Male mate preferences in a gynogenetic species complex of Amazon mollies

MICHAEL J. RYAN, LAURIE A. DRIES, PUJA BATRA & DAVID M. HILLIS

Department of Zoology, University of Texas

Abstract. Female Amazon mollies, *Poecilia formosa*, are gynogenetic and mate with males of a sexual species, *P. latipinna* or *P. mexicana*, for successful reproduction. It was found that both species of males are able to distinguish between conspecific females and Amazon mollies, and preferentially mate with the former. Male mate preference per se is not an evolved response to avoid mating with Amazon mollies; male *P. latipinna* that are from populations currently allopatric with Amazon mollies also prefer conspecifics. The strength of this preference, however, is stronger in *P. latipinna* from populations sympatric with Amazon mollies than in *P. latipinna* from populations that are allopatric with the gynogen, suggesting that reproductive character displacement in mating preferences has occurred. Male size did not influence mating decisions; thus, the hypothesis was rejected that the gynogens are usually mated by smaller, younger males, which have yet to learn to discriminate between females. Unlike some previous studies, it was shown that *P. mexicana* can discriminate between conspecific females and Amazon mollies, although the strength of preference might be weaker in *P. mexicana* than in *P. latipinna*. Male *P. latipinna* and *P. mexicana* differed in their choices between females of the two heterospecific species in the complex. Male *P. latipinna* were reluctant to mate at all when given a choice between the two heterospecific females, *P. mexicana* and *P. formosa*. *Poecilia mexicana* males, alternatively, showed high mate attraction to *P. formosa* when these females were paired with *P. latipinna* females. This result is intriguing, given that it is thought that female *P. mexicana* and male *P. latipinna* were involved in the original hybridization event that gave rise to *P. formosa*. Therefore, the strong attractiveness of *P. formosa* to *P. mexicana* may be related to a maternally derived genetic component.

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Trivers (1972) indicated that females should be more discriminating than males in mate choice owing to differences in parental investment. This might be especially true in discriminating between conspecifics and heterospecifics, a situation in which the relative costs of discrimination error (i.e. a heterospecific mating) will be much higher for females than males (Dobzhansky 1937; Andersson 1994; Wiley 1994). Selection against heterospecific matings is sometimes thought to result in reproductive character displacement in courtship signals in areas of sympatry (e.g. Blair 1974; Coyne & Orr 1989), but might also result in displacement of mating preferences (Gerhardt 1994).

Although less common, male mate choice occurs (Andersson 1994), usually when males

Correspondence: M. J. Ryan, Department of Zoology, University of Texas, Austin, TX 78712, U.S.A. (email: mryan@mail.utexas.edu).

have greater than typical parental investment (e.g. pipefish, *Nerophis ophidion*: Rosenqvist 1990; jacanas, *Jacana spinosa*: Jenni 1974) or when there is considerable, often size-related variation in female fecundity (e.g. weevils, *Brentus anchorago*: Johnson 1982).

A reproductive system in which male mate choice should play a pivotal role is gynogenesis. Gynogenetic species are all-female and reproduction is clonal, but females must mate with males of closely related species, because the male's sperm provides the stimulus necessary for initiation of embryogenesis. One such system that has been the focus of a variety of studies is that of the Amazon molly, *Poecilia formosa*. First described by Hubbs & Hubbs (1932), the Amazon molly is a gynogen that reproduces by apomixis and is dependent on the sperm of males of either *P. latipinna* or *P. mexicana* (reviewed in Balsano et al. 1989). Molecular analysis suggests that *P. formosa* was

derived from a hybridization event between *P. latipinna* and *P. mexicana* (Balsano et al. 1989), with the maternal contribution from *P. mexicana* (Avise et al. 1991; Schartl et al. 1995).

Poecilia formosa ranges along the eastern coast of northern Mexico into southern-most Texas. It also has been introduced into a number of sites in central Texas. *P. formosa* is sympatric with *P. mexicana* in most inland sites in Mexico, and is sympatric with *P. latipinna* along the Gulf coast in northern Mexico, in the Rio Grande Valley and in introduced populations elsewhere throughout the rest of its range in Texas. In some Mexican sites, all three species occur, as well as some triploid gynogenetic forms. Both of the sexual species have populations that are allopatric to *P. formosa*; *P. latipinna*, for example, extends along the Gulf coast into Florida and north along the eastern seaboard to southern Virginia.

Considerable attention has been given to the ability of male mollies to discriminate between conspecific females and Amazons. Previous studies of male mate choice in these mollies have suggested that male *P. latipinna* readily favour mating with the conspecific over the gynogen, with the suggestion that the strength of discrimination is stronger in sympatry than in allopatry (Hubbs 1964). It has further been suggested that the small *P. latipinna* males are more likely to mate with *P. formosa*, either because these males are younger and have yet to learn how to discriminate, or because they are subordinates in a dominance hierarchy (Woodhead & Armstrong 1985). Alternatively, other studies have suggested that *P. mexicana* males are not able to discriminate between conspecific females and Amazons in all (Balsano et al. 1985) or some situations (Schlupp et al. 1991).

The purpose of our study was to examine male mate choice in both species of males, and in males from populations that are allopatric and sympatric with *P. latipinna*, under a consistent experimental paradigm to more readily facilitate comparisons between species and localities. We also examined the male's ability to discriminate between females of the two heterospecifics in the species complex.

We investigated heterospecific preferences for two reasons. In some localities all three species are sympatric: thus discrimination between both heterospecifics might be a task that confronts males in nature. More interesting, perhaps, is the peculiar genetic relationship of the gynogen to the sexual species. *Poecilia formosa* is genetically more similar to each of the purported parental sexual species than each of those sexual species are to each other. The relationship of the gynogen to each of the sexual species differs, however, since the maternal contribution in the initial hybridization event is known to be from *P. mexicana*. If the relative attractiveness of a heterospecific is merely related to the overall genetic similarity of the two species, we predicted that, for both *P. latipinna* and *P. mexicana*, their strength of attraction to *P. formosa* would be intermediate to their attraction to the conspecific and heterospecific. If the attraction of a heterospecific is maternally linked, however, then *P. latipinna* should show the same low levels of response to *P. mexicana* and *P. formosa*, but *P. mexicana* males should find *P. formosa* females relatively attractive.

We addressed the following questions: do males of both sexual species discriminate between conspecific females and gynogens? Is discrimination stronger between males from populations sympatric with the gynogens, suggesting reproductive character displacement of mating preferences? Are small males more likely to mate with gynogens? Does the maternal component of the Amazons influence their attractiveness to males?

METHODS

Localities

Fish were collected in the field from populations in which the sexual species, *P. latipinna* and *P. mexicana*, were either sympatric or allopatric with *P. formosa*. For ease of description, we will refer to males from populations allopatric with *P. formosa* as simply 'allopatric' and males from populations sympatric with *P. formosa* as 'sympatric'. Fish were transported to our laboratory in Austin, Texas for use in the behavioural studies. Some fish were collected and then maintained in large artificial ponds at the Brackenridge Field Laboratory in Austin, Texas, prior to use. Either these adults or their offspring were tested.

Poecilia latipinna and *P. formosa* were collected from sites of sympatry in Brownsville, Texas (Table I). *Poecilia formosa* were also collected in San Marcos, Texas; this species was introduced to central Texas in 1955 from Brownsville (Hubbs

Table I. The species, origins of males and females, and sizes of females used in the mate discrimination experiments

Male		Female			*t*	*N*	*P*	Female			Size (>3 mm)	
Species	Locale	Species	Locale	Mean (SE) standard length				Species	Locale	Mean (SE) standard length	Female 1	Female 2
latipinna	BR, TX	*latipinna*	BR, TX	32.5 (0.55)	−2.35	9	0.047	*formosa*	BR, TX	36.0 (1.6)	0	3
latipinna(a)	FL	*latipinna*	FL	47.0 (1.76)	−1.79	9	0.11	*formosa*	SM, TX	49.0 (1.4)	1	3
latipinna(b)	GV, TX	*latipinna*	BR, TX	32.4 (0.64)	−1.87	7	0.11	*formosa*	BR, TX	33.8 (0.54)	0	1
latipinna(c)	FL	*latipinna*	BR, TX	39.9 (1.0)	−2.65	11	0.024	*formosa*	SM, TX	42.7 (0.9)	0	7
mexicana	MX	*latipinna*	BR, TX	40.5 (1.27)	−0.70	10	0.50	*formosa*	SM, TX	40.7 (1.33)	0	0
latipinna	BR, TX	*mexicana*	MX	44.0 (1.79)	0.50	10	0.632	*formosa*	SM, TX	43.8 (1.78)	0	0
mexicana	MX	*mexicana*	MX	42.9 (1.9)	−0.22	14	0.084	*formosa*	SM, TX	43.1 (1.61)	2	4

BR, TX: Brownsville, Texas, sympatric population; GV, TX: Galveston, Texas, allopatric population; FL: Florida Keys, Florida, allopatric population; MX: Tamaulipas, Mexico and San Luis Potosi, Mexico, sympatric population; SM, TX: San Marcos, Texas, sympatric population. Also presented is a statistical comparison of the size of the two species of females used in each experiment, by a Student's *t*-test, and a summary indicating the number of tests in which female 1 or female 2 was the larger (>3 mm) of the pair. The letters in parentheses following *latipinna* refer to the results presented in Fig. 2.

1964). We collected *P. latipinna* from populations that were allopatric with *P. formosa* from two sites: Galveston, Texas, which is about 550 km from the northern-most naturally occurring population of the range of *P. formosa*, and from the Florida Keys, where populations are much further from any known *P. formosa* populations (Table I). The preference for the conspecific versus gynogen was not confounded by the preference of familiar versus unfamiliar females, because in some tests the male and female *P. latipinna* were from different sites (Florida and Texas; Table I). We also tested male *P. latipinna* from Florida, an allopatric site, with conspecific females from the same population versus *P. formosa*; in this experiment, choice between conspecifics and gynogens was confounded with familiar versus unfamiliar females, but the male responses did not differ from the other tests with allopatric males (see below).

Poecilia mexicana were collected from the Rio Coy in the state of San Luis Potosi, Mexico, and Rio Tigre at Aldama, Tamaulipas, Mexico where they are sympatric with *P. formosa*.

Size and Condition of Fish

Some evidence suggests that different levels of receptivity of females, estimated by the number of days since a brood was last dropped, can affect male preferences (Schlupp et al. 1991; Sumner & Johnson 1994). We attempt to standardize female condition by isolating females from males for at least 30 days prior to testing. Since inter-brood interval in these fish is about 1 month, although females store sperm, the isolation ensured that most females had not been inseminated since their last brood and presumably had similar levels of receptivity. In tests with females subject to the same treatment, Foran & Ryan (1994) showed that females of *P. latipinna* and *P. formosa* aggressively interacted for access to males, indicating that under such treatment females were motivated to mate. Males also were isolated from females for a similar period prior to experimentation.

Female size also can influence male mating preferences; males prefer larger females (Bergland et al. 1986). We attempted to match females for size; i.e. within 1 mm standard length, whenever possible. When the females differed in size, the gynogenetic female was chosen to be the larger of the pair. Comparisons of female standard length and the results of the preference tests (Table I; Figs 1–3) show that any size difference between stimulus females did not confound male mating preferences.

Male Mate Choice Experiments

We conducted mate choice experiments in 19-litre aquaria divided into three sections of equal size by clear glass plates that allowed for some water flow throughout the aquarium. We placed the male in the centre section and a female on either side. The fish acclimated for 7 days, during which time they were fed Tetramin flake food. We initiated an experiment by removing the dividers that separated the male from the females. We used mating attempts, that is gonopodial thrusts, as the bioassay for male mate preference. All fish were used only once. The fish were observed for 15 min, during which time the number of gonopodial thrusts by the male to each female was counted.

We included in our analyses only those males who gave more than five total thrusts; males giving fewer thrusts were judged to be unmotivated to mate. This criterion was based on examining the frequency distribution of gonopodial thrusts for all experiments which revealed a large discontinuity in the distribution at five thrusts. An exception is in the experiments in which male *P. latipinna* were given a choice between two heterospecific females, when most males gave few thrusts. These males were then immediately presented with two conspecific females and the average number of thrusts was determined. If a male gave more than five thrusts to conspecific females, we assumed that his lack of interest in mating with heterospecifics was due to lack of a sufficient stimulus rather than lack of motivation.

Statistical Analysis

Data were analysed with non-parametric statistics. Male mate preference was assessed by comparing the number of gonopodial thrusts to each species of female using a Wilcoxon signed-ranks test. We compared the strengths of preference between experiments by comparing the proportion of total thrusts directed towards *P. formosa* with a Mann–Whitney *U*-test or Kruskal–Wallis test. We determined whether male
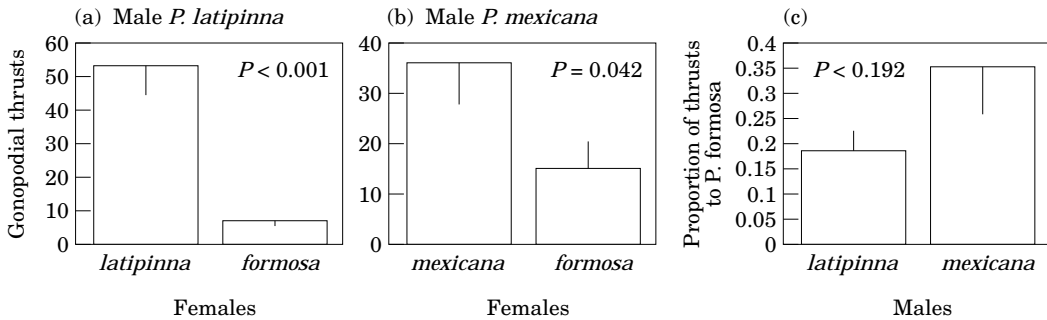
Figure 1. Results of male mate discrimination tests showing the mean+SE number of gonopodial thrusts directed to the conspecific and gynogenetic female. Probabilities in (a) and (b) are one-tailed probabilities of a Wilcoxon signed-ranks test. (c) Presents a two-tailed probability from a Mann–Whitney U-test. (a) The number of thrusts by male *P. latipinna* directed to females of *P. latipinna* and *P. formosa*. (b) The number of thrusts by male *P. mexicana* to females of *P. mexicana* and *P. formosa*. (c) The proportion of total thrusts directed to *P. formosa* by males of *P. latipinna* and *P. mexicana*.

size influenced mate preference by determining the Spearman rank correlation coefficient for size and strength of preference.

We used one-tailed probabilities in testing for significant differences in the preference for conspecific versus heterospecific females, since there is a clear a priori prediction for the direction of the difference. For all other tests, we used two-tailed probabilities, although it could be argued that a one-tailed test is more appropriate for comparing the strength of preference between male *P. latipinna* that are sympatric versus allopatric with *P. formosa*.
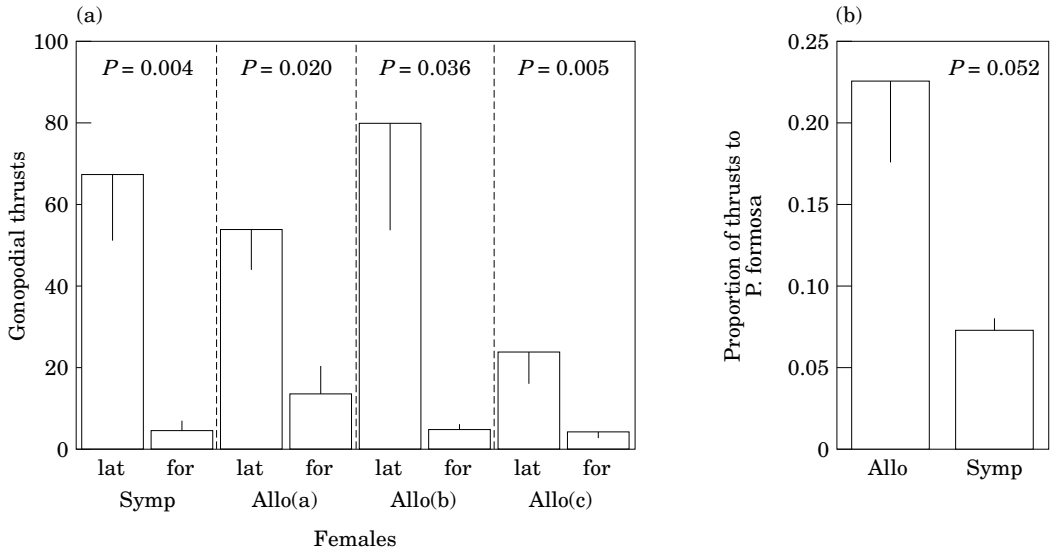
RESULTS

When the tests from all four experiments of *P. latipinna* are combined, males showed significant preference to attempt copulation with conspecific females over Amazons ($z=3.69$, $P<0.001$; Fig. 1). Male *P. mexicana* also preferred conspecific to Amazon females, although the preference was not as pronounced as in *P. latipinna* males ($z=1.73$, $P=0.042$; Fig. 1). Even though *P. latipinna* males appeared to have a stronger preference for conspecific females than did *P. mexicana* males, the two did not significantly differ in the proportion of thrusts directed to the *P. formosa*; that is, their strengths of preference were not significantly different (Mann–Whitney U-test$=182$, $P=0.129$; Fig. 1).

We compared the preference of each of the three populations of *P. latipinna* for conspecific versus Amazon females (Table I). Each population showed a significant preference for the conspecific (sympatric $z=2.67$, $P=0.004$; allopatric (a) $z=1.95$, $P=0.020$; allopatric (b) $z=1.86$, $P=0.036$; allopatric (c) $z=2.59$, $P=0.005$; Fig. 2). There was no difference between the males from allopatric sites in their strength of preference; i.e. in the proportion of thrusts directed toward Amazon females (Kruskal–Wallis $H=0.37$, $P=0.829$, two-tailed test). When comparing the strength of preference in the sympatric population to that of the combined results for the allopatric populations, however, there was a significant difference. The conspecific preference of male *P. latipinna* from populations sympatric with *P. formosa* was stronger than the conspecific preference of *P. latipinna* males from populations allopatric with *P. formosa* ($U=174$, $P=0.052$, two-tailed test; Fig. 2).

It had been suggested that in the *P. latipinna*–*P. formosa* association, *P. formosa* are more likely to mate with small males (Woodhead & Armstrong 1985). In examining the data from the experiments with sympatric *P. latipinna* males, the hypothesis of a negative relationship between male size and the proportion of thrusts directed to the gynogen is rejected (Spearman rank $r_S=0.55$, $N=9$, $P>0.05$). We also tested this hypothesis using the combined data for all allopatric *P. latipinna*, because these populations did not show significant differences in the strength of preference. Combining the data from allopatric males provides a larger sample size, thus
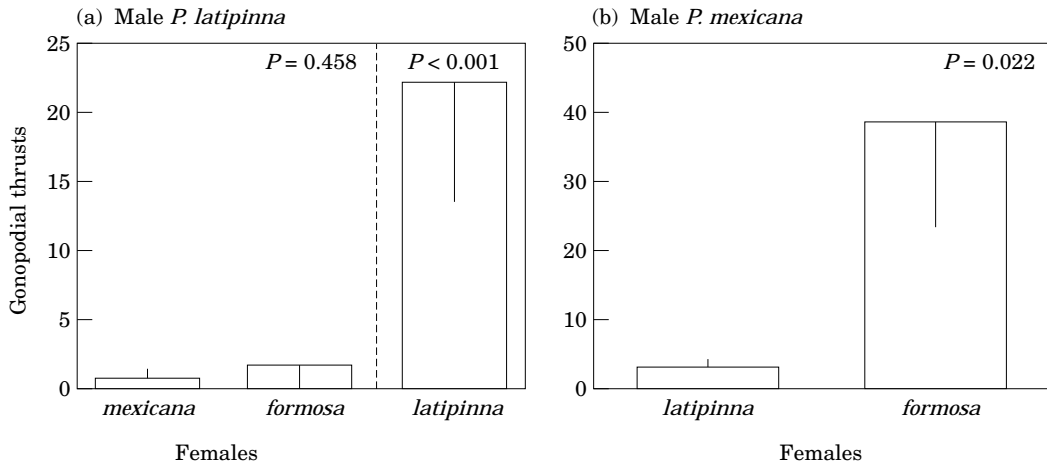
(a)

(b)

Figure 2. Results of male mate discrimination tests showing the mean ± SE number of gonopodial thrusts directed to the conspecific and gynogenetic female. Probabilities in (a) are one-tailed probabilities of a Wilcoxon signed-ranks test. (b) presents a two-tailed probability from a Mann–Whitney *U*-test. (a) The number of thrusts by male *P. latipinna* directed to females of *P. latipinna* and *P. formosa* for four populations. In one population males are sympatric (symp) with *P. formosa*; in three populations they are allopatric (allo). The letter following 'allo' indicates the origins of the fish used, as indicated in Table I; 'lat' is *P. latipinna* and 'for' is *P. formosa*. (b) The proportion of total thrusts directed to *P. formosa* by males of *P. latipinna* that are sympatric or allopatric with *P. formosa*.

reducing the probability of a Type II error, although it could be argued that the hypothesis only makes predictions for males from populations sympatric with *P. formosa*. Nevertheless, there was no significant relationship between male size and the strength of preferences as estimated by the proportion of thrusts directed towards female *P. formosa* (r_S=0.122, N=27, P<0.05).

Male *P. latipinna* and *P. mexicana* did not show similar preferences when confronted with the two heterospecifics. *Poecilia latipinna* did not discriminate between the two species, which appeared to be due to a lack of the males' interest in females of either species (z=0.73, P=0.458; Fig. 3). In these experiments, data were used only if males subsequently gave at least five thrusts to a conspecific female, our minimum criterion for male responsiveness. Males gave significantly fewer total thrusts in the experiments with the two heterospecific females (2.37 ± 1.18, N=8) than they did in the experiments with one conspecific and one heterospecific female (59.19 ± 7.8, N=36; U=284,

P<0.001; Fig. 3). Thus, the male's lack of interest appears to be due to a lack of a sufficient stimulus (i.e. female) rather than lack of motivation. These results contrast with the responses of male *P. mexicana* in the analogous experiments. *Poecilia mexicana* males were responsive to the heterospecific females, and thus the controls used in *P. latipinna* to test for motivation were not necessary. *Poecilia mexicana* males gave an average of 41.8 ± 15.5 total gonopodial thrusts (N=10), which was not significantly different than the average number of total thrusts in experiments with one conspecific and one heterospecific female (51.4 ± 8.06, N=14; U=94, P=0.151). *Poecilia mexicana* males preferred *P. formosa* (50.57 ± 21.0, N=7) to *P. latipinna* females (3.85 ± 2.24, N=7; z=2.29, P=0.022; Fig. 3).

The species preferences demonstrated here were not confounded by mate preference for larger females. In only two cases were the size differences between females statistically significant, and in both cases males preferred the smaller rather than the larger females (Table I).

Figure 3. Results of male mate discrimination tests showing the mean ± SE number of gonopodial thrusts directed to the sexual heterospecific versus the gynogenetic female. Probabilities are two-tailed probabilities of a Wilcoxon signed-ranks test. (a) The number of thrusts by male *P. latipinna* to females of *P. mexicana* and *P. formosa*. These same males were then tested with a female *P. latipinna*, and the number of gonopodial thrusts to the conspecific is shown. (b) The number of thrusts by male *P. mexicana* directed to females of *P. latipinna* and *P. formosa*.

DISCUSSION

Our results bear on three questions generally relevant to the evolution and function of mate recognition and, more specifically, to how sexual–asexual species complexes are maintained. First, we discuss the male's ability to discriminate between conspecific and heterospecific, and given this ability to discriminate, we ask how these mating systems might be maintained. Studies have examined male mate discrimination in this species complex of mollies with varied and sometimes contradictory results. To interpret our studies in the proper context, it is necessary to review these studies briefly. Second, we review our evidence suggesting that there has been reproductive character displacement in male mate discrimination. Third, we discuss the differences in responses of male *P. latipinna* and *P. mexicana* to sexual heterospecific and gynogenetic females relative to the hypothetical origin of *P. formosa*.

Male Mate Discrimination: Conspecific Versus Gynogen

Poecilia latipinna *mate preference*

Hubbs (1964) examined preferences of male *P. latipinna* for conspecific versus *P. formosa* females in an experimental paradigm similar to ours with similar results. He showed that males from sites that were both sympatric and allopatric with *P. formosa* preferred conspecific females to female *P. formosa*. Hubbs also suggested that the preference might be stronger between sympatric males than allopatric males but did not statistically compare the strength of preference. The responsiveness of the fish in Hubbs' study, as indicated by the number of gonopodial thrusts, was high and similar to the responsiveness shown in our studies. One of the purposes of the present study was to replicate the experiments of Hubbs, extend them to more allopatric populations and to experiments involving *P. mexicana*, and to do so with an experimental procedure that was duplicated, and thus comparable, with other studies of mate choice in the species complex.

Woodhead & Armstrong (1985) also examined the ability of *P. latipinna* (and *P. sphenops*) males to discriminate between conspecific and *P. formosa* females. Males were raised in conditions that were intended to mimic situations of sympatry and allopatry in order to assess the role of learning in the acquisition of male mating preferences. They assayed a variety of male mating behaviours and conducted experiments under conditions in which all fish were free-ranging, as in our study, and in which males had only visual access to females.

The effects of rearing conditions on mate preference are not entirely clear. Male *P. latipinna* raised with only *P. formosa* females had a stronger preference for conspecific females than did males raised with conspecifics alone or with conspecific and gynogenetic females. It is difficult to interpret the relevance of this result to the questions that we address here, but they do suggest that the role of early experience might be worthy of investigation.

Woodhead & Armstrong also examined mate preference as a function of male size in small groups of males with conspecific and gynogenetic females. Small males spent less time engaged in sexual activities than large males and were more likely to mate with gynogens than were larger males. Woodhead & Armstrong concluded that small males are younger and therefore have not yet learned to perfectly discriminate between the females. In contrast, our results do not show a relationship between the strength of preference of *P. latipinna* and male size.

We question Woodhead & Armstrong's interpretation of the role of male size in gynogenetic mating for several reasons. First, in several poeciliids male size can be influenced by variation at the pituitary locus due to its effect on the timing of sexual maturation (Kallman 1989). Since male poeciliids usually cease or at least drastically reduce growth at the onset of maturity, early maturing males are smaller but not necessarily younger than larger males; size of mature males does not predict age (e.g. Morris & Ryan 1990). Travis (1989) has argued for such an effect in *P. latipinna*. Second, the assessment of preference by Woodhead & Armstrong based on time spent in sexual activities includes time spent on courtship, which is usually a large male strategy. In many species of poeciliids (e.g. Ryan & Causey 1989), including *P. latipinna* (Travis & Woodward 1989), small males do not court but instead quickly chase after females in an attempt to force copulations. Thus the amount of time that small males devote to mating is less than that spent by large males because of their different mating strategies. Third, learning requires feedback. It is not clear how males could learn that they are mating with heterospecific females. Our results suggest that there is not an intrinsic bias in male preference for Amazon females related to male size, as implied by Woodhead & Armstrong. These authors, however, tested groups of males. It is possible that the size-based mating strategies

that occur in *P. latipinna* (Travis & Woodward 1989) could result in smaller males being more likely to mate indiscriminately when in competition with larger males.

Poecilia mexicana *mate preference*

Balsano et al. (1985) assessed mate preferences in recently field-caught male *P. mexicana* utilizing dichotomous choice tests with *P. mexicana* and *P. formosa* females as stimuli. They concluded that male *P. mexicana* were not able to discriminate between conspecific and gynogenetic females. This lack of discrimination, they suggested, was because of a skewed operational sex ratio: there were more males than receptive females at any one time. They also concluded that lack of discrimination is reinforced by a higher level of aggressiveness in unreceptive conspecific females than unreceptive gynogenetic females; that is, female access rather than male mate choice might have a greater influence on the probability of mating. The interaction between *P. mexicana* and *P. formosa* females is contrary to Foran & Ryan's (1994) study showing that *P. formosa* females were more aggressive than *P. latipinna* females.

In contrast to Balsano's study, our results show that male *P. mexicana* are able to discriminate between conspecific and gynogenetic females. The difference in our results and those of Balsano et al. (1985) might derive from several methodological differences. First, there is no indication that Balsano et al. attempted to standardize female receptivity. Second, their sample sizes are rather small (four males). Third, the males might not have been sufficiently motivated; the average number of thrusts to each female was less than 13, substantially smaller than the average in our tests.

Schlupp et al. (1991) showed that male *P. mexicana* can sometimes discriminate between conspecific and gynogenetic females. *Poecilia mexicana* males preferred conspecifics when only visual cues were available, but this preference could be confounded by the presence of chemical and tactile cues. These tests, however, were conducted using highly inbred lines and the number of gonopodial thrusts was quite small, suggesting poorly motivated males and thus accounting for low levels of discrimination.

Maintenance of the Asexual–Sexual Complex

Our results are consistent with those of Hubbs (1964), Woodhead & Armstrong (1985) and

Schlupp et al. (1991) in their demonstration that conditions exist under which male *P. latipinna* and *P. mexicana* can discriminate between their own conspecific females and females of the gynogenetic Amazon molly, *P. formosa*. This raises the question, however, of how the asexual–sexual species complex is maintained, since it requires males to mate with gynogens.

Conspecific male mating preferences predict that obtaining mates should be a problem for *P. formosa*, and the evidence supports this prediction. Hubbs (1964) showed that, although *P. latipinna* and *P. formosa* had the same rates of ovulation, *P. formosa* females were less likely to be gravid, more likely to be only partially gravid (have only some of the eggs fertilized) and had a smaller proportion of their total eggs fertilized. All of these results, as Hubbs indicates, are consistent with male preference for conspecifics. Balsano et al (1985) showed a similar but weaker pattern in *P. mexicana* and *P. formosa*. In some parts of the year, the reproductive output of the bisexual and unisexual females was similar, but when there were differences, *P. mexicana* were more likely to have fertilized eggs.

An underlying assumption to this paradox of maintaining gynogenesis is that males derive all costs and no benefits from mating with gynogens. This assumption, however, might be unwarranted. Schlupp et al. (1994) showed that there is mate copying in the *P. latipinna*–*P. formosa* complex. Laboratory studies show that when male *P. latipinna* mate with female *P. formosa*, they increase their attractiveness to conspecific females. Since these species occur in mixed schools, it is assumed that there is the opportunity for mate copying in nature. Thus, although males might incur a cost for mating with heterospecifics, there appears to be some potential for benefit as well. Whether these benefits more than outweigh the costs is not known.

Another recent study questions a major assumption about the asexual–sexual mollie species complex: that of no genetic exchange between males and gynogens. Schartl et al. (1995) demonstrated that subgenomic amounts of DNA are transmitted as microchromosomes from *P. mexicana* and *P. latipinna* into *P. formosa*. They argued that such genetic leakage might compensate for the mutational load that results from asexual reproduction (i.e. Muller's ratchet), and thus contributes to the continued mainten-

ance of the gynogens. For this mechanism to operate across all genes, each portion of the genome would have to be occasionally transmitted in microchromosomes, a possibility that has not been demonstrated. It should also be clear that this occasional genetic exchange between males and gynogens will not alleviate the cost of mating with heterospecifics. From the point of view of a selfish gene, this might appear to be true, but such genetic exchange does not increase the fitness of these males if fitness is measured as the relative production of *conspecific* descendants (see also Schlupp et al. 1991).

Although several studies have now shown that male mollies discriminate against gynogens, we suggest that this result should not be over-interpreted to mean that female *P. formosa* do not regularly mate in nature. These studies do not show an absence of mating between males and gynogens. Within a mixed species group, we would expect females to differ in size and in receptivity, and Schlupp et al. (1991) suggested that differences in receptivity can override conspecific preferences. Furthermore, none of these studies addresses in detail the role of frequency dependence. Male discrimination abilities as well as the potential advantages to mate copying could be influenced by the relative proportion of sexual and asexual females.

Studies of mate discrimination in this complex have focused on males, but selection also can act on the female gynogens. For example, Foran & Ryan (1994) have shown that *P. formosa* are more aggressive in excluding *P. latipinna* females from males than are *P. latipinna* females in excluding *P. formosa* from males. Because selection appears to have strengthened male mating preference in areas of sympatry, there might be an arms race between male mating discrimination and the behaviour patterns of female *P. formosa*. All of these factors (variance in female size and receptivity, frequency-dependent effects and the evolution of female mating strategies to circumvent male mating preferences) might interact to result in *P. formosa* gaining the requisite matings for reproduction. These factors are amenable to analysis using field observations, population manipulations and controlled behavioural experiments.

Reproductive Character Displacement

Reproductive character displacement and/or reinforcement (Butlin 1987) occurs in response to

selection to maximize species differences and thus to minimize the chances of heterospecific matings in areas of sympatry (Dobzhansky 1937). Much of the evidence in support of character displacement is from comparisons of male courtship signals between areas of sympatry and allopatry (reviewed in Andersson 1994). Mate recognition and discrimination is a problem in animal communication, and in any communication system, the integrity of information transfer can be enhanced by changes in the receiver as well as in the signal. Few studies, however, have examined reproductive character displacement in the receiver. One recent exception is a study by Gerhardt (1994) showing some differences in preferences for male advertisement calls in female grey treefrogs, *Hyla chrysoscelis*, from populations that are sympatric and allopatric with the closely related *H. versicolor*.

Our results show that the ability of male *P. latipinna* to discriminate between conspecific and Amazon females per se appears not to be an evolutionary response to selection to avoid mating with Amazon females. Males in populations allopatric with *P. formosa*, and thus having had no opportunity for interaction with these females, show strong preference for conspecific females over *P. formosa*. One can rarely absolutely exclude the possibility of sympatric interactions in the evolution of preferences in males from allopatric populations. It is always possible that populations of *P. latipinna* currently allopatric with *P. formosa* were once sympatric with this species. Another alternative is that this mating preference evolved in sympatry, and its presence in allopatry is either the result of gene flow, or of the species expanding its range from one initially sympatric with *P. formosa*. There is no evidence to suggest that either of these alternatives is likely. Also, these explanations for the lack of sympatric interactions in the evolution of conspecific mating preferences are not peculiar to this study but apply to any studies that reject the hypothesis of reproductive character displacement.

Although we argue that the preference for conspecific versus *P. formosa* females might be an incidental consequence of a more general conspecific preference, the strength of this preference is stronger in sympatry than in allopatry. This strengthening of the male preference in sympatry is consistent with the hypothesis that male mating preferences in *P. latipinna* have been influenced

by selection for reproductive character displacement. We caution that although we tested males from three allopatric populations, we have examined males from only one sympatric population. Our conclusions are further strengthened, however, by their consistency with an earlier study by Hubbs (1964) in which males from sympatric and allopatric populations preferred female *P. latipinna* to *P. formosa*, and the strength of preference was greater in the sympatric population.

Male Preference for Heterospecifics

Poecilia latipinna and *P. mexicana* males differ in their responses to sexual heterospecific females and gynogenetic females. *Poecilia latipinna* are reluctant to mate at all when given this choice, but *P. mexicana* choose to mate with gynogenetic females in preference to *P. latipinna* females. This result can be interpreted in the context of the initial hybridization event that gave rise to *P. formosa*, which is thought to have been between a *P. mexicana* female and a *P. latipinna* male (Avise et al. 1991). Thus *P. mexicana* males might be attracted to a phenotypic character whose expression results from some maternally contributed, conspecific-derived, genetic component. This interpretation warrants experimental verification.

High levels of heterozygosity in hybrids are generally presumed to result in an overall intermediate phenotype. However, some fish hybrids show differential expression of parental alleles (Whitt et al. 1977). Hybrid males from crosses between *P. mexicana* females and *P. velifera* males preferred *P. mexicana* females when given a choice between females of the parental species, although the males showed the complete sexual display of the *P. velifera* fathers (Parzefall 1989).

If *P. formosa* differentially expresses mate recognition characters that result from the genetic contribution of the maternal species, *P. mexicana*, it would be expected that male *P. mexicana* would be attracted to *P. formosa* in situations in which comparison with conspecific females is not possible. We can test this hypothesis using hybrid males and females of reciprocal crosses in a series of mate choice experiments. If there is a mating rule that takes into account maternal inheritance, one would expect *P. latipinna* males to prefer

hybrid females whose mothers were *P. latipinna* and *P. mexicana* males to prefer females whose mothers were *P. mexicana*.

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