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## Asexuals looking for sex: conflict between species and mate-quality recognition in sailfin mollies (*Poecilia latipinna*)

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**Abstract** When two closely related species are sympatric the process of species recognition (identifying conspecifics) and mate-quality recognition (increased fitness benefits) can yield a conflict when heterospecifics resemble high-quality conspecifics. Conflict in species versus mate-quality recognition may serve as a possible mechanism for the persistence of unisexual, gynogenetic Amazon mollies (*Poecilia formosa*). Amazon mollies require sperm from closely related species (e.g., sailfin mollies, *P. latipinna*) to start embryogenesis but inheritance is strictly maternal. When choosing mates, male sailfin mollies from populations sympatric with Amazon mollies may rely on traits indicating species identity rather than those indicating mate quality. Conversely, males from allopatric populations may rely more on traits indicating mate quality. Previous work has found that male sailfin mollies in sympatry exhibit a significantly greater mating preference for female sailfin mollies over Amazon mollies compared to males in allopatry. In addition, male sailfin mollies prefer to associate with and produce more sperm in the presence of larger conspecific females, which are more fecund. We hypothesized that male sailfin mollies experience a conflict in species recognition and mate-quality recognition in the presence of Amazon mollies that are relatively larger than female sailfin mollies. To test this hypothesis, we paired males from sympatric and allopatric populations with a larger Amazon molly and a smaller female sailfin molly. We scored the number of mating attempts that males directed to conspecific and heterospecific females. Males in most

sympatric and allopatric populations demonstrate no clear preference for conspecifics. In addition, we found some evidence for a difference in mating preference between allopatric and sympatric populations with males from allopatry showing a greater heterospecific mate preference. These results indicate a conflict between species and mate-quality recognition. In sympatry this conflict may contribute to the persistence of gynogenetic Amazon mollies.

**Keywords** Gynogenetic · Mate choice · *Poecilia formosa* · *Poecilia latipinna* · Species recognition

### Introduction

Mate choice requires two potentially overlapping processes: species recognition, in which conspecific individuals are identified, and mate-quality recognition in which high-quality mates are selected (Ryan and Rand 1993; but see Abt and Reyer 1993). The processes of species recognition and mate-quality recognition may result in a conflict when high-quality conspecifics resemble heterospecifics (Pfennig 1998). In response to this conflict, selection should favor mate preferences that minimize costs associated with heterospecific mating or mating with low-quality conspecifics. One outcome may be that individuals forgo one type of recognition for the other (Pfennig 1998). For example, spadefoot toads (*Spea multiplicata*) are distributed across both sympatric and allopatric populations with *S. bombifrons*. Pfennig (2000) found that male *S. multiplicata* that enhance female fertilization success are characterized by an extreme call resembling that of heterospecific males. Female *S. multiplicata* from populations that are allopatric with *S. bombifrons* prefer this extreme conspecific call, and this leads to higher fertilization success. Females from populations sympatric with heterospecifics, however, prefer calls of males that are less extreme and are closest to the mean for their population. Therefore, female *S. multiplicata* in sympatric populations, on average, exhibit reduced fertilization success and thus compromise

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potential mate-quality benefits to ensure conspecific matings (Pfennig 2000).

Although female choice is most often examined, male choice can be important in some contexts. One such case involves mating systems where unisexual females of one species are reliant on sperm from males of a bisexual species to reproduce. In this situation, males may incur the costs of sperm production/expenditure as well as time/energy expended mistakenly courting heterospecific females. In these systems males may experience a conflict in species recognition and mate-quality recognition.

Heterospecific mating can be costly for males with limited resources. When mating, males may spend time finding and courting a mate, risk greater exposure to predators, and expend energy through sperm production (reviewed in Andersson 1994; reviewed in Wedell et al. 2002). Recent evidence indicates that sperm production costs may be higher than previously thought (Wedell et al. 2002). Aspbury and Gabor (2004) found that the presence of females positively affects the amount of sperm male sailfin (*Poecilia latipinna*) mollies have available to transfer indicating that there may be constraints on sperm production in males. Additionally, this response was strongest when large females were available to males, indicating that males may be conserving energy resources in the absence of high-quality female stimuli.

A well-studied system where species recognition and mate-quality recognition may conflict is the unisexual–bisexual complex of mollies. The Amazon molly, *P. formosa*, is a unisexual species that reproduces through gynogenesis and genetic transmission is clonal (Hubbs and Hubbs 1932; Balsano et al. 1989). However, sperm from male sailfin mollies, *P. latipinna*, or male Atlantic mollies, *P. mexicana*, is needed to initiate embryogenesis (Hubbs and Hubbs 1946; Kallman 1962; Darnell et al. 1967; Schlupp et al. 2002). From an evolutionary perspective, mating with heterospecifics has no apparent direct fitness benefits to males (but see Schlupp et al. 1994 for a possible indirect benefit), and therefore, this behavior should be selected against. However, Amazon mollies have successfully persisted for up to 100,000 years (Avisé et al. 1991; Scharl et al. 1995), suggesting that male sailfin and Atlantic mollies do at least occasionally mate with Amazon mollies.

Male sailfin mollies prefer to associate with larger female sailfin mollies (Ptacek and Travis 1997; Gabor 1999), and also direct more gonopodial thrusts at larger female sailfin mollies (Ptacek and Travis 1997). In addition, females that have broods from multiple sires have higher fecundity than average for their body size (Trexler et al. 1997). Male sailfin molly preference for large conspecific females, indicates that body size is a cue males use to assess conspecific mate quality. Male sailfin mollies can also discriminate between conspecific and heterospecific females, with sympatric males showing a stronger mating preference for conspecific females than allopatric males (Hubbs 1964; Ryan et al. 1996; Gabor and Ryan 2001). Consequently, if there is a conflict between species recognition and mate-quality recognition individuals from populations

allopatric to Amazon mollies may rely more on traits indicating mate quality rather than those indicating species identity while males from populations that are sympatric with Amazon mollies may rely more on traits indicating species identity rather than those indicating mate quality.

We examined whether male sailfin mollies mate preference for larger females conflicts with males' abilities to discriminate between heterospecifics and conspecifics (Amazon mollies and sailfin mollies, respectively). We tested male-mating preferences using males from allopatric and sympatric populations. Males were simultaneously presented with a large Amazon molly and a smaller female sailfin molly, and the number of mating attempts directed at each female was counted. Males are expected to prefer conspecifics. If there is no conflict between species and mate-quality recognition, then we predict males will continue to show a strong preference for conspecific females. However, if a conflict exists, then we predict that males would no longer demonstrate conspecific mating preference as they had in previous studies when female sailfin and Amazon mollies were size matched (Ryan et al. 1996; Gabor and Ryan 2001). In addition, males from allopatric populations may be more likely to mate with larger Amazon mollies than males from sympatric populations based on the lack of selection to avoid heterospecific matings.

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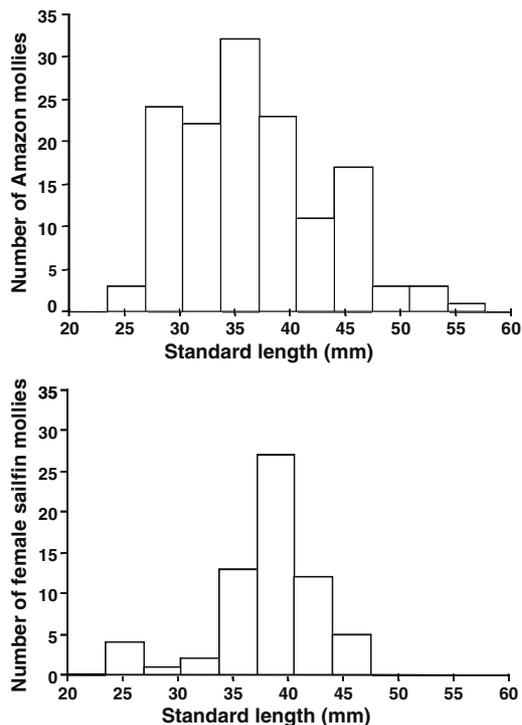
## Methods

### Natural history

Mollies are livebearing fishes. Females typically have a 30-day ovarian cycle, and are usually more receptive to males for 1–2 days after parturition (Liley 1966). Insemination takes place after a male inserts his gonopodium (modified anal fin used to transfer sperm) into a female's gonopore. This mating behavior is referred to as gonopodial thrusting and may or may not be preceded by courtship displays (Travis and Woodward 1989).

Amazon mollies are native to streams and coastal lagoons ranging from southern Texas to Vera Cruz, Mexico and are introduced in areas of Central Texas from populations located in Brownsville, Texas (Hubbs 1964). Sailfin mollies are found along the North American Gulf coast from North Carolina, USA to the Yucatan Peninsula. They are also found inland in Florida, Louisiana, and Texas in ponds, streams, drainage ditches and saltwater marshes.

The hybrid origin of Amazon mollies has resulted in a morphology intermediate between the parental species (Hubbs and Hubbs 1946; Dries 2003). Amazon mollies have fewer dorsal fin rays (10–12) than sailfin mollies (13–15), and the dorsal fin is positioned more posteriorly in Amazon mollies than in sailfin mollies (Hubbs and Hubbs 1932). There is also a lateral spot pattern present on the body of sailfin mollies that Amazon mollies lack. Thus far, no studies have determined if these visual differences are important in species recognition by male sailfin mollies. In the sympatric populations that we have sampled, the standard length (SL) of Amazon mollies is



**Fig. 1** Size distributions of (a) *Poecilia formosa* ( $n = 139$ ) and (b) female *P. latipinna* ( $n = 64$ ) from sympatric populations in the wild. Standard length is measured from the tip of the snout to the end of the musculature in the caudal peduncle

significantly smaller than female sailfin mollies ( $n_{\text{female sailfin}} = 64$  (mean  $\pm$  SE =  $38.3 \pm 0.58$ );  $n_{\text{Amazon}} = 139$  (mean  $\pm$  SE =  $36.5 \pm 0.55$ ); unpaired  $t$ -test  $t = -2.0$ ,  $P = 0.047$ ; Fig. 1). However, Amazon mollies show significantly more variation in SL (Kolmogorov-Smirnov  $D = 18.2$ ;  $P = 0.0002$ ; SL range female sailfin molly = 25.2 – 47.0 mm; Amazon molly = 23.5–57.7 mm; Fig. 1). In other words, although female sailfin mollies are on average larger, Amazon mollies have greater variation in size such that frequently Amazon mollies are larger than female sailfin mollies.

#### Collection and maintenance

All fishes were collected from eight natural populations by seine and dip-net. The following five populations of sailfin mollies are sympatric with Amazon mollies (date collected): Venus, Mexico (May 2002); Alfred Bonfil, Mexico (May 2002); Vicente Guerrero, Mexico (March 2003); Comal, Texas (May 2002); and Martindale, Texas (May 2002). All Mexican populations are from the Tamaulipas region of Mexico and are naturally sympatric. The Martindale, Texas and Comal, Texas sites represent introduced sympatry, with fish coming from populations in Florida and Louisiana in the 1930's (Brown 1953). The populations farthest south in Mexico are considered to be in 'deepest sympatry' with Amazon mollies. They are closest to the geographical origin of Amazon mollies

and may have a longer evolutionary history with Amazon mollies. Allopatric populations of sailfin mollies were collected from three sites: Aquarena Springs, Texas (May 2002); Lafayette, Louisiana (March 2003); and Destin, Florida (1997). Aquarena Springs, Texas is an allopatric population, introduced in 1944, with fish originating from populations in Louisiana and Florida (Brown 1953). All populations were wild-caught except for the allopatric Destin, FL population, which has been maintained in the lab from a wild caught population. The Amazon mollies used in tests of allopatric populations were lab reared from populations collected at Rio Tigre, Mexico (1989; 1998) while female sailfin mollies used in these trials originated from the same native population as the males. For sympatric trials, both native female sailfin and Amazon mollies were used when possible. However, for the Venus, Mexico and Martindale, Texas populations, native female sailfin mollies were unavailable and Aquarena Springs, Texas female sailfin mollies were used instead.

Fishes were transported by car to Texas State University at San Marcos, Texas, where they were maintained in a laboratory in 38 l aquaria ( $54 \times 29 \times 33$  cm) and 53 l aquaria ( $76 \times 32 \times 32$  cm). Fishes were maintained at a constant temperature ( $22$ – $25^\circ\text{C}$ ) and on a 14 L:10 D cycle with UV fluorescent lighting that simulates daylight (40 W Coralife Day-Max Aquarium daylight, 40 W Coralife Actinic Blue, 40 W Coralife 10,000 k high intensity purified super daylight, and 40 W General Electric). Fishes were fed Spirulina and Freshwater flake food (Ocean Star International Inc.) twice a day and supplemented with live and freeze-dried brine shrimp. Populations were housed separately and within each population we separated sex and species for at least 30 days before testing. The isolation of females from males for at least 30 days increases the chance that the females will be at a similar stage in the brood cycle. As females have a 30-day ovarian cycle, most females will have dropped any broods that they may have been carrying (Farr and Travis 1986; Snelson et al. 1986; but see Hubbs and Dries (2002) for variation in interbrood interval). Only mature males, identified by the fusion of the anal fin into the gonopodium, were used in trials.

#### Mating trials

Mating trials were conducted in a 38 l aquarium ( $54 \times 29 \times 33$  cm) that contained tan gravel and 24 cm of aerated and filtered water. A 15 W Sun Glo full spectrum fluorescent light (General Electric) was placed directly on top of the aquarium. Three sides were covered with black plastic to prevent test fishes 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 fishes during observation. All fishes were fed prior to testing. We conducted trials from 0900–1500 h, 17 June 2002 to 30 September 2002 and 25 April 2003 to 27 August 2003.

Mature males (mean  $\pm$  SE = 32.76 $\pm$ 0.62 mm; range = 20.9–54.0 mm) were selected haphazardly as test fish. Testing was performed as in Gabor and Ryan (2001) except in each trial, Amazon mollies (mean  $\pm$  SE = 47.16  $\pm$  0.52 mm) were at least 10 mm SL  $\geq$  female sailfin mollies (mean  $\pm$  SE = 33.75  $\pm$  0.35 mm) whereas Gabor and Ryan (2001) used size-matched females. For each trial, one male sailfin molly was first placed under a clear plastic cylinder (12 cm diameter  $\times$  30 cm) in the middle of the aquarium. Females (one sailfin molly, one Amazon molly) were simultaneously placed in the aquarium and allowed to swim freely. Stimulus females may have been used in more than one trial when there were limited numbers of females in a population. However, stimulus females were not paired together more than once and all females were visually inspected to further increase the chances of having similar brood stages. After a 10 min habituation period and careful removal of the plastic cylinder, we recorded the number of gonopodial thrusts that males directed towards each female for 10 min after the first gonopodial thrust. If males performed less than five gonopodial thrusts within the 10 min period, or did not thrust at all within 30 min, they were returned to their home tank to be re-tested in the future. Recording the number of gonopodial thrusts is important as these are actual mating attempts by males. We use this method because it has been found that for sailfin mollies, recording the time spent with a female may not indicate mate preference (Gabor 1999; but for exceptions in other poeciliids see Bisazza et al. 1989; Herdman et al. 2004). One caveat of this method is that females also interact with each other (Foran and Ryan 1994). As with Gabor and Ryan (2001) we rarely observed aggressive interactions between females. In addition, this design more accurately reflects natural conditions in which males and females interact with one another. The outcome of male choice in nature will be affected by female interactions.

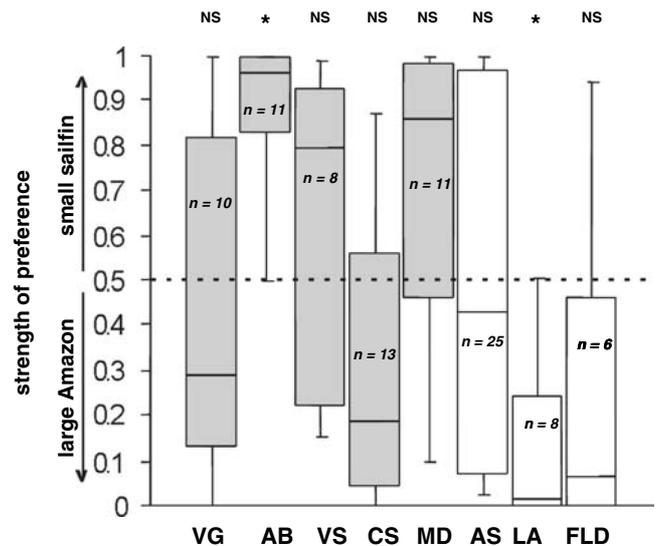
### Statistical analysis

We used a two-tailed Wilcoxon signed-rank test to compare the number of gonopodial thrusts directed toward conspecific and heterospecific females within populations. We then calculated the strength of preference for each male as the proportion of the total gonopodial thrusts directed towards sailfin mollies. A Mann-Whitney  $U$  test was used to compare the strength of preference of all males from all sympatric and from all allopatric populations. This test was also used with the pooled median strength of preference for each population because it could be argued that each population is a data point and not each individual within a population. Non-parametric tests were used because our data were non-normally distributed after all attempts at transformation. Sample sizes vary for a few reasons: (1) we could not collect the same number of males from each population, (2) not all males responded, and (3) some males died in the lab before testing. All analyses were two-tailed with  $\alpha=0.05$ .

## Results

Males from four sympatric populations (Vincente Guerrero, Mexico; Venus, Mexico; Martindale, Texas; and Comal Springs, Texas) showed no significant preference for either conspecific or heterospecific females (Table 1; Fig. 2). Males from the naturally sympatric Alfred Bonfil, Mexico population directed significantly more thrusts towards conspecific females than towards heterospecifics (Table 1; Fig. 2). Males from two of the three allopatric populations (Aquarena Springs, Texas and Destin, Florida) showed no significant mating preference for heterospecifics or conspecific females (Table 1; Fig. 2). Males from the allopatric population from Lafayette, Louisiana, however, directed significantly more thrusts towards heterospecific females than towards conspecific females (Table 1; Fig. 2).

When the strength of preference of all males from the allopatric and the sympatric populations were pooled, males from allopatry showed a significantly greater strength of preference for heterospecifics than did males from the sympatric populations ( $n_{\text{allopatry}} = 40$  (mean  $\pm$  SE =



**Fig. 2** Box plots representing male *Poecilia latipinna* strength of preference estimated by the number of gonopodial thrusts directed towards female *P. latipinna* divided by the total number of gonopodial thrusts when the SL of *P. formosa* are  $\geq 10$  mm than female *P. latipinna*. The middle horizontal lines represent the medians for each population and the upper and lower lines of the boxes represent the first and third quartiles. The whiskers represent the range (Sokal and Rohlf 1995). Sample sizes are as indicated in Table 1. Population designations are as follows: VG = Vicente Guerrero, MX; AB = Alfred Bonfil, MX; VS = Venus, MX; CS = Comal Springs, TX; MD = Martindale, TX; AS = Aquarena Springs, TX; LA = Lafayette, LA; FLD = Destin, FL. Significance levels correspond to  $P$ -values from Wilcoxon signed-rank tests comparing the number of thrusts directed at sailfin molly and Amazon molly females: \*= $P < 0.05$ ; n.s. =  $P \geq 0.05$ . Data above the dashed no-preference line ( $y=0.5$ ) indicate increasing strength of preference for female sailfin mollies, data below the line indicate an increasing preference for Amazon mollies. Shaded populations indicate sympatry and white populations indicate allopatry. The left-most population is the population in 'deepest sympatry.' Populations to the right appear in order of increasing distance from deepest sympatry

**Table 1** Comparison of gonopodial thrusts among populations of male *Poecilia latipinna* as directed at female *P. latipinna* and *P. formosa*. Results of Wilcoxon signed-rank tests comparing mean number of thrusts directed at female sailfin mollies, *P. latipinna*, and Amazon mollies, *P. formosa*, for males of each population tested

Population		Mean $\pm$ SE number of thrusts directed at		<i>n</i>	<i>Z</i>	<i>T+</i>	<i>P</i>
		<i>P. latipinna</i>	<i>P. formosa</i>				
Sympatry	Vincente Guerrero, MX	22.20 $\pm$ 14.34	6.80 $\pm$ 3.07	10	-0.05	27	0.95
	Alfred Bonfil, MX	48.46 $\pm$ 13.60	8.27 $\pm$ 5.22	11	-2.31	59	<b>0.02</b>
	Venus, MX	31.44 $\pm$ 13.04	11.00 $\pm$ 4.07	8	-0.14	26	0.88
	Comal Springs, TX	21.62 $\pm$ 8.76	35.39 $\pm$ 11.70	13	-1.50	50	0.13
	Martindale, TX	76.55 $\pm$ 21.89	37.27 $\pm$ 12.48	11	-1.51	24	0.13
Allopatry	Aquarena Springs, TX	30.96 $\pm$ 8.78	31.96 $\pm$ 8.68	25	-0.03	163.5	0.98
	Lafayette, LA	8.75 $\pm$ 7.38	51.88 $\pm$ 11.61	8	-2.10	4	<b>0.03</b>
	Destin, FL	9.00 $\pm$ 4.55	29.50 $\pm$ 9.43	6	-1.57	3	0.11

0.38 $\pm$ 0.06);  $n_{\text{sympatry}} = 55$  (mean  $\pm$  SE = 0.58 $\pm$ 0.05); Mann-Whitney  $U = 795$ ,  $n=95$ ,  $P=0.0296$ ). However, there was no significant difference in the median strength of preference between the allopatric and the sympatric populations (median<sub>allopatry</sub> = 0.16, median<sub>sympatry</sub> = 0.68; Mann-Whitney  $U=13$ ,  $n_{\text{allopatry}} = 3$ ,  $n_{\text{sympatry}} = 5$ ,  $P=0.1011$ ).

## Discussion

Results of this study, when viewed in conjunction with previous studies, provide evidence for a conflict in mate quality and species recognition in male sailfin mollies. Although a conspecific preference has previously been shown across sympatric populations (Hubbs 1964; Ryan et al. 1996; Gabor and Ryan 2001; but for variation in male preferences see Balsano et al. 1985; Woodhead and Armstrong 1985; Schlupp et al. 1991), in our study male sailfin mollies from sympatry showed no significant preference for conspecific females, except in one population (Alfred Bonfil, Mexico; Fig. 2). Males in allopatry also showed no significant conspecific mating preference, and one population (Lafayette, Louisiana; Fig. 2) showed a significant heterospecific mating preference. Our results are especially interesting when compared to trials of mollies from populations in the same general geographic regions as ours using size-matched females (Gabor and Ryan 2001). Whereas Gabor and Ryan (2001) found that all six of their populations from sympatry showed a conspecific preference when the female sailfin and Amazon mollies were size-matched, only one of our sympatric populations showed a conspecific preference when female Amazon mollies were larger than sailfin mollies (Fig. 2). Further, Gabor and Ryan (2001) found that three of five allopatric populations also showed a significant conspecific preference, while none of our three allopatric populations demonstrated significant conspecific preferences with males from one allopatric population showing a significant preference for larger heterospecifics (Fig. 2).

Our results support the prediction from Pfennig (1998) that when high-quality conspecifics resemble heterospecifics there should be no significant mating preferences when given a choice between a conspecific and a heterospecific, indicating a conflict in species and mate-

quality recognition (Pfennig 1998). The data may also support a second prediction from Pfennig (1998) that there is a difference in the use of species recognition versus mate-quality recognition between sympatric and allopatric populations. When the data from all males from sympatric populations and all males from allopatric populations are pooled, males from sympatry show a significantly greater strength of preference for conspecifics than males from allopatry. However, when the median strength of preference for each population is compared there is no significant difference between sympatric and allopatric populations. This second analysis, however, is limited by the small sample size of total number of populations.

Our results are similar to those found for flat lizards (*Platysaurus spp.*). Wymann and Whiting (2003) found that the allopatric species *Platysaurus broadleyi* and *P. capensis* use size as a mate-quality cue and exhibited a loss of conspecific preference when presented with a larger heterospecific female. Whereas, Pfennig (2000) found that female *S. multiplicata* in sympatric populations compromise potential mate-quality benefits to ensure conspecific matings.

An alternative explanation of our results is that larger Amazon mollies are more aggressive than smaller female sailfin mollies and are thus able to exclude female sailfin mollies from males. However, female sailfin mollies are significantly more aggressive than Amazon mollies in the presence of a male (Foran and Ryan 1994). In addition, in this study we only once observed the behaviors Foran and Ryan (1994) described as aggressive performed by females of either species in our trials. While it is still possible that female interactions may affect our results, we feel that this is an aspect of the natural conditions that can influence male-mating mistakes. Another explanation of our results could be due to small sample sizes. However, we view our results as reliable as our sample sizes are similar to those of other studies concerning male-mating behavior in this species (Ryan et al. 1996; Gabor and Ryan 2001). A final alternative explanation for our lack of population level preferences may be that males within a population express preferences that differ based on their phenotype. In male sailfin mollies, size is correlated with sexual behaviors. As male size increases, so do rates of courtship while rates of forced insemination attempts decrease (Farr

et al. 1986). Woodhead and Armstrong (1985) indicated that smaller males were more likely to mate with Amazon mollies. However, in our study, we found that male size was not associated with male strength of mating preference (linear regression,  $r^2=0.008$ ,  $n=95$ ,  $P=0.3928$ ); therefore, the relative importance of species and mate-quality recognition does not appear to differ for males of different sizes.

Gabor and Ryan (2001) found similar strengths of preference for conspecific females as Ryan et al. (1996) and suggested that male preferences have remained relatively constant over time across populations. However, compared to the previous work by Ryan et al. (1996) and Gabor and Ryan (2001) using size-matched females, our study demonstrates a lack of clear male-mating preferences. This indicates that a change in male-mating preferences for conspecifics in most populations is a result of the treatment where Amazon mollies were larger than the female sailfin mollies.

We tested populations that are the same (AS, LA, and FLD) or near to the original geographic regions as used by Gabor and Ryan (2001). In Gabor and Ryan (2001) the allopatric populations of AS and LA showed a significant conspecific preference, where as in this study they did not and LA actually showed a strong heterospecific preference. In our study, FLD lost its heterospecific preference as seen previously but the results from Gabor and Ryan (2001) were unexpected. Gabor and Ryan (2001) did not publish their results for CS because they tested this population after submitting the manuscript. They found that males from CS thrusted towards female sailfin mollies significantly more than towards Amazon mollies ( $n=9$ ;  $P=0.01$ ). In our current results, the sympatric population Vincente Guerrero no longer shows a conspecific preference when compared to VR. The same pattern is seen with our Venus population in comparison to the VH population from Gabor and Ryan (2001). Similar to EM, our results for Alfred Bonfil still show strong conspecific preferences.

The strongest demonstration of no preference among the allopatric populations occurred in the population with the largest sample size ( $n=25$ ). A different allopatric population had much smaller sizes ( $n=8$ ), and statistically significant preferences for heterospecific females were detected. Even though we typically assume that detected significance at a small sample size would translate into a greater significance at a larger sample size, it may not be so in this context. It is possible that individual males vary in the strength of their preferences, or preference of each male is context dependent. We can not examine this with our data, but if this is true, then it is possible that large sample sizes may not reveal strong conspecific preference, but could demonstrate no preference in this context.

Though most populations lack significant mating preferences when the heterospecific female was larger, two populations showed significant preferences in the expected directions based on our predictions. The Alfred Bonfil, Mexico site, which is similar to the EM site in Gabor and Ryan (2001) which previously showed a strong conspecific preference. This population is deep within sympatry and therefore close to the origin of Amazon mollies (Fig. 2), these

males preferred small conspecifics to larger heterospecifics unlike males from all other populations. Thus, males from this population may sacrifice their emphasis on mate quality for conspecific matings. These males may avoid larger females and consequently avoid mating with heterospecifics. In contrast, by preferring to mate with larger heterospecifics to conspecific females, males from the geographically distant allopatric site in Louisiana may be emphasizing mate-quality recognition over species recognition.

When a conflict in species recognition and mate-quality recognition occurs, it may be resolved in different ways depending on the relative importance of both. The resolution will depend on the costs of mating with heterospecifics and the frequency in which heterospecifics are encountered (Pfennig 1998, 2000). There may be a compromise of one type of cue for another but this is not a long-term solution. Selection will ultimately favor individuals that minimize recognition mistakes such as mating with heterospecifics and/or mating with low-quality mates (Ryan et al. 2003) especially when sperm is limited as it is in sailfin mollies (Aspbury and Gabor 2004). One outcome of such selection may yield traits used for species recognition differing from those used for mate-quality recognition. In this unisexual–bisexual species complex of mollies, we expect high-quality sailfin females to produce species recognition cues that are distinct from Amazon mollies. However, Dries (2003) suggests that Amazon mollies may garner matings due to the genes they share with sailfin mollies as a result of their hybrid origin. This may limit the amount of morphological divergence that can occur between the species due to their shared ancestry. Thus, males may require alternative mechanisms to discriminate between females. Another evolutionary result of the conflict in cues may be for males to assess multiple species-specific cues, where different cues give different information about mate quality (Candolin 2003). Thus, male recognition of high-quality females would increase while avoiding the risk of mating with a heterospecific (Pfennig 1998; Hankison and Morris 2003).

Male sailfin mollies are likely to be under strong selection to resolve this conflict in species and mate-quality recognition based on the frequency of unisexuals and bisexuals in sympatric populations (Fig. 1). Frequency hypotheses have been modeled for the unisexual–bisexual *Poeciliopsis* complex and for *P. formosa* with their *P. mexicana* host (Moore and McKay 1971; Kawecki 1988). For both complexes, a density dependant mechanism may regulate the ratio of unisexuals to bisexuals. Thus, in populations with high proportions of unisexuals and high densities of males, there will be high proportions of copulations with unisexuals (Moore and McKay 1971; Kawecki 1988). If a similar mechanism exists for the *P. formosa*/*P. latipinna* complex, males sailfin mollies will often encounter Amazon mollies and therefore often face this conflict between species and mate-quality recognition. This data still needs to be collected.

In this experiment, we examined male preference for conspecific and heterospecific females when the heterospecific females were larger. Naturally occurring variation in female size may be influenced by many factors: (1) Amazons that are larger in size may be older as both Amazons and female

sailfin mollies continue to grow after reaching maturity. (2) Foraging behaviors and interspecific competition may influence female size. (3) Amazon mollies may have a faster growth rate, allowing them to grow larger throughout their lifespan than female sailfin mollies and thus, fully exploit male preference for large size. However, we do not currently have the data needed to differentiate between these hypotheses.

Size is widely regarded as indicating mate quality in both males and females (review in Andersson 1994) although it may also be important to consider conflicts involving mate-quality recognition other than size. Thus far, no study has identified what cues, other than size, male sailfin mollies use for identifying high-quality mates or for identifying conspecifics. There is some evidence that female sailfin mollies are, on average, smaller in sympatric populations than in allopatric populations (Gabor unpublished data). Thus, males from sympatry would be expected to recognize smaller females as conspecifics as they have in the sympatric population in Alfred Bonfil, Mexico. These males may be using small size as a species recognition cue and thus avoid large Amazon mollies. Amazon mollies would alternatively be expected to evolve to become bigger than sailfin mollies if large size increases their mating success. Alternatively, other selection forces may work against large size. For example, larger fish are prone to greater predation pressures (Trexler et al. 1994). Finally, Amazon mollies may be unable to evolve a larger average size than sailfin mollies due to morphological constraints as a consequence of hybrid origin or due to the slow rate of evolution in clonal species (Dries 2003).

In conclusion, we found evidence for a conflict in species recognition and mate-quality recognition used by male sailfin mollies in both sympatry and allopatry. This conflict may affect the evolution of male preferences for conspecific mates, sexually selected characters, and may lead to important evolutionary differences between sympatric and allopatric populations. Understanding male preferences and female cues will lead to a better understanding of mate choice processes and may help to explain the persistence of unisexual, gynogenetic Amazon mollies as they may be exploiting this conflict and thus garner necessary matings.

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