

Variation in male sailfin molly, preference for female size: does sympatry with sexual parasites, drive preference for smaller conspecifics?

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Abstract Male sailfin mollies (*Poecilia latipinna*) are sexually parasitized by gynogenetic Amazon mollies (*Poecilia formosa*). In areas of sympatry, Amazon mollies are frequently larger than female sailfin mollies. In sympatry, selection may favor males that prefer smaller conspecific mates (avoid mismating with Amazon mollies), or selection may favor males that prefer larger conspecific mates (higher fecundity). To explore this potential species and mate-quality recognition conflict, we examined male preference variation across populations. Males from one sympatric population showed stabilizing preference functions, whereas in another sympatric population, males showed directional preference functions. Variation across sympatric populations may be related to the length of time of co-evolution with Amazon mollies. In the allopatric populations, we found flat preference functions. Variation in male preferences could have important ramifications for the maintenance of Amazon mollies, as well as for the evolution of female size.

Keywords Geographic variation · Live-bearing fish · *P. latipinna* · *Poecilia formosa* · Preference function · Species recognition · Unisexual–bisexual mating system

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Introduction

Mate preference is a trait that describes how an individual evaluates mates, and is affected by an individual's preference function, which describes an individual's preference across a range of traits possessed by potential mates (Wagner 1998). Most preference functions describe the average population level preference in terms of the type of selection that they generate on the opposite sex as compared to individual preference functions that describes the specific preference of individuals (Chenoweth and Blows 2006). Here, we focus on the former type of preference function. Preference functions can be linear or nonlinear (sensu Lande and Arnold 1983). Linear preference functions can be directional (i.e., increasing preference for more extreme traits), stabilizing (i.e., greatest preference for intermediate trait values), or disruptive (increased preference for traits at both high and low extreme values; Wagner 1998; Cotton et al. 2006). Individuals may also have a flat preference function, indicating no variation in preference for a range of values for a particular trait, an absence of preference, or a polymorphism in preference (Morris et al. 2003).

Mate choice can be the direct outcome of mate preference if environmental or social effects do not preclude an individual from expressing their mate preference (Heisler et al. 1987; Cotton et al. 2006). When closely related species occur in sympatry, mate choice consists of species recognition and identification of high-quality conspecific mates (Andersson 1994). In sympatry, when heterospecifics resemble high-quality conspecifics, an antagonistic relationship may occur between species and mate-quality recognition and can result in individuals favoring conspecifics with intermediate trait values that are not preferred in allopatry (Pfennig 1998, 2000). Such differences in population mean mating prefer-

ences between sympatric and allopatric populations of a species (i.e., reproductive character displacement: Brown and Wilson 1956; Howard 1993) has been found in a number of taxa (review in Gabor and Ryan 2001; Höbel and Gerhardt 2003; Albert and Schluter 2004; Jang and Gerhardt 2006a, b).

In addition to variation between sympatric and allopatric populations in mating preferences, variation can also be detected across populations, regardless of whether or not the populations are sympatric with or allopatric to closely related heterospecifics. Variation between populations in mating preferences could be due to different ecological characteristics, such as predator regimes or habitat differences that lead to differences in signal discrimination abilities (Endler and Houde 1995; review in Jennions and Petrie 1997; Boughman 2001; Simmons et al. 2001). Geographic variation in mate preferences can lead to divergence and speciation (review in Ptacek 2000; Boughman 2001; Simmons et al. 2001; Slabbekoorn and Smith 2002; Kwiatkowski and Sullivan 2002; Rundle et al. 2005).

We examined male mating preference variation across populations using a well-studied unisexual–bisexual mating complex of mollies. Mollies are live-bearing fish where male sailfin mollies, *Poecilia latipinna*, occur in both allopatry from and sympatry with the clonal, all female, gynogenetic Amazon molly, *Poecilia formosa*. Amazon mollies are of hybrid origin and require sperm from their parental species (*P. latipinna* and *Poecilia mexicana*) to start egg development, but the male genes are not incorporated into the embryo (Hubbs and Hubbs 1932, 1946). Male sailfin mollies show reproductive character displacement in mating preferences: males from sympatric populations show a significantly greater preference to mate with conspecific females over Amazon mollies than do males from allopatric populations (Gabor and Ryan 2001). In sympatric populations, Amazon mollies are significantly larger than female sailfin mollies (Heubel 2004; Table 1). While male preference functions for larger conspecific over smaller conspecific females has yet to be examined (see within), male sailfin mollies from some allopatric populations prefer to associate with larger conspecific females (Travis 1994; Gabor 1999), and to mate with larger conspecific females (Ptacek and Travis 1997) and produce more sperm in the presence of larger conspecific females

(Aspbury and Gabor 2004). Body size is an important mate-quality signal in many species (Andersson 1994), and larger female sailfin mollies have larger broods (Travis et al. 1990; Robinson et al. unpublished data). Therefore, male sailfin molly preferences for larger body size could be due to the direct fitness benefits accrued via increased reproductive output (Ryan and Keddy-Hector 1992; Andersson 1994). However, when closely related heterospecifics resemble larger conspecific mates (high-quality conspecifics), individuals may not be able to engage in both species and mate-quality recognition based on body size alone, and may need to rely on multiple cues (Pfennig 1998; Hankison and Morris 2003).

Given the evidence that in some populations male sailfin mollies prefer larger conspecifics, male sailfin mollies in sympatry could encounter a conflict in mate-quality recognition and species recognition and more frequently mismatch when Amazon mollies are larger than female sailfin mollies. This hypothesis was tested by Gumm and Gabor (2005). When male sailfin mollies were given a choice between mating with larger Amazon mollies and smaller female conspecifics their preferences differed depending on the population examined: male sailfin mollies from four out of five populations sympatric with Amazon mollies that preferred conspecifics over heterospecifics when females were size matched, no longer preferred conspecifics. Males from these four sympatric populations face a conflict between species recognition and mate-quality recognition when evaluating a female's size (Gumm and Gabor 2005). One prediction from these prior results is that males from allopatric populations will show directional preference functions (preferring larger females). We examined preference functions for conspecifics based on varying female body size of male sailfin mollies from four different populations (two allopatric and two sympatric with Amazon mollies). We used models, rather than live females to assess the preference functions. Models have been used previously to examine both male and female molly preferences, and they allow the isolation of a single trait (size) which is difficult to control when using live stimuli (MacLaren et al. 2004; Gumm et al. 2006; MacLaren and Rowland 2006a, b; MacLaren 2006; Kozak et al. 2008). We also tested male mate choice for larger versus smaller

Table 1 Mean \pm SE SL (mm) and minimum (Min) and maximum (Max) SL of females (field caught and lab) from the two allopatric (SC and AS) and sympatric (AB and VG) populations used in the experiments

Population	Species	<i>n</i>	Mean SL (mm)	Min	Max
Sympatric 1 (AB)	<i>Poecilia formosa</i>	124	41.40 \pm 0.51	29.7	57.7
Sympatric 1 (AB)	<i>Poecilia latipinna</i>	104	39.42 \pm 0.64	26.0	53.7
Sympatric 2 (VG)	<i>Poecilia formosa</i>	68	42.45 \pm 0.71	30.1	55.6
Sympatric 2 (VG)	<i>Poecilia latipinna</i>	85	38.60 \pm 0.50	29.7	54.8
Allopatric 1 (SC)	<i>Poecilia latipinna</i>	39	37.26 \pm 1.19	27.0	52.7
Allopatric 2 (AS)	<i>Poecilia latipinna</i>	105	35.86 \pm 0.53	29.7	56.6

females in the same four populations using live fish to determine if mating preference assayed as association time translates into mate choice (Gabor 1999).

Methods

Fish maintenance and collection

In the following two experiments, we tested male mate preference and male mate choice for males from two sympatric and two allopatric populations. We collected fish from two sympatric populations in Tamaulipas, Mexico in 2006: Vincente Guerrero (henceforth VG; 25.07 N, 98.02 W) and Alfred Bonfil (henceforth AB; 25.30 N, 97.86 W) and from two allopatric populations in Texas: Salt Creek in Aransas County, Texas (henceforth SC; 28.33 N, 97.24 W) in 1998 and Aquarena Springs at Spring Lake, the headwaters of the San Marcos River, Hays County, Texas (henceforth AS; 29.89 N, 97.82 W) between 2004 and 2007. Fish were brought to Texas State University-San Marcos where they were maintained in large outdoor tanks (1,800 l) as randomly mating populations until ready to be used in the laboratory. When we brought the fish into the laboratory they were maintained in aerated and filtered 38-l or 53-l aquaria with tan gravel substrate. The fish were maintained at 24–27°C on a 14:10 light:dark cycle using full spectrum fluorescent lighting and were fed Ocean Star International Freshwater Flake mixed with Ocean Star International Spirulina Flake food twice daily until satiation and supplemented with live brine shrimp once per day.

Experiment 1: male preference functions

Model trials

We tested males from two populations that are sympatric with Amazon mollies (VG, $n=30$; AB, $n=26$), and males from two allopatric populations (AS, $n=30$; SC, $n=30$). Each male was tested in simultaneous, dichotomous choice tests in three different treatments: (1) 32 vs. 38 mm (6 mm difference); (2) 38 vs. 50 mm (12 mm difference); and (3) 32 vs. 50 mm (18 mm difference; Fig. 1). These treatments allowed us to evaluate male preference functions for female size differences in increments of 6 mm. The models were created from photos of live female sailfin mollies.

Fish were tested in a 38-l aquarium (54×29×33 cm) under similar conditions to those presented by Gumm et al. (2006). Briefly, the models were animated using the motorized pulley system and tank set-up as described and used by Gumm et al. (2006). A motor, attached to the center of a board, moved two round disks simultaneously

clockwise on the left and right outer sides of the tank such that the fish appeared to be “swimming” (see Gumm et al. 2006). For these trials tan gravel was placed on the outside left and right of the aquarium (35 cm) to create a similar background to the inside of the aquarium. The aquarium contained 15.2 cm of water. The front of the aquarium was covered with one-way film to minimize disturbance of the fishes by outside activity. The testing aquarium was visually divided into three sections. The outer sections (9 cm of each end of the aquarium) were the choice sections while the inner section (36 cm) was the no choice zone and the acclimation zone. All fishes were fed 15 min prior to testing. Mature males, differentiated by fusion of the gonopodium, were isolated for at least 3 days before testing. Male test fish were selected haphazardly and were not reused after being tested in all three treatments. Trials were conducted from June–September 2007, and May–June 2009 from 0800–1600 h.

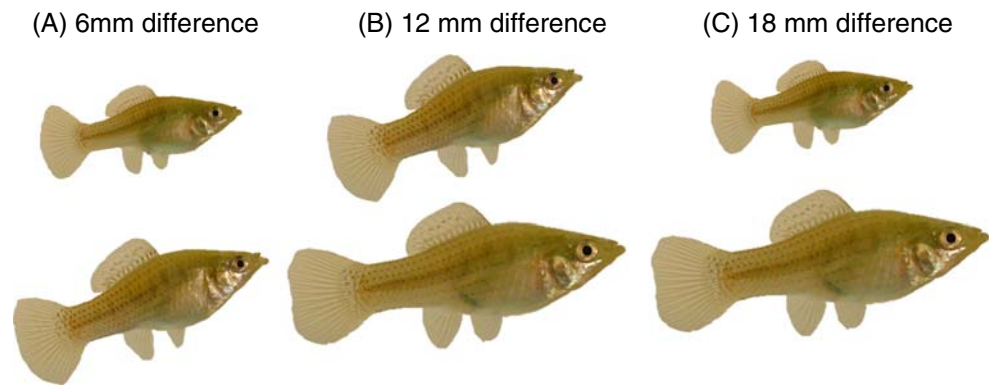
Males were tested in all three treatments on the same day with 5 min between treatments. The order of the treatments was randomized for each male. For each treatment, a male test fish was placed in the center of the aquarium under a clear plastic cylinder (12 cm diameter×15 cm) and allowed to acclimate for 10 min with the models rotating on the right and left outer sides of the aquarium. After 10 min, we released the male and recorded for 10 min the time spent by the male in each of the choice sections where they were viewing the females outside of the aquarium. At the end of the 10 min, the sides of the models were reversed to account for potential side biases. We then re-acclimated the male for 10 min with the models rotating. Following this, we released the male and recorded for 10 min the time spent by the male in each of the choice sections. The initial left–right position of the transparencies was randomized between tests and each transparency was randomly selected from the 18 models of each class size with no two pairs being reused. Therefore, each pair of models was a novel set of stimuli. Once the male had been tested in the three different treatments, we measured male standard length (SL).

Model construction

We used digital photos previously created by Gumm et al. (2006) using 18 live female sailfin mollies from the sympatric VG population. Males from all populations responded to the models of VG females, and the male behaviors most commonly observed during the model presentations from each of the populations included erected large dorsal fin, sigmoid curving of the body, and flexing of the gonopodium. These actions are attributed to male mating behavior (Farr et al. 1986).

Only non-postpartum females were used as models. All photos were taken in water when fishes had their dorsal fins

Fig. 1 Male sailfin mollies, *Poecilia latipinna*, preference functions were measured across three treatments using model female sailfin mollies: **a** 32 mm vs. 38 mm models, **b** 38 mm vs. 50 mm models, and **c** 32 mm vs. 50 mm models



extended. Using Adobe Photoshop, we selected the side of each fish that had the highest image quality and then created the mirror image of that side of the fish. The two images per fish were printed onto transparencies using a Hewlett Packard 7350 printer. We printed and then cut out images of the same fish, without their fins, on white paper so that the shape of the body of each fish matched with the transparency images. The white paper was placed between the two transparencies to create a two-dimensional model with an opaque body and transparent fins (see Gumm et al. 2006 for more details of construction). To control for initial size differences, each image was “free transformed” to 32, 38, and 50 mm. To make the variation in size between treatments relative we chose the 6 mm increment. These sizes are biologically relevant as 32-mm females are close to the minimum size of mature females, 38 mm is close to the mean size of female sailfin mollies across the four populations we tested and 50 mm is close to the maximum size of conspecifics in these populations ($n=333$; mean \pm SE=37.8 \pm 0.33 mm; range 26 to 56.6 mm). Transforming female size did not change the relationship between female dorsal fin area and SL (an isometric relationship between these two traits in female sailfin mollies was confirmed by testing the hypothesis that the slope of the relationship between the ln-transformed SL and ln-transformed dorsal fin area = 2.0. This was tested by measuring the SL and dorsal fin area of $n=10$ female sailfin mollies from the VG population and using a simple linear regression to estimate the slope and confidence interval; slope = 2.4, 95% confidence interval [1.43, 3.37]).

Statistical analyses

To determine if there were mate preferences for female size we compared male preference for the larger or smaller model fish, for each treatment and population, based on the amount of time test individuals spent in the choice section on the right-hand side of the tank between trials, within each treatment using paired t tests. To examine the strength of mating preferences we calculated a male’s strength of

preference (SOP) for larger female size as the time on the right side of the aquarium with the larger female divided by the time on the right side with the smaller and larger female (see Gabor 1999 for discussion of independence of time data in paired association preference tests). To examine variation in SOP for larger female size across the three treatments, we used repeated measures ANOVA on arcsine transformed SOP with population as the between subject factor and treatment as the repeated measure. We used post-hoc paired t tests to compare the mean arcsine transformed SOP across treatments within each population (Bonferroni corrected $\alpha=0.05/3=0.017$).

To examine male preference for specific female sizes, we determined the total time males from each population spent with a given size female model (e.g., 32, 38, 50 mm) by combining the time that males spent with that size female model in the two treatments (e.g., 32-mm models in treatment 1 and 3). We then compared the total time males spent with the 32-, 38-, and 50-mm female models for each population using repeated measures ANOVA on the total time with each size model with population as the between subject factor and model size as the repeated measure. All data sets analyzed met the assumptions of the analyses used.

Experiment 2: male mate choice

We tested male mate choice in a 20 l aquarium that contained tan gravel and aerated and filtered water with a 15 W full spectrum fluorescent light (General Electric) placed directly on top of the aquarium. Three sides were covered with black plastic to prevent test fish from being distracted by the environment around the tank. The front of the tank was covered with one-way film to minimize disturbance of the fish during observation. Mature males that were not used in the association preference experiment were selected haphazardly as test fish. Females were isolated for a minimum of 30 days before being used in single sex tanks. All fish were fed prior to testing. We

Table 2 Mean \pm SE SL (mm) of females used in the mating preference experiment

Population	Large female size	Small female size	<i>W</i>	<i>P</i>
Sympatric 1 (AB)	49.15 \pm 1.28	37.94 \pm 1.22	-68.0	<0.0001
Sympatric 2 (VG)	43.30 \pm 0.88	32.08 \pm 1.18	-52.5	<0.0001
Allopatric 1 (SC)	46.14 \pm 0.77	34.14 \pm 0.50	-68.0	<0.0001
Allopatric 2 (AS)	53.14 \pm 0.84	40.91 \pm 0.92	-85.5	<0.0001

P values are for comparing the mean sizes of the large and small female using Wilcoxon signed-rank test

AB Alfred Bonfil, VG Vicente Guerrero, AS Aquarena Springs, SC Salt Creek

conducted trials from March–September 2004, April–August 2005, and March 2006 from 0900–1500 h.

We tested males from the same populations that are sympatric with Amazon mollies (VG, $n=15$; AB, $n=16$), and allopatric populations (AS, $n=18$; SC, $n=16$). Testing was performed as in Gumm and Gabor (2005) except in each trial only female sailfin mollies were used where the larger female was at least 10 mm SL greater than or equal to the smaller female (female sizes are given in Table 2). A male sailfin molly was first placed in a clear plastic cylinder (12 cm diameter \times 30 cm) in the middle of the aquarium and then the two female sailfin mollies were simultaneously placed in the aquarium and allowed to swim freely. After a 10 min acclimation period we carefully removed the plastic cylinder and recorded the number of gonopodial thrusts (mating attempts) that males directed towards each female for 10 min (after the first gonopodial thrust). If males performed less than five gonopodial thrusts they were considered unresponsive and no data were recorded.

Statistical analyses

We compared male mate choice for larger vs. smaller females based on the number of gonopodial thrusts

directed at larger or smaller females in each test using Wilcoxon signed-ranks tests for each population. We then calculated male SOP to mate with larger females as the number of thrusts to the larger female divided by the number of thrusts to both the larger and smaller females. We examined variation in SOP to mate with larger females between the four populations with an ANOVA on arcsine transformed SOP, and used post-hoc paired *t* tests to compare the mean SOP across populations (Bonferroni correction $\alpha=0.05/6=0.008$).

Analyses of female body size distributions

We measured the size distributions for all populations using females collected both in the field and females that were in the laboratory from previous collections. To investigate the degree of variation between Amazon mollies and sailfin mollies in body size, we used ANOVA with type III sums of squares (Sokal and Rohlf 1995) on the SL measurements of females from the four populations used in this experiment, with population, species, and the population \times species interaction as the effects.

Table 3 Mean \pm SE time (s) male sailfin mollies spent with larger and smaller female sailfin mollies across three treatments

Treatment	Population	<i>n</i>	Mean \pm SE (s) larger female	Mean \pm SE (s) smaller female	<i>t</i>	<i>P</i>
32 vs. 38 mm	Sympatric 1 (AB)	26	273.62 \pm 22.62	63.81 \pm 6.47	-8.765	<0.0001
	Sympatric 2 (VG)	30	228.10 \pm 21.40	167.47 \pm 13.73	-2.092	0.045
	Allopatric 1 (SC)	30	127.45 \pm 28.92	77.03 \pm 17.73	-1.932	0.063
	Allopatric 2 (AS)	30	182.70 \pm 23.29	166.97 \pm 18.92	-0.524	0.604
38 vs. 50 mm	Sympatric 1 (AB)	26	131.89 \pm 26.76	201.77 \pm 16.00	1.869	0.073
	Sympatric 2 (VG)	30	278.50 \pm 17.25	171.30 \pm 22.52	-3.231	0.003
	Allopatric 1 (SC)	30	156.42 \pm 24.94	134.94 \pm 28.65	-0.666	0.510
	Allopatric 2 (AS)	30	176.4 \pm 18.16	147.67 \pm 15.61	-1.772	0.087
32 vs. 50 mm	Sympatric 1 (AB)	26	133.15 \pm 29.48	196.54 \pm 25.73	1.288	0.210
	Sympatric 2 (VG)	30	274.97 \pm 18.91	142.10 \pm 19.07	-4.016	0.0004
	Allopatric 1 (SC)	30	121.68 \pm 27.97	122.39 \pm 25.37	0.026	0.979
	Allopatric 2 (AS)	30	196.77 \pm 22.77	198.93 \pm 22.64	0.109	0.914

AB Alfred Bonfil, VG Vicente Guerrero, SC Salt Creek, AS Aquarena Springs

Table 4 Results of repeated measure ANOVA on arcsine transformed male strength of preference (SOP = time spent associating with large female/total time associating with larger and smaller females combined)

Source	Num DF	Den DF	<i>F</i>	<i>P</i>
Population	3	79	6.622	0.0005
Treatment	2	78	5.111	0.0080
Population × treatment	6	156	7.865	0.0001

Significant *P* values are in bold

Results

Experiment 1: male preference functions

In one sympatric population (VG), males significantly preferred the larger female than the small female in all treatments (Table 3). In the other sympatric population (AB), males preferred the larger female in the 6-mm difference (38-mm female) treatment, but showed no significant preferences in the 12- or 18-mm difference treatments (Table 3). In all three treatments there was no significant difference in the time males associated with the larger female model and the time males associated with the smaller female model for both allopatric populations (Table 3).

There was a significant effect of treatment (6, 12, and 18 mm size difference) and of population of males on SOP, as well as a significant treatment × population interaction (Table 4; Fig. 2). In one sympatric population (AB), males had a significantly greater SOP for the larger female in the 6-mm difference treatment than in the 12- or 18-mm difference treatments (Fig. 2). Males in the other sympatric

population (VG) showed no difference in SOP across the treatments (Fig. 2). In one allopatric population (SC), males had a higher SOP for larger females in the 6-mm difference treatment than in the 18-mm difference treatment (Fig. 2). There were no differences in SOP across treatments in the other allopatric population (AS; Fig. 2).

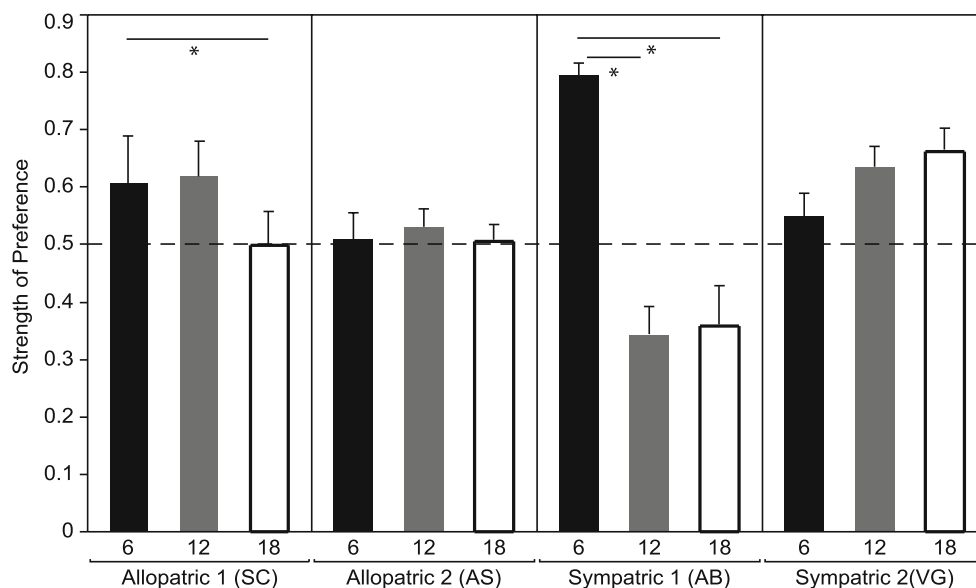
There was a significant effect of female size (32, 38, or 50 mm), population, and a significant female size × population interaction on time in association (repeated measures ANOVA, size, $F_{2, 229} = 10.808$, $p < 0.0001$; population, $F_{3, 230} = 13.7878$, $p < 0.0001$; size × population, $F_{2, 229} = 7.630$, $P < 0.0001$). Males from one sympatric population (VG) spent significantly more time associating with 50-mm than with 32- and 38-mm females, and also spent more time with 38-mm females than with 32-mm females (Fig. 3). Males from the other sympatric population (AB) spent significantly more time with the 38-mm females than with the 32- or 50-mm females (Fig. 3). Males from both allopatric populations did not spend significantly more time with 32-, 38-, or 50-mm females (Fig. 3).

Experiment 2: male mate choice

Males from one sympatric population (VG) mated significantly more often with larger females than with smaller females ($n = 15$; $Z = -60$; $P < 0.0001$; Fig. 4) as did males from both allopatric populations (SC; $n = 16$; $Z = -64$; $P = 0.0002$; AS; $n = 18$; $Z = -48.5$; $P = 0.030$; Fig. 4). However, males from the other sympatric population showed no mating preference for larger or smaller females (AB; $n = 16$; $Z = -30.5$; $P = 0.120$; Fig. 4).

There was significant variation in the mating SOP across populations (ANOVA, $F_{3, 61} = 9.51$, $P < 0.0001$). Males from

Fig. 2 Mean ± SE strength of preference of male sailfin mollies for the larger female model in each of the three treatments (size differences of 6, 12, and 18 mm) for males from two sympatric (AB and VG) and two allopatric populations (AS and SC). Values below the dotted line indicate a preference for smaller females, whereas values over the dotted line indicate a preference for larger females. *Indicates a significant difference in SOP between the treatments, within each population (post-hoc paired *t* tests, $P \leq 0.008$)



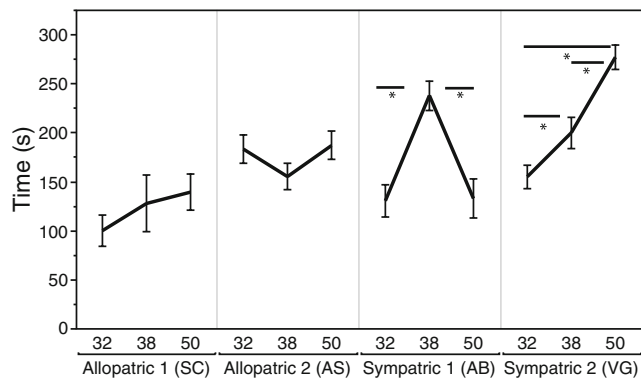


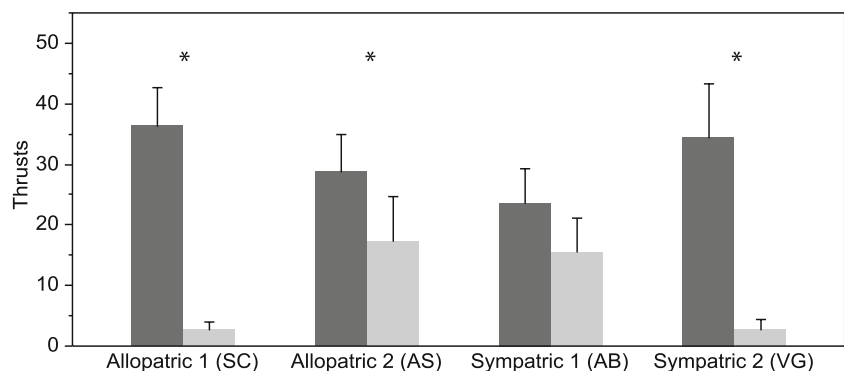
Fig. 3 Mean \pm SE time (s) that male sailfin mollies spent associating with the 32, 38, and 50 mm female models for males from two sympatric (AB and VG) and two allopatric populations (AS and SC). *Indicates a significant difference between time with the different size models within each population (post-hoc paired t tests, $P < 0.0001$)

one sympatric population (VG) showed a significantly greater SOP for larger females than males from the other sympatric population (AB; $P < 0.0001$) and than the males from one allopatric population (SL; $P = 0.0008$). Males from the allopatric SC population also showed a significantly greater SOP for larger females than males from one sympatric population (AB; $P = 0.0002$) and than the males from the other allopatric population (AS; $P = 0.0024$).

Analysis of female body size

There was significant variation across populations, as well as an population \times species interaction in female SL (Table 1; ANOVA: population, $F_{3,528} = 6.539$, $P = 0.0002$; species, $F_{1,528} = 0.765$, $P = 0.382$; population \times species, $F_{3,528} = 3.291$, $P = 0.020$). Post-hoc paired comparisons (paired t tests; $t = 1.965$, $P < 0.05$) revealed that Amazon mollies are significantly larger than the sailfin mollies in both the sympatric populations, and that the female sailfin mollies from the sympatric populations are significantly larger than those from the allopatric populations.

Fig. 4 Mean \pm SE mating attempts (thrusts) that male sailfin mollies directed at the larger (dark gray bars) and smaller (light gray bars) female sailfin mollies for males from two sympatric (AB and VG) and two allopatric populations (AS and SC). *Indicates a significant difference (Wilcoxon signed-rank test, $P < 0.05$)



Discussion

In this study, we were able to address several questions about mating preferences for female size by males of the sailfin molly. Male preferences based on female size produce a conflict between choosing a fecund female and choosing a conspecific female. This study documented variation in the preference functions for conspecific female size across populations of male sailfin mollies that are both allopatric from and sympatric with the sexually parasitic Amazon molly. In addition, we found that mating preferences are correlated with mate choice in some populations, but not all.

Regardless of the size difference between females, males from the sympatric VG population preferred larger females. Males from this population also preferred to mate with larger females than smaller females. The increase in the preference as the stimuli increased in size differences (Fig. 3) also suggests that these males have directional preference functions. This increasing preference for larger conspecific female size could have implications for both the evolution of female size in the population, as well as for the maintenance of the Amazon mollies. The female sailfin mollies in this population are significantly larger than the females from the allopatric populations. This character displacement in female size could have arisen via the strong male preference for larger females in this sympatric population, but other ecological factors (e.g., food availability, predation, temperature), or differences in the social environment (e.g., frequency of heterospecifics and conspecifics) could also account for such differences. Given that Amazon mollies are significantly larger than female sailfin mollies in this population (Table 1), along with the observation that males in this population show the highest preference for the largest females (50 mm), males in this population may have a conflict between species recognition vs. mate-quality recognition. This hypothesis is further supported by Gumm and Gabor (2005), who showed that males from this population showed no significant preference for females when given the choice to mate with a larger Amazon molly and a smaller

female sailfin molly. This unresolved conflict could provide a mechanism for the continued maintenance of Amazon mollies in this population.

Males from the other sympatric population (AB) showed very different patterns of preferences than the males from the first sympatric population (VG). Males from the AB population preferred to associate with the larger female in the 6 mm size difference only. In this treatment males spent significantly more time with the 38-mm female than with the 32-mm female. In the 12-mm size difference treatment, males preferred to associate with the smaller female, which was also the 38-mm fish. Males spent significantly more time associating with female models that were 38-mm than with the 32- or 50-mm female models. Furthermore, males showed the highest SOP for the preferred female in the 6 mm treatment and the 12 mm difference. The peak in preference for these medium-sized females suggests that these males have stabilizing preference functions. In the mating experiment, males from the sympatric AB population showed no significant mate choice for larger females. This result further supports the hypothesis that these males have stabilizing preference functions, as the mean size of the large female in this experiment was close to the size of the largest female in the mate preference experiment. Taken together the results of the preference function and mating experiments for males from the sympatric AB populations support the hypothesis that males from this population have resolved the conflict in species recognition vs. mate-quality recognition, as suggested by Gumm and Gabor (2005). Gumm and Gabor (2005) found that when given the choice to mate with a conspecific female, or a larger Amazon molly, males preferred to mate with the smaller conspecific female. In this population the average size of Amazon mollies is greater than the size of females that males from this population prefer (38 mm), and Amazon mollies are significantly larger than female sailfin mollies (Table 1). Therefore, it is possible that by exhibiting a preference for the 38-mm females, males may decrease costs associated with mating with Amazon mollies.

In both of the allopatric populations (AS and SC), males showed no significant preference for the larger or the smaller female in any of the treatments during the mate preference time trials. Furthermore, males from both the allopatric populations did not differ in the amount of time they associated with the different size female models. However, males from both of these populations preferred to mate with larger conspecific females than with smaller conspecific females in the mating trial experiments. Previously, when males from the allopatric AS population were tested for mate choice for a larger Amazon molly vs. a smaller conspecific female, they showed no significant preference (Gumm and Gabor 2005), whereas when females were size matched, males from this population

preferred to mate with conspecific females over Amazon mollies (Gabor and Ryan 2001). Furthermore, males from this allopatric population also produce more sperm when in the presence of conspecifics than they do when in the presence of Amazon mollies (Aspbury and Gabor 2004). The combination of these results suggests that males from AS recognize conspecifics over heterospecifics but that large size is a general cue that males respond to. In Gabor and Ryan (2001), males from the SC population did not show a conspecific mate preference with size-matched females yet it is still possible that this population also uses large size as a general signal, which is found in many species (Ryan and Keddy-Hector 1992). Despite the potential for selection via male mate choice for larger females in the two allopatric populations, female sailfin mollies from these populations are significantly smaller than the female sailfin mollies found in the sympatric populations. This result suggests that other factors affecting female body size exert stronger selection than male mate choice. For example, Trexler et al. (1994) showed that wading bird predators preferentially prey on larger sailfin mollies.

It is unclear why the males from allopatry showed mate choice for larger females, but lacked mating preferences when given access to visual cues only. It is possible that these males rely on non-visual cues to assess conspecific quality. We have previously found that males from the allopatric AS population do not show a preference for conspecific females over Amazon mollies when given access to only visual or only chemical cues, but they do prefer conspecific females when given access to both types of cues (Aspbury et al. 2009). In another poeciliid fish, the guppy (*Poecilia reticulata*), males express mate preferences for larger females when given access to multiple cues, but not when given access to visual cues alone (Herdman et al. 2004).

Preference functions describe the relationship between the strength of a choosing individual's preference and the variation in the traits of the individuals being chosen. In this study, we have found variation in this relationship across populations that differ in several ways, including whether or not the males are in sympatry with or are allopatric to the sexually parasitic Amazon molly. However, we did not find evidence for reproductive character displacement in male preference for conspecific female size. Males from the two different sympatric populations differed in the nature of their preference for female size. One hypothesis to explain the differences in the shape of the preference functions between the sympatric populations is that the sympatric VG population may not have had sufficient co-evolutionary time with Amazon mollies. Although we cannot test this hypothesis with our data, examining preference functions from across the range of sympatry, as well as biogeographic

investigations into the age of different sympatric populations could help shed light on this hypothesis.

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