system and have parallels with end-organ tuning such that they may lead to sensory biases even in the absence of end-organ tuning. Research in humans has shown that short-term perceptual learning can result in fast, potentially permanent effects on visual sensory processing (Kami and Sagi 1993). Weinberger (1993) showed that classical conditioning using a tone as the conditioned stimulus can lead to changes in receptors of the primary and secondary auditory cortex favoring the frequency of the conditioned tone.

Like sensory tuning at the periphery, learning about important cues in the environment could also influence mate preferences through perceptual biases. Perceptual learning, or learning to better detect and discriminate critical cues in the environment through experience, leads to changes at different levels of sensory processes (Goldstone 1998). The increase in selective attention resulting from learning to associate a cue with a critical aspect of an animal's environment could act like sensory tuning by increasing the detectability of certain cues (Goldstone 1998, Dayan *et al.* 2000). Learning an association between a color and food may be sufficient to increase detectability of a similarly colored male trait due to this increase in selective attention. However, studies that directly test for learned preference transfer are rare.

One study demonstrated that a learned preference for a particular food color can influence mate preference much as the sensory tuning at the periphery can shape preferences. Amcoff *et al.* (2013) tested the effect of food color on attraction to a male ornament in the swordtail characin (*Corynopoma riisei*). This ornament acts as a lure, attracting females by mimicking a food item. In their study, females were fed either red or green food and then tested for their response to both live and animated males with natural ornaments or artificially red-colored ornaments. Females that had been fed red food showed a preference for males with red ornaments but those that had been fed green food did not. Importantly, this does not show a transfer of preferences across domains but transfer to different objects that females perceive as related to food. While learning leads to some similar outcomes as sensory end organ tuning, including increased detectability of certain cues in the environment, it is not known how learned preferences outside of a mating domain (e.g. foraging) may influence mate preferences.

In this study we test whether females transfer a learned preference for a food color to a preference for a male with the same color trait using the sailfin molly (*Poecilia latipinna*). Sailfin mollies are live-bearing fish that inhabit fresh and brackish water from North Carolina to Texas and down along the Atlantic coast of Mexico to the Yucután Peninsula (Burgess 1980, Gabor and Ryan 2001). Sailfin molly females and closely related Atlantic mollies (*Poecilia mexicana*) have been shown to exhibit a preference for novel orange coloration on males that is thought to have arisen from a pre-existing bias in the sensory periphery for orange in a foraging context (Schlupp *et al.* 1999). We use an associative learning paradigm to condition females to associate either green or blue with food. We then test female preference for playbacks showing animated males with novel green and blue spots on their dorsal fins. We predict that if females can transfer learned preferences across domains, they should prefer the male with the dorsal fin spot color to which they have been conditioned.

METHODS

Subjects

Female sailfin mollies were collected from stock ponds at Brackenridge Field Laboratory in Austin, Texas from populations originating in Olmito, Texas. Subjects were housed in the lab in groups of 10 to 15 females in one of nine 10-gallon tanks (50.8 x 25.4 x 26.7cm). Each group tank was randomly assigned to one of two treatment groups, blue (*n*=4) or green (*n*=5), for conditioning. A total of 81 females were included in the post-conditioning color preference test (blue-trained: *n*=27, green-trained: *n*=54). Twenty-four subjects (*n*=12 from each treatment), including at least one subject from each group tank, were included in the final mate choice tests (SL=33.26 +/- 4.3mm).

Stimuli

Conditioning stimuli

Conditioned stimuli (CS) consisted of videos of identical-paired stimuli, one blue and one green, against a black background presented on iPads on either side of the group tanks. The images consisted of identical blue and green shapes (triangle, rectangle, circle) of two different sizes (large and small) selected each day according to a randomized schedule. We used different shapes and sizes of CS to ensure that females associated the color of the CS, and not other aspects of the images, with the unconditioned stimulus (US).

Color preference test stimuli

To test for conditioning status after group conditioning we presented each female with blue and green novel-shaped stimuli. These stimuli were similar to those used for conditioning trials except that they had the size and shape of the male dorsal fin spots encountered in the subsequent mate choice trials.

Mate choice stimuli

Video playbacks were composed of a repeating 60 second clip of an animated male (SL=44.0mm), shown against a light gray background with a computer-generated gravel bottom. In each clip the animated male entered the screen, performed courtship-like behaviors involving quick back and forth swimming, and then departed via the other side of the screen. Animated males differed only in the color of the spot on their dorsal fin, which was either blue or green and matched the color of the CS. The dorsal fin spot was identical to the spot used as the color preference test stimuli.

Video playbacks of animated males were used for mate choice tests so that identically sized and shaped spots could be added to the fins of male stimuli that were the exact colors as those used for the CS. By using animations we controlled for differences in male size, body coloration, and behavior that might influence female preference in this species (size: Ptacek and Travis 1997, MacLaren *et al*. 2004, coloration: Schlupp *et al.* 1999). We used anyFish software to create a three-dimensional animation of a courting sailfin molly male (Veen *et al*. 2013). In a prior study we tested female response to an animation identical to the one used in this study except lacking the dorsal fin spots. We found that 77% of females significantly preferred the male animation to a playback of the background without animation (*n*=26).

Conditioning

Conditioning was performed once per day for 40 consecutive days in group tanks during January and February 2014 between 0900 and 1800 h. Tanks were covered on all sides. Lighting in the room was provided by a combination of overhead fluorescent and natural light. Each day training stimuli were presented to each group using two iPad computer tablets (24.1 x 18.5cm), one on either side of the tank. Thirty seconds after iPads were put in place an identical shape, the CS, appeared on each of the screens, one in blue and one in green. Food, the US, was immediately placed near the color to which the group was being trained. Females were allowed to eat with the shape remaining on the screen for 30 s after which the iPads were removed.

Post-conditioning tests

All post-conditioning tests were conducted between late February and mid-March, 2014 between 0900 and 1700 h. The test tank used for all post-conditioning tests was similar to the group tanks in size and appearance and located in the same room to maintain consistency between training and testing contexts. The tank was divided into three equal sections, two choice zones on either side and a neutral zone in the middle, by vertical lines drawn on the front of the tank (Fig. 5).

Color preference test

To ensure that subjects were conditioned to the color to which they had been trained, we tested each female's response to novel blue and green shapes that were identical to the fin spots on the male animation (Fig. 5). Two iPads with videos of either a blue or green spot were placed on either side of the tank according to a randomized schedule. Each female was placed in a clear Plexiglas cylinder in the middle of the test tank and allowed to acclimate for 5 min. After that time the shapes appeared on the screens and the female was allowed to observe them for one minute. We then released the female from the cylinder and recorded the time it spent in either choice zone for one minute starting after it first left the neutral zone. Only females that investigated the color to which they had been conditioned and did not approach the incorrect choice zone were used in the mate choice trials.

Mate choice test

After successfully completing the color preference tests, each subject was placed back under the cylinder in the same test tank. We covered the iPads with white boards, removed them, and replaced them with the male animation videos loaded. Screens were black while the subject acclimated for 10 min. After that time, videos began playing the animations and the subject was allowed to observe them for one minute (Fig. 5). We then released the subject and recorded the time it spent in each choice zone for 10 min starting after it first left the neutral zone. We then placed the subject back under the cylinder, switched the videos, and repeated the procedure with the videos on opposite sides to test for side bias. We omitted any females from analysis that spent more than 80% of their time on one side of the tank (Schlupp *et al*. 1999).

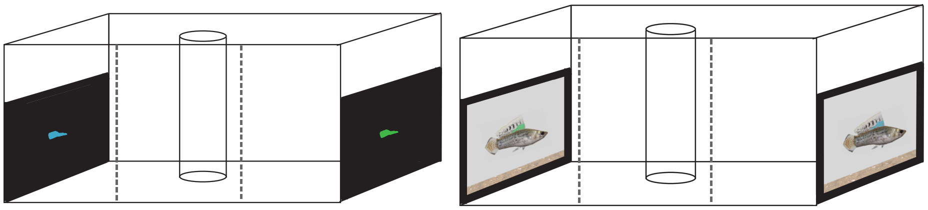

Figure 5: Test tank set up for the color preference test (left) and mate choice test (right). Stimuli are enlarged relative to tank to show detail.

Statistical analysis

We compared female response to blue and green spots in the color preference test using Fisher's exact test. We used two-tailed, paired Student's t-tests to compare the time that blue-trained females and green-trained females spent in each choice zone. Using two-tailed t-tests, we tested whether the two groups differed in the time they spent with the blue-spot male compared with the green-spot male. All analyses were performed using the programming language R (R Development Core Team 2010).

RESULTS

We found differences in response to conditioning between the blue-trained and green-trained females in the post-conditioning color preference test (Fig. 6). Blue-trained females showed a stronger preference for blue spots than green-trained females showed for green spots (Chi-squared = 8.94, P = 0.003). Blue-trained females preferred blue spots to green (P = 7.629e-05) and showed a stronger preference for blue spots than did green-trained females (Chi-squared = 4.90, P = 0.027). Green-trained females showed a stronger preference for green spots than did blue-trained females (Chi-squared = 6.00, *P* = 0.014). However, green-trained females as a group did not show a preference for either green or blue spots (*P* = 0.618).

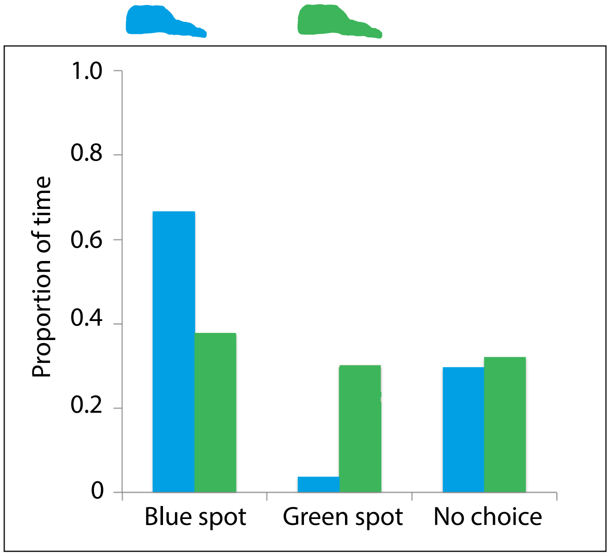

Figure 6: Proportion of blue-trained females (left bars) and green-trained females (right bars) that preferred the blue spot, green spot, or did not show a preference during testing.

For the mate choice tests we compared the total amount of time that blue-trained and green-trained females spent in each choice zone. We found that neither blue-trained females nor green-trained females significantly preferred one animation over the other (Fig. 7. for blue-trained females, t = -0.72, df = 11, *P* = 0.486; for green-trained females, t = 0.42, df = 11, *P* = 0.682). Blue- and green-trained groups of females did not differ in the amount of time they spent with the blue-spot male animation compared with the green-spot animation (Fig. 8. two-tailed t-test: t = -0.81, df = 22, *P* = 0.429).

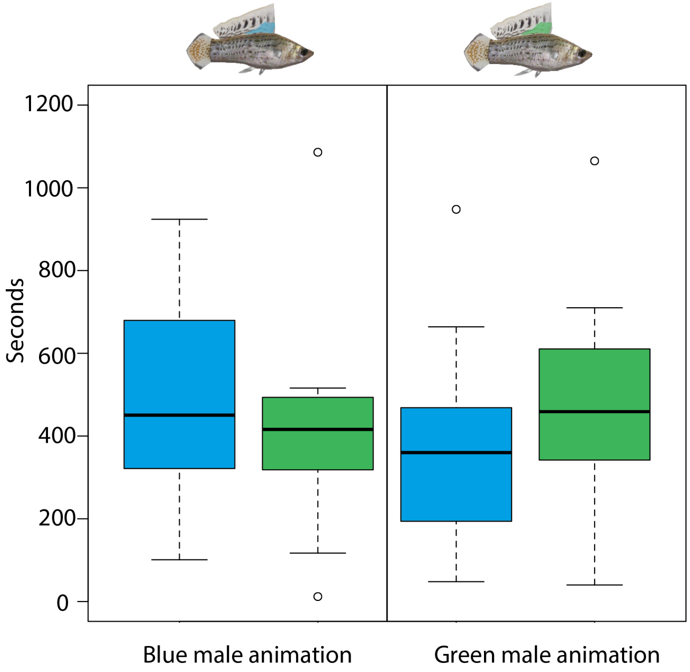

Figure 7: Boxplots showing the total time spent in the blue male animation choice zone (left panel) and the green animation choice zone (right panel) by blue-trained females (bars on left) and green-trained females (bars on right).

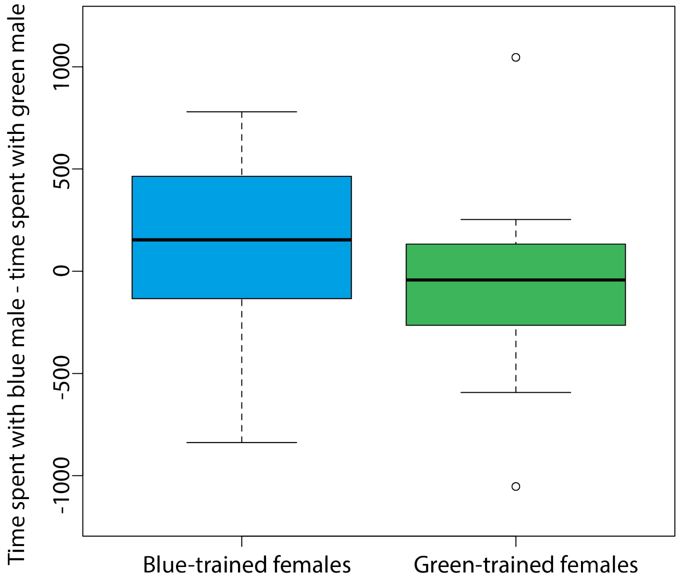

Figure 8: Boxplots showing the difference in time spent in the blue male animation choice zone compared to the green animation choice zone for blue-trained females (bar on left) and green-trained females (bar on right) in seconds.

DISCUSSION

Preference transfer

We found no evidence that a color preference acquired in a feeding domain is transferred to a mating domain. Training color did not influence the amount of time a female spent on the side with the male displaying its trained color spot compared to the other male. There was no difference between the green-trained group and the blue-trained group in their preference for one male over the other.

One explanation for our results is that we may not have been able to achieve sufficient power to detect an effect due to the use of animations to detect potentially weak preferences. One drawback to using animations is that female responses to animations are generally weaker than their responses to real males (Schlupp 2000). In a preliminary study we found that 77% of females tested significantly preferred a video of the animation to a video of the background without the animation. We found variation across females in the total amount of time they spent in the animation choice zones in the current study. However, we did not find a difference in response pattern between females that showed stronger preferences for the animations and those that showed weaker preferences. Females that spent more time in the choice zones did not show stronger preferences for the male with the color spot to which they had been conditioned than did the females that spent less time.

Although there was no correlation between total time spent in animation choice zones and preference for either male, weaker female preferences for male animations compared to real males may have made it difficult to detect an effect of conditioning. Compounding this problem, the preference under investigation—preference for a male trait arising from a food preference—may itself be weak (Fuller 2009). Considering these challenges we may have needed much larger samples sizes to detect an effect of conditioning on mate preference.

It is also possible that our results demonstrate that learned preferences are not readily transferred from a non-mating domain to a mating domain. Studies in humans have shown that mating-related preferences can be transferred from the mating domain to the food domain, but only in the case of hedonic foods (Faraji-Rad *et al*. 2013, Festjens *et al*. 2013). It is not known whether this process can work in reverse. One study testing for an effect of learned food preference on mate preference in zebrafish did not find evidence for a transfer of preference. Owen *et al*. (2012) fed young female zebrafish (*Danio rerio*) either green or red food and then tested their preference for normal mates versus red transgenic mates as adults. They found that regardless of the color of food they were fed during rearing, female zebrafish preferred novel transgenic red males to normal males.

It may be informative to compare our results and the study of Owen *et al*. (2012) to a study that did find an effect of food color preference on mate response for a male ornament. Although these two studies, ours and Owen *et al*. (2012), are by no means conclusive as to the role of learning in the transfer of preferences across domains, they offer an interesting foil for the swordtail characin study of Amcoff *et al*. (2013). In Amcoff *et al*. (2013) females that had been fed red food preferred males with red ornaments but females that had been fed green food did not. A critical difference between the swordtail characin system and the other two is that female mate response in the swordtail characin is a feeding response and therefore does not require a transfer of a preference from a feeding domain to a mating domain. These results could have implications for the types of ornaments that may be more likely to evolve due to learned preferences in other domains.

Learning may influence preference transfer in our system, however, it may be difficult to find evidence experimentally for the transfer of preferences regardless of whether those preferences are learned or not. Fuller and Noa (2010) investigated the transfer of preferences from a foraging domain to a mating domain for biases originating in the sensory periphery in the bluefin killifish (*Lucania goodie*). They measured female preferences for males with blue, red, and yellow anal fins and pecking preferences for blue, red, and yellow colored objects in different lighting environments to look for preference correlations. They found that pecking preference, a proxy for foraging preference, and mating preference did not correlate across the different environments as would be expected under the sensory bias hypothesis (Fuller 2009, Fuller and Noa 2010).

Taking a different approach, Fuller (2009) trained artificial neural networks to evolve preferences for a certain color of food and examined correlated responses of mate preferences arising from a shared sensory system. Although she found a small overall increase in female preference for males that bore traits the color of the food to which the network population had been trained, she found large amounts of variation among the populations, with many populations showing negative correlated responses.

Although both the theoretical and empirical literature on this topic are limited, there are some reasons to think that learning may be an important mechanism to consider for sensory bias processes. Learning influences both food and mate preferences in many species. In some cases food and mate preferences are tightly linked such as in *Drosophila*. Learning shapes *Drosophila* mate choice, courtship behavior, and food substrate preferences (Kim *et al*. 1996, and reviewed by Dukas 2008). Female preferences for males are strongly tied to diet, with females preferring males reared on the same diet as themselves. These preferences have been found to be due to the influence of gut microbiota on the pheromone composition of male flies (Sharon *et al*.

2010). Learning may be particularly likely to influence sensory biases in species that learn their food preferences.

Learning may be involved in the development of preferences for male traits that evolve as sensory traps, like preferences for the food lure ornament in the swordtail characin. Male traits that exploit female foraging biases through sensory traps trick females into responding to traits that mimics prey. Male water mites mimic the vibrations made by copepod prey by tapping their legs on the surface of the water to attract females. Hungry females are more likely to respond than satiated females demonstrating that female preference for this trait remains situated in the feeding domain (Proctor 1991).

Discrimination learning processes, like peak shift, could be a mechanism by which a feeding response to a food-mimicking ornament is transferred into a mating preference independent of a feeding preference. Responding to sensory traps can come with costs to females through suboptimal mating (reviewed by Ryan and Cummings 2013). Discrimination learning can result in differentiation between the mimic and the real thing. One case that may illustrate this process is the evolution of female preference for terminal yellow bands (TYBs) on male tails in a group of goodiine fishes. In this group female feeding response to TYBs, which resemble prey, predates the evolution of female mating preferences for males with TYBs (Macías Garcia and Ramirez 2005). Females of species in which males have more elaborate TYBs show weaker feeding responses towards the TYB yet maintain mate preferences for males with elaborate TYBs. This suggests an increased female ability to discriminate between prey and the TYB. This study shows that a mating preference for a trait may arise as a feeding preference and later become emancipated from this initial preference.

Learning bias

We found marked differences in the conditioning response of females trained to associate blue with food and those trained to associate green with food. As a group blue-trained females showed a clear preference for blue over green after conditioning. Green-trained females, however, did not prefer green to blue. Because they could reach food soon after seeing either shape due to the size restrictions of the tank, females could have developed an association between food and either a shape appearing on the screen in general, the correct color shape, or the wrong color shape. Given our results from the conditioning test, it seems that all three of these conditioning responses occurred at different frequencies in each treatment group. During testing females either pecked repeatedly at the correct color spot, failed to choose one spot over the other and instead swam back and forth between the two, or pecked repeatedly at the wrong color spot for the entire length of the test.

Differences in conditioning ability between groups could arise because females are not likely to develop a food association with green or because females exhibit a learning bias for blue over green. Learning to associate green with food could be difficult for fish due to the prevalence of green in their environment. The ability to successfully associate two events depends on the number of other associations each element has with other events (Domjan 2009). Alternatively, sailfin mollies could possess a learning bias for blue that is strong enough to overwhelm a conditioned response to green in our conditioning paradigm. Bumblebees (*Bombus terrestris*) show violet and blue flower color biases that may be driven by selection on foraging preferences to favor profitable local flower colors (Raine and Chittka 2007). Bias for blue flowers has been shown to affect the ability of bumblebees to learn new profitable flower colors (Jones *et al.* in prep.). Zebrafish show a bias for pecking at red objects, a behavior considered a proxy for food color preference. Spence and Smith (2008) demonstrated that zebrafish can learn to associate other colors (blue, white, and green) with food but maintain a stronger preference for red compared to their trained color.

It is important to consider existing color biases when designing stimuli for a study investigating learned preference transfer. Owen *et al.* (2012), in their investigation of learned preference transfer, found female preference for red transgenic males over normal males regardless of food color treatment. Color bias may have been one reason that they did not find an effect of conditioned food preference on mate preference because a strong bias would have made it difficult to detect an effect. We chose colors for our conditioned stimuli to which we thought females would not show a bias. Although investigations into the interaction between color bias and the ability to transfer preference would be interesting, future studies should start out by testing for color bias before conducting learned transfer investigations to eliminate this as a confounding factor.

Color biases have implications for what types of male traits are more likely to evolve through sexual selection by female choice. Finches have been found to prefer males with red leg bands while avoiding those with blue or green leg bands (Burley *et al.* 1982). Westerman (2012) found that naïve female butterflies (*Bicyclus anynana*) show a mating bias against males with enhanced wing ornamentation. When they are exposed once to males with novel, enhanced wing decoration prior to mating, however, they show increased preferences for that type of male. Similar exposure to males with decreased wing ornamentation compared to normal males does not result in preferences for that type of male. This demonstrates a strong experience-based bias favoring enhanced over reduced ornamentation.

Future directions

Future research investigating the transfer of learned preferences should include both theoretical models and empirical studies. A useful study would be to simulate the transfer of learned preferences from a foraging domain to a mating domain using artificial neural networks as Fuller (2009) did with not-learned preferences. Empirical studies should focus on investigations using animal model systems for learning, animals known to learn their food preferences, and sensory trap systems. The Japanese quail (*Coturnix coturnix japonica*) is a model organism for a large body of research on appetitive conditioning, including sexual and food conditioning (Mills *et al*. 1997, Cusato and Domjan 2000). Male Japanese quail respond vigorously to both live females and models of females in associative learning paradigms. One could test learned preference transfer by constructing an association between a conditioned stimulus, such as color, with food, and determining if the presentation of that color on a female influenced subsequent sexual response. Pigeons are also a model organism for learning and reproduction studies and could be used to test transfer of preferences in females. Animals that show learned food preferences, like hummingbirds, could also be good systems with which to investigate transfer of learned preferences. Investigations into sensory trap systems could also help uncover under what circumstances learned preferences transfer. This study contributes to a body of literature in great need to theoretical and empirical studies in the fields of learning, behavior, and evolution.

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