Non-repeatable mate choice by male sailfin mollies, *Poecilia latipinna*, in a unisexual-bisexual mating complex

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Most studies of repeatability examine female mate choice, but male mate choice may have significant evolutionary consequences when males of a sexual species are sexually parasitized by heterospecific gynogenetic females as is the case for sailfin mollies, *Poecilia latipinna*. Amazon mollies, *Poecilia formosa*, are all female gynogens that require sperm from *P. latipinna* for initiation of embryogenesis, but inheritance is strictly maternal. We examined repeatability and consistency of male mate choice for female sailfin versus Amazon mollies in a series of 4 experiments. We measured male association preference for 1) size-matched female sailfin versus Amazon mollies, 2) smaller female sailfin mollies versus larger Amazon mollies, and 3) larger female sailfin mollies versus smaller Amazon mollies. We also examined 4) actual male mate choice for size-matched sailfin versus Amazon mollies. Male mate preference was not repeatable in any of our studies, and male sailfin mollies were consistent in their mate preference for conspecific females over heterospecific females only when the conspecific female was larger than the Amazon and when males could actually mate with females of both species. In all experiments, males that showed the highest conspecific preference also showed the most consistency in their preference across the 2 days of testing. The lack of repeatability of male mate preference may contribute to the persistence of Amazon mollies. *Key words:* Amazon molly, gynogenetic, live-bearing fish, *Poecilia formosa*, species recognition. *[Behav Ecol 19:871–878 (2008)]*

Sexual selection via mate choice traditionally is approached from the perspective of female choice of potential male mates. This focus is a result of the general understanding that females are usually the limiting sex in that females invest more in reproduction (Trivers 1972), and operational sex ratios are often male biased (Emlen and Oring 1977). However, in species with polygynous mating systems and conventional sex roles, males should exhibit mate choice when females vary in quality (Parker 1983). Additionally, male choice is expected when closely related species are found in sympatry and males are expected to recognize and prefer to mate with conspecific over heterospecific females (Pfennig 1998; Gabor and Ryan 2001). A conspecific mate preference should be consistent with repeated access to the female stimuli. However, most studies emphasize variation in mate choice between individuals or populations, and less emphasis is placed on within-individual variation and consistency within individuals in mate choice (Widemo and Sæther 1999). Most studies of individual variation in mate choice emphasize repeatability in female mate choice (e.g., Boake 1989; Bakker 1993; Godin and Dugatkin 1995; Kodric-Brown and Nicoletto 1997; Brooks and Endler 2001; Aspbury and Basolo 2002; Morris et al. 2003; Cummings and Mollaghan 2006). Much less work has been done on male mate choice in general (for exceptions, see Ptacek and Travis 1997; Gabor 1999; Bisazza et al. 2000; Gabor and Ryan 2001; Herdman et al. 2004; Gumm and Gabor 2005; Wong et al. 2005), and only one study to date has examined repeatability of male mate choice (Hoysak and Godin 2007; and see Travis and Woodward 1989).

One system where within-individual variation in male mate choice may have significant evolutionary consequences is when males of a sexual species are sexually parasitized by heterospecific gynogenetic females. Gynogens require sperm from closely related males for initiation of embryogenesis, but inheritance is strictly maternal, such that none of the sexually reproducing male's genes are passed on to the offspring of the gynogens (Dawley 1989). In such systems, males that fail in species recognition and mate with only females of the unisexual heterospecific species will not pass on their genes, and thus, variation in male mate choice might become limited or nonexistent. If there is a genetic basis to male mate choice, elimination of males that consistently mate with heterospecific females and fixation in the population of males that consistently mate with conspecific females could result in the extinction of the unisexual species. One well-studied unisexual–bisexual mating system consists of a bisexual species, the sailfin molly, *Poecilia latipinna*, and a unisexual (all female) gynogenetic species, the Amazon molly, *Poecilia formosa*. Amazon mollies are of hybrid origin resulting from a mating between a sailfin molly and an Atlantic molly, *Poecilia mexicana* (Hubbs and Hubbs 1932; Kallman 1962; Darnell et al. 1967; Avise et al. 1991; Schartl et al. 1995). Amazon mollies require sperm from one of their parental species to start the development of their eggs, but inheritance is completely maternal (Hubbs and Hubbs 1932; Balsano et al. 1989). Amazon mollies have existed for up to 100 000 years (Avise et al. 1991; Schartl et al. 1995; but see Dries 2003), suggesting that variation must still exist in male mating preferences, such that male sailfin mollies continue to mate with Amazon mollies. However, Gabor and Ryan (2001) have demonstrated that males from populations sympatric with Amazon mollies have significantly stronger mating preferences for conspecific females than do males from populations allopatric with Amazon mollies. Given this reproductive character displacement, male sailfin mollies from sympatric populations are expected to be consistent in their mating preferences across repeated trials.

Male sailfin mollies prefer to mate and associate with larger conspecific females (Ptacek and Travis 1997; Gabor 1999),

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which are presumably more fecund than smaller females (guppies, *Poecilia reticulata*: Reznick and Endler 1982; Herdman et al. 2004; sailfin mollies, *P. latipinna*: Aspbury A, Gabor GR, Robinson DM, Konkin-Garcia T, unpublished data). In addition, males also produce more sperm prior to mating when in the presence of larger female conspecifics than when in the presence of smaller female conspecifics (Aspbury and Gabor 2004a). However, size as a mate-quality cue can cause a conflict in mate choice when the trait overlaps with traits of heterospecifics. Based on sampling in 3 sympatric natural populations, there are frequently larger Amazon mollies than female sailfin mollies; yet, female sailfin mollies have significantly larger standard length (SL) than Amazon mollies. However, Amazon mollies show significantly more variation in size such that they often grow much larger than female sailfin mollies (Gumm and Gabor 2005). Gumm and Gabor (2005) found that when males from populations sympatric with Amazon mollies were paired with Amazon mollies that were larger than sailfin mollies, males from 3 of 4 populations showed no mating preference for sailfin mollies. This result contrasts with the results of Gabor and Ryan (2001), in which males from sympatric populations showed a mating preference for conspecifics when the females were size matched. Taken together, these results suggest a conflict between mate-quality recognition and species recognition when making mating decisions.

Although the results from previous studies of male mating preferences in sailfin mollies indicate that males are expected to be consistent in their mate choice, given different circumstances, there may be variation both within and between males in mate choice. Repeatability is a measure that can be used to partition variation within and between males (Boake 1989). In the following experiments, we measured repeatability in male association and mating preferences when males were given a choice between a female conspecific and an Amazon molly. Low levels of repeatability could indicate random choice for conspecific or heterospecific females, with either high variation both within and between males or high variation within males and low variation between males. Alternatively, a low value of repeatability could indicate that male choice for conspecific or heterospecific females is consistent and unanimous (low variation within and between males) (Howard and Young 1998; Widemo and Sæther 1999). Conversely, a high value of repeatability indicates that male choice is consistent but not unanimous across individuals and results from low within- and high between-individual variation (Howard and Young 1998; Widemo and Sæther 1999). Because low values of repeatability can arise either because of consistent mate choice or because of random choice, low values of repeatability could be uninformative. A complimentary approach to measuring repeatability, as suggested by Cummings and Mollaghan (2006), is to evaluate the "consistency" of mating choices, which is a measure of variation in an individual's choices that is independent of population-level variation. The coefficient of variation (CV) is the sample population standard deviation divided by the mean of the sample population. Because the measure of variation is divided by the population mean, comparisons can be made between 2 sample populations even if the populations differ in their means (Sokal and Rohlf 1995). This attribute makes it possible to use the CV to compare the consistency of individual mating choices when individuals differ in their mean responses to stimuli.

In this study, we examined repeatability and consistency of male sailfin molly association preference when tested repeatedly in a dichotomous choice design. We first examined repeatability and consistency of male association behavior when a conspecific female was paired with a size-matched Amazon molly. Based on prior studies of male sailfin molly mate preferences (Ryan et al. 1996; Gabor and Ryan 2001), we expected

that males would show consistent and/or repeatable association preference for conspecifics. We next examined repeatability and consistency of male association preference when males from one sympatric population were given a choice between paired larger Amazon mollies and smaller female sailfin mollies. This experiment was conducted to examine repeatability when there is potential conflict between mate-quality and species recognition (Gumm and Gabor 2005). In this experiment, there is no a priori expectation that males would show repeatable or consistent association preferences. In a third experiment, larger female sailfin mollies were paired with smaller Amazon mollies to examine repeatability of male association behavior in the absence of a conflict between species and mate-quality recognition. In this case, female size would be a supportive cue because larger conspecific females may be higher quality mates, and we expected to find repeatable and consistent association preference for conspecific females. In a fourth experiment, we examined repeatability of male mate choice when females were size matched and males were allowed to actually mate with these females. Again, as with the first experiment, we expected to find repeatable and consistent male mating preference for conspecific females.

METHODS

We used sailfin mollies and Amazon mollies originating from 2 sympatric populations in Tamaulipas, Mexico: Alfred Bonfil and Vincente Guerrero (field collected in 2005 and 2006) for the first 3 experiments. Males from both populations were only tested with females of both species from their original population. For the fourth experiment, we used males only from the Vincente Guerrero population, and, due to constraints on the number of fish we had in the laboratory, we tested males with female sailfin mollies from Spring Lake, TX, the San Marcos River headwaters (field collected 2006), and laboratory-reared Amazon mollies originating from another population sympatric with sailfin mollies collected at Rio Tigre, Mexico (field collected 1989 and 1998). We maintained the fish at Texas State University in 38-L (54 × 29 × 33 cm) and 75-L aquaria (76 × 32 × 32 cm) on a 14:10 h light:dark cycle using full spectrum UV lighting to simulate daylight. We fed the fish Ocean Star International Inc. Spirulina Flake mixed with Ocean Star International Inc. Freshwater Flake food twice daily until satiation, and the fish were supplemented daily with live brine shrimp. In all experiments, we used nongravid females that were separated from males for at least 30 days. Males were haphazardly chosen 24 h before the experiment and placed individually into a 21-L tank to standardize their behavior prior to testing.

Experiments 1–3: repeatability of male association preference

In experiment 1, we tested repeatability of male association preference for size-matched (±2 mm) female sailfin mollies and Amazons mollies using both the Alfred Bonfil (N = 36) and the Vincente Guerrero (N = 35) populations. In experiment 2, we used fishes from Alfred Bonfil (N = 36) to examine repeatability in male association preference for smaller conspecific females (≤10 mm SL) than heterospecific females. In experiment 3, we used fishes from Vincente Guerrero (N = 36) to test repeatability of male association preference for larger conspecific females (≥10 mm SL) than heterospecific females. Trials were conducted from March to October 2005 and from March to August 2006 between 0800 and 1700 h.

Males were tested by placing them in the center of a 57-L aquarium (61 × 30.5 × 30.5 cm) split into 5 sections. The outer

2 sections (10 cm) on either side were separated by sealed Plexiglas dividers that allowed visual cues only to eliminate potential variance in chemical cues across days. A stimulus female of each species (sizes depended on the experiment; Table 1) was placed in the outer sections. Males were acclimatized for 10 min under a cylinder in the nonchoice section (20 cm). After the acclimatization period, the cylinder was carefully lifted and the time spent associating with a female on either side of the aquarium (within 10 cm of divider) was recorded for each male for 10 min. After this trial, each male was replaced in his individual tank and the females were also separated in individual tanks until the next day when they were retested using the same protocol. We tested the fish on the second day within an hour of the testing time the prior day. The side of the tank that each species of female was placed in was randomized for each trial with the limitation that half the males were tested with female sailfin mollies on the right side of the tank on the first day and on the left side of the tank on the second day. The other half of the males were tested with the reverse placement of females. We calculated male strength of preference as the time associating with female sailfin mollies minus the time spent associating with Amazon mollies.

Experiment 4: repeatability of male mate choice

In experiment 4, we used males from Vincente Guerrero ($N = 13$) to test the repeatability of male mate choice when females were size matched (± 2 mm) and the males could actually mate with either species of female (Table 1). Males were separated for 24 h before testing. Trials were conducted from August to October 2006 from 0800 to 1700 h.

Males were placed in an acclimatization chamber with a size-matched female sailfin molly and an Amazon molly in a 21-L aquarium for 10 min. After this acclimatization period, the male was released and allowed to interact with both species. We counted the number of gonopodial thrusts directed at each species (methods used by Gabor and Ryan 2001), after the first gonopodial thrust occurred, for 10 min. Each male was replaced in the individual tank, and the females were also placed in an individual tank separated from the male until the next day when all 3 fish were retested using the same protocol. Testing occurred within an hour of the time they were tested on the previous day. Strength of preference is defined as the proportion of the total number of gonopodial thrusts directed at female sailfin mollies.

Statistical analyses

Repeatability was calculated from the mean square (MS) among groups (males) and the MS between groups (error) from a 1-way analysis of variance on the strength of preference for female sailfin mollies across both days of testing (Lessells and Boag 1987). The standard error (SE) associated with this

measure of repeatability was calculated as the square root of the sampling variance of the intraclass correlation (Becker 1984). We used paired t-tests to determine whether males preferred to associate with sailfin mollies over Amazon mollies on each of the 2 days they were tested. We also measured consistency of male preference by examining the CV for the proportion of time spent associating with conspecific females across the 2 repeated trials (Cummings and Mollaghan 2006). Because the CV is calculated on a per individual basis, it gives information about individual variation independent of differences between individuals in their mean responses to different stimuli. A lower CV indicates less individual variation and therefore higher consistency. The CV combined with strength of preference provide an indication of overall consistency, such that males that spend >75% of their time with conspecifics (strength of preference) indicates a strong preference and <75% strength of preference indicates a weak preference for conspecifics. All data met the assumptions of parametric statistics. All tests were 2 tailed, with $\alpha = 0.05$.

RESULTS

Experiment 1: repeatability of male association preference for size-matched females

When females of both species were size matched, there was no significant repeatability in individual male association preference for males from Alfred Bonfil ($F_{35,36} = 0.877$; $P = 0.650$; repeatability $\pm$ SE $= -0.003 \pm 0.169$). Lack of significant repeatability could have been due to either habituation (i.e., reduced total time in association on the second day of testing) or a side bias (i.e., males spending most of their time in association on either the left or the right side of the tank). In this experiment, there was a significant decrease in the total time males spent associating with the females between the 2 days of testing (mean total time associating on day 1 (s) $\pm$ SE $= 492.11 \pm 13.47$, mean total time associating on day 2 (s) $\pm$ SE $= 447.31 \pm 17.24$; paired t-test: $t = -3.89$; $P = 0.0004$), but there was no evidence of side bias (mean total time associating on left side of tank (s) $\pm$ SE $= 449.94 \pm 40.85$, mean total time associating on right side of tank (s) $\pm$ SE $= 489.47 \pm 41.00$; paired t-test: $t = 0.516$; $P = 0.609$).

There was also no correlation between the strength of preference for sailfin mollies on day 1 and day 2 ($F_{1,34} = 0.207$, $P = 0.650$, $r^2 = 0.006$; Figure 1a), and males showed no significant preference to associate with sailfin mollies or Amazon mollies on either day (Table 2). When we analyzed the relationship between the CV and the average proportion of time males spent with female sailfin mollies, we found that the more males preferred female sailfin mollies the more consistent their preference ($F_{1,34} = 7.372$, $P = 0.010$, $r^2 = 0.178$; Figure 2a). Specifically, males that showed the greatest strength of preference also showed the greatest consistency in their preference ($N = 6$, mean CV $= 0.213$), whereas those that showed weaker strength

Table 1

Sample size (N) and mean $\pm$ SE SL of female and male fish used in the experiments

Experiment	Population	N	Mean $\pm$ SE male sailfin SL	Mean $\pm$ SE Amazon SL	Mean $\pm$ SE female sailfin SL
(1a) Size-matched females (association)	AB	36	33.97 $\pm$ 1.16	40.57 $\pm$ 0.55	40.51 $\pm$ 0.55
(1b) Size-matched females (association)	VG	35	33.40 $\pm$ 1.23	42.73 $\pm$ 0.49	42.84 $\pm$ 0.45
(2) Larger Amazon molly (association)	AB	36	36.11 $\pm$ 1.19	49.36 $\pm$ 0.37	37.40 $\pm$ 0.47
(3) Larger sailfin molly (association)	VG	36	38.28 $\pm$ 0.91	33.12 $\pm$ 0.56	46.50 $\pm$ 0.47
(4) Size-matched females (mating)	VG	13	40.57 $\pm$ 1.64	47.43 $\pm$ 0.80	47.22 $\pm$ 0.55

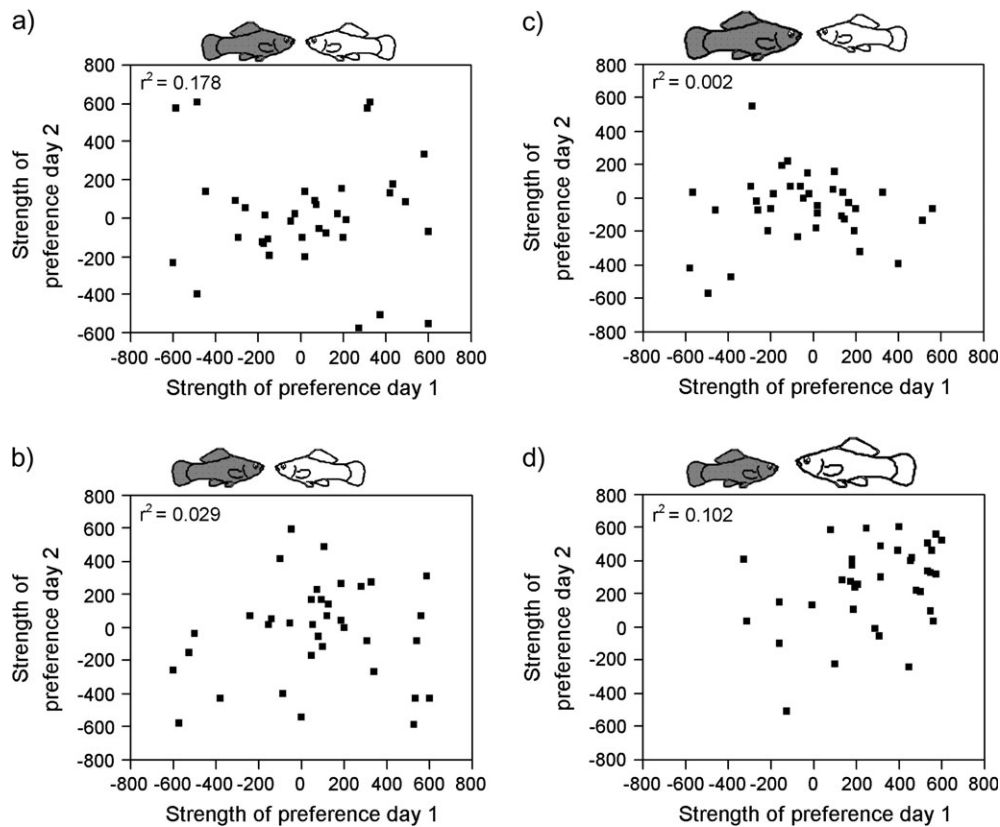AB, Alfred Bonfil; VG, Vincente Guerrero.

Figure 1
Repeatability of male association and mating preferences: relationship between the strength of preference to associate with female sailfin mollies in trials repeated on successive days for males from (a) the Alfred Bonfil population when the females were size matched, (b) the Vincente Guerrero population when the females were size matched, (c) the Alfred Bonfil population when the Amazon mollies were larger, and (d) the Vincente Guerrero population when the female sailfin mollies were larger. Images denote relative size of female sailfin mollies (unfilled image) and Amazon mollies (shaded image).

of preference showed lower consistency in their preference ($N = 30$, mean CV = 0.541).

Similar to males from Alfred Bonfil, there was no significant repeatability in individual male association preference for males from Vincente Guerrero ($F_{34,35} = 1.169$, $P = 0.320$, repeatability $\pm$ SE = 0.005 $\pm$ 0.169). In this experiment, there was no evidence of habituation or of side bias (mean total time associating on day 1 (s) $\pm$ SE = 471.97 $\pm$ 19.76, mean total time associating on day 2 (s) $\pm$ SE = 439.67 $\pm$ 15.88, paired t-test: $t = -1.479$; $P = 0.148$; mean total time associating on left side of tank (s) $\pm$ SE = 455.50 $\pm$ 37.26, mean total time associating on right side of tank (s) $\pm$ SE = 445.86 $\pm$ 39.47, paired t-test: $t = 0.137$; $P = 0.892$).

There was also no correlation between the strength of preference for sailfin mollies on day 1 and day 2 ($F_{1,33} = 0.320$, $P = 0.580$, $r^2 = 0.009$; Figure 1b). Males did not show a preference to associate with female sailfin mollies over Amazon mollies on either day (Table 2). When we analyzed the relationship between the CV and the average proportion of time males spent with female sailfin mollies, we again found that the more males prefer female sailfin mollies the more consistent their preference ($F_{1,33} = 23.841$, $P < 0.0001$, $r^2 = 0.419$; Figure 2b). Specifically, males that showed the greatest strength of preference for conspecifics also showed the greatest consistency in their preference ($N = 5$, mean CV = 0.191), whereas those that showed weaker strength of preference for

Table 2

Mean $\pm$ SE time (s) male sailfin mollies spent with conspecifics females and Amazon mollies

Experiment	Population	N	Day	Mean $\pm$ SE (s) sailfin	Mean $\pm$ SE (s) Amazon	t	P
(1a) Size-matched females	AB	36	1	262.78 $\pm$ 29.49	229.33 $\pm$ 28.31	-0.595	0.556
		36	2	224.08 $\pm$ 26.37	223.22 $\pm$ 24.66	0.018	0.990
(1b) Size-matched females	VG	35	1	272.12 $\pm$ 29.48	199.81 $\pm$ 28.51	1.330	0.190
		35	2	202.56 $\pm$ 24.49	237.11 $\pm$ 27.62	-0.700	0.492
(2) Larger Amazon molly	AB	36	1	195.75 $\pm$ 24.12	239.31 $\pm$ 24.77	-0.930	0.359
		36	2	183.11 $\pm$ 18.22	252.19 $\pm$ 21.36	-1.970	0.057
(3) Larger sailfin molly	VG	36	1	365.89 $\pm$ 26.96	89.92 $\pm$ 18.78	-6.300	**<0.0001**
		36	2	348.14 $\pm$ 24.87	107.22 $\pm$ 21.43	5.574	**<0.0001**

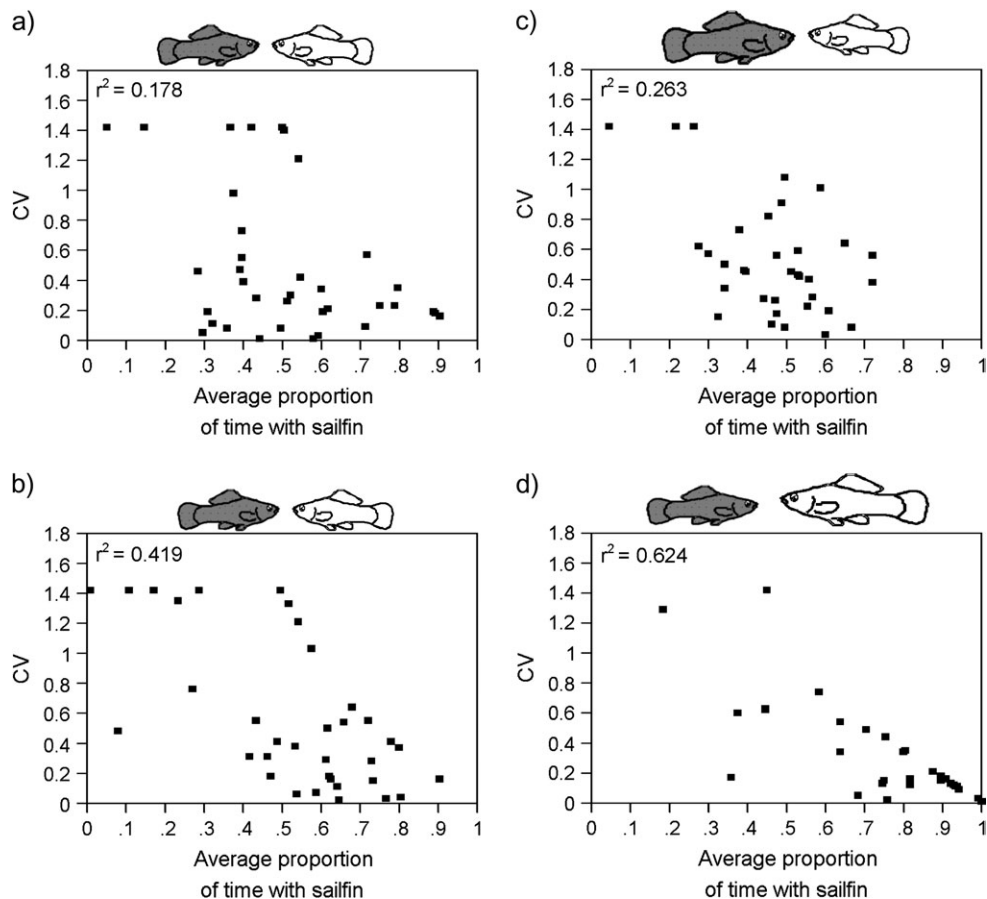AB, Alfred Bonfil; VG, Vincente Guerrero. Significant P values are in bold font.

Figure 2
Consistency of male association and mating preferences: relationship between the CV and average proportion of male association time with the female sailfin mollies across 2 repeated trials for males from (a) the Alfred Bonfil population when the females were size matched,
(b) the Vincente Guerrero population when the females were size matched, (c) the Alfred Bonfil population when the Amazon mollies were larger, and (d) the Vincente Guerrero population when the female sailfin mollies were larger. Images denote relative size of female sailfin mollies (unfilled image) and Amazon mollies (shaded image).

conspecifics showed lower consistency in their preference ($N = 30$, mean CV = 0.623).

Experiment 2: repeatability of male association preference when Amazon mollies were larger than sailfin mollies

When Amazon mollies were larger than sailfin mollies, we found that there was no significant repeatability in individual male association preference ($F_{35,36} = 1.105$, $P = 0.38$, repeatability $\pm$ SE = 0.0003 ± 0.169). There was no evidence of habituation or of side bias (mean total time associating on day 1 (s) $\pm$ SE = 435.06 ± 14.11, mean total time associating on day 2 (s) $\pm$ SE = 435.31 ± 18.61, paired t-test: $t = 0.017$; $P = 0.987$; mean total time associating on left side of tank (s) $\pm$ SE = 448.00 ± 34.69, mean total time associating on right side of tank (s) $\pm$ SE = 437.69 ± 28.34, paired t-test: $t = -0.190$; $P = 0.851$).

There was no correlation between the strength of preference for sailfin mollies on day 1 and day 2 ($F_{1,34} = 0.056$, $P = 0.81$, $r^2 = 0.002$; Figure 1c). These males also did not show a significant preference to associate with female sailfin mollies over Amazon mollies on either day (Table 2). When we analyzed the relationship between the CV and the average proportion of time males spent with female sailfin mollies, we again found that the more males prefer female sailfin mollies the more consistent their preference ($F_{1,34} = 11.41$, $P = 0.0019$,

$r^2 = 0.263$; Figure 2c). However, no males had very strong strength of preference.

Experiment 3: repeatability of male preference when sailfin mollies were larger than Amazon mollies

There was no significant repeatability in male preference when sailfin mollies were larger than Amazon mollies ($F_{35,36} = 2.119$, $P = 0.014$, repeatability $\pm$ SE = 0.03 ± 0.169). In this experiment, there was no evidence of habituation or of side bias (mean total time associating on day 1 (s) $\pm$ SE = 455.81 ± 15.50, mean total time associating on day 2 (s) $\pm$ SE = 455.36 ± 16.94, paired t-test: $t = -0.023$; $P = 0.982$; mean total time associating on left side of tank (s) $\pm$ SE = 415.78 ± 23.79, mean total time associating on right side of tank (s) $\pm$ SE = 488.11 ± 27.09, paired t-test: $t = 1.687$; $P = 0.101$).

There was a positive correlation between the strength of preference for sailfin mollies on day 1 and day 2 ($F_{1,34} = 4.83$, $P = 0.03$, $r^2 = 0.099$; Figure 1d), and males significantly preferred to associate with larger sailfin mollies over smaller Amazon mollies on both days of testing (Table 2). When we analyzed the relationship between the CV and the average proportion of time males spent with female sailfin mollies, we again found that the more males prefer female sailfin mollies the more consistent their preference ($F_{1,34} = 54.63$, $P < 0.0001$, $r^2 = 0.623$; Figure 2d). Specifically, most of the

males showed a very strong strength of preference for conspecifics and these males had the greatest consistency in their preference ($N = 26$, mean CV $= 0.121$), whereas those few males that showed weaker strength of preference for conspecifics also showed lower consistency in their preference ($N = 10$, mean CV $= 0.617$).

Experiment 4: repeatability of male mate choice

We found that there was no significant repeatability in individual male mate choice in males from Vincente Guerrero ($F_{12,13} = 1.26$, $P = 0.34$, repeatability $\pm$ SE $= 0.02 \pm 0.012$). There was no correlation between the strength of preference on day 1 and day 2 ($F_{1,11} = 0.11$, $P = 0.75$, $r^2 = 0.01$; Figure 3a). Males significantly preferred to mate with female sailfin mollies (mean $\pm$ SE gonopodial thrusts—day 1: 74.69 ± 48.24; day 2: 38.85 ± 33.41) than with Amazon mollies (mean $\pm$ SE gonopodial thrusts—day 1: 4.23 ± 4.14; day 2: 2.62 ± 5.69) on both days of testing (day 1: $N = 13$, $t = -5.47$, $P = 0.0001$; day 2: $N = 13$, $t = -3.80$, $P = 0.003$). When we analyzed the relationship between the CV and the average mating preference for female sailfin mollies, we again found that the more males prefer sailfin females the more consistent their preference ($F_{1,11} = 199.87$, $P < 0.0001$, $r^2 = 0.948$; Figure 3b). Specifically, most of the males showed a very strong strength of preference for conspecifics and these males had the greatest consistency in their preference ($N = 12$, mean CV $= 0.06$), whereas the one male that showed weaker strength of preference for conspecifics also showed low consistency in his preference ($N = 1$, CV $= 1.06$).

DISCUSSION

This study demonstrates that there is no detectable repeatability in male sailfin molly association or mating preferences in any of the dichotomous choice scenarios presented in our experiments. This result is surprising, given evidence from diverse taxa for repeatable female mating preferences (barn swallows, *Hirundo rustica*: $r = 0.57$, Møller 1994; field crickets, *Gryllus integer*: $r = 0.59$, Wagner et al. 1995; sticklebacks, *Gasterosteus aculeatus*: $r = 0.65$, Bakker 1993) and in poeciliid fish (guppies—*P. reticulata*: $r = 0.577$, Godin and Dugatkin 1995; $r = 0.399$, Brooks and Endler 2001; $r = 0.05$ live stimuli, $r = 0.47$ video stimuli, Kodric-Brown and Nicoletto 1997; least killifish—*Heterandria formosa*: $r = 0.722$, Aspbury and Basolo 2002; *Xiphophorus cortezi*: $r = 0.5$ live stimuli, $r = 0.86$ video stimuli, Morris et al. 2003). Despite this evidence for repeatable female mating preferences, only one other study besides ours has examined repeatability of male association or mating behavior. Male mosquitofish (*Gambusia holbrooki*) show no repeatability in association preference for large over small females when not allowed full contact but do have significant repeatability in association behavior ($r = 0.36$) and mating attempts ($r = 0.33$) when allowed to fully interact and mate with the females (Hoysak and Godin 2007).

There are several processes that could explain the low measures of repeatability in male sailfin molly association time and mating that we observed in this study. First, our low measures of repeatability could indicate random choice for conspecific or heterospecific females, with either high variation both within and between males or high variation within males and low variation between males. In both cases, individual males would not be expected to be consistent in their choices. This is a likely explanation for the results of experiments 1 and 2 when males were given the choice to associate with either size-matched females or a larger heterospecific versus a smaller conspecific. In both experiments, we found no significant male association preferences. Furthermore, in these experiments, very few
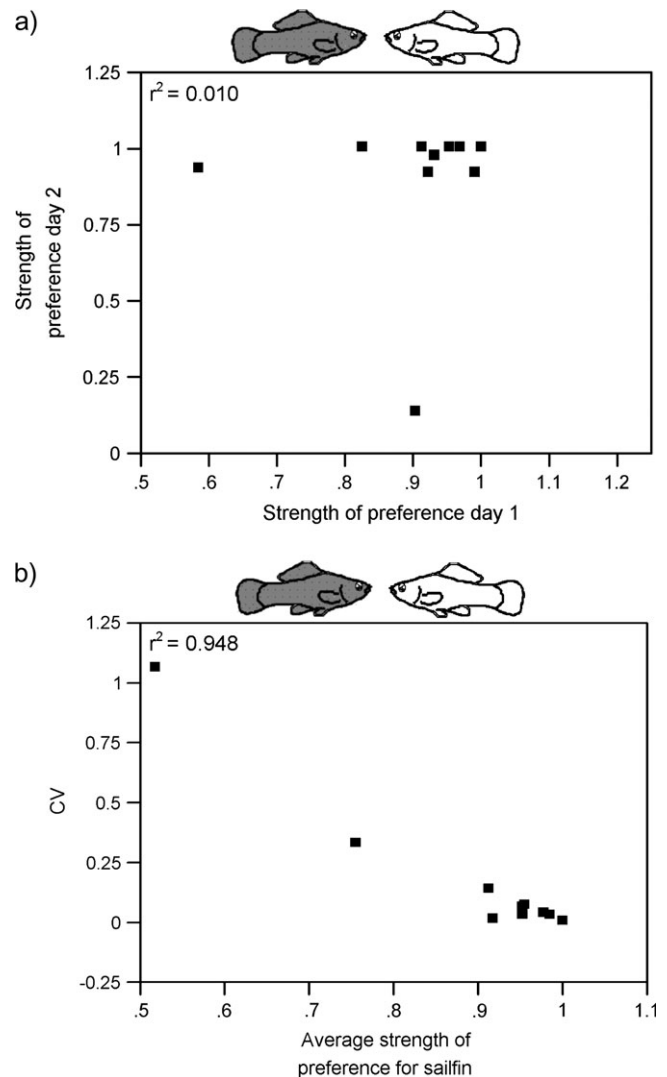

Figure 3
Repeatability and consistency in male mating preferences for males from the Vincente Guerrero population. (a) Relationship between the strength of preference to mate with size matched female sailfin mollies in trials repeated on successive days and (b) relationship between the CV and average strength of preference for female sailfin mollies across 2 repeated trials. Images denote relative size of female sailfin mollies (unfilled image) and Amazon mollies (shaded image).

males showed consistency. Alternatively, our low measurement of repeatability could indicate that male choice for conspecific females is consistent and unanimous (Widemo and Sæther 1999), suggesting that most males in a population prefer conspecific females, and individual males usually prefer conspecific females. Our data from experiments 3 and 4 suggest that this process is likely occurring when males are given the choice to associate with a conspecific female that is larger than a heterospecific female (experiment 3) and when males are allowed to interact and mate with size-matched conspecific and heterospecific females (experiment 4). In both of these experiments, males showed strong conspecific preferences on both days of mating (Table 2), and most of the males had very strong consistency in their choices (low CV). Taken together, our results suggest that individual males are more likely to be consistent in mate choice when either given direct mating access to females or the conspecific visual cue is larger than

that of the heterospecific cue and that these scenarios also lead to unanimous choice among males.

In 2 of our experiments (experiments 1 and 2), we found that individual males were not consistent in their association preferences. One possible explanation is males need physical contact with females, and/or access to visual and chemical cues, or be given the choice between relatively larger visual conspecific cues to differentiate between species. In our association tests, we did not permit chemical cues through the barriers separating the aquarium sections. Prior studies have varied results as to whether male sailfin mollies are able to choose between conspecific and heterospecifics based on visual cues alone. Schlupp and Ryan (1997) found that male sailfin mollies preferred conspecifics over heterospecifics based on visual cues only. Using models that provided only visual cues, Gumm et al. (2006) found that male sailfin mollies from one of the sympatric populations used in the current study preferentially associated with conspecific over heterospecific females. However, using a sequential testing design, Heubel and Schlupp (2006) found that male sailfin mollies did not prefer to associate with conspecific females over Amazon mollies when given access to only visual cues. In our experiments, male mate choice was more consistent than male association preference, which may suggest that visual, chemical, and possibly tactile cues provide the required information for males to assess species identity. The roles multiple cues and multiple modalities play in determining male sailfin molly preferences are currently being investigated.

Our low measures of repeatability indicate that the male mating traits we measured may not have a genetic basis. However, the relationship between repeatability and heritability is not always clear due to a variety of environmental and experimental reasons (Widemo and Sæther 1999). For example, Brooks and Endler (2001) examined the relationship between repeatability and heritability of female preference in guppies and found that female responsiveness to males was significantly repeatable ($r = 0.39$) and yet had a low heritable component ($h_s^2 = 0.26$). The biological basis for low measures of repeatability in our study may not be the lack of heritable variation in the traits measured but could instead arise due to constraints on the expression of these behaviors. It is possible that the expression of association time and mating attempts in sailfin mollies are both constrained by other factors related to mating that may themselves be repeatable. Travis and Woodward (1989) found that some components of male courtship behavior in male sailfin mollies are repeatable within male size classes. Male sailfin mollies may exhibit repeatability in more costly traits involved in mating, other than association time or mating attempts, such as sperm production (Aspbury and Gabor 2004a, 2004b). The relationship between repeatability and heritability needs to be examined in sailfin mollies in order to fully understand the sources of variation that underlie variation in male mate choice.

The results of experiment 4, in which males were allowed to mate with the females, are similar to the results of Gabor and Ryan (2001). In each study, males from sympatry preferred to mate with conspecific females over heterospecific females that were size matched. Our results also support prior work on the potential for mate-quality and species recognition conflict in sailfin mollies. When males were given a choice between a smaller female conspecific and a larger Amazon molly (experiment 2), we found that although there was a correlation between consistency and association preference, no males showed a greater than 75% preference for female sailfin mollies. These results are consistent with those of Gumm and Gabor (2005) who found that males lost their strong conspecific mating preference for female sailfin mollies when Amazon mollies were larger. The results of experiment 3 show that when males were given a choice between a larger female conspecific and a smaller Amazon molly, there was a strong correlation between consistency and association preference where most of the males showed a very strong strength of preference for larger conspecific females. This suggests that when mate-quality cues and species recognition cues are not antagonistic, male association preference is much more consistent. Therefore, in natural populations, the relative size distributions of female sailfin and Amazon mollies may contribute to the maintenance of Amazon mollies via males making mating "mistakes." As reported in Gumm and Gabor (2005), in some sympatric populations (including the 2 used in this study), although Amazon mollies are not on average larger than female sailfin mollies, Amazon mollies show a wider range of variation in SL than do female sailfin mollies, such that frequently larger Amazon mollies are found in natural populations.

In conclusion, whereas male mate choice was not repeatable in any of our studies, male sailfin mollies were consistent in their mate preference for conspecific females over heterospecific females when males could actually mate with females of both species and when there was no conflict in species recognition. The consistency in male behavior may have contributed to the evolution of reproductive character displacement of male sailfin molly mate choice in this unisexual–bisexual mating system. On the other hand, the lack of repeatability and consistency of male association preference when Amazon mollies are larger than sailfin mollies may contribute to the persistence of Amazon mollies.

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