

Repeatability of female preferences in a unisexual–bisexual mating system

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ABSTRACT

Background: A gynogenetic species is a hybrid species in which females must mate with males of their parental species to initiate egg development, but inheritance is strictly maternal.

Question: Is the mating preference of an all-female, gynogenetic fish and its parental species repeatable?

Organisms: The Amazon molly, *Poecilia formosa*, is a livebearing gynogenetic fish of hybrid origin. Its parental species are *P. latipinna* (paternal) and *P. mexicana* (maternal).

Methods: We examined and compared the repeatability in preferences of *P. formosa* and female *P. latipinna* for larger vs. smaller *P. latipinna* males. We also examined the repeatability of female *P. mexicana* preference for large male *P. mexicana*. Repeatability relates variation within individuals to the variation between individuals. We related measures of consistency to individual preferences to examine whether female preferences were unanimous.

Conclusions: Females of all three species showed population-level mean preferences for larger males. Repeatability of the preference was low for Amazon mollies even though they are a clonal species. The maternal parental species, *P. mexicana*, also showed low repeatability but females of *P. latipinna* showed high repeatability in preference for large males, but their preferences were not unanimous.

Keywords: livebearing, mate choice, *Poecilia formosa*, *Poecilia latipinna*, *Poecilia mexicana*, repeatability.

INTRODUCTION

Most of the predictions of models developed to explain the evolution and maintenance of female mating preferences, and any subsequent effects on male trait evolution have been tested in sexually reproducing species (Haesler and Seehausen, 2005; Schielzeth *et al.*, 2010). However, females of unisexual species that rely on sperm for reproduction also exhibit mating biases [hybridogenetic frogs (Roesli and Reyer, 2000, Engeler and Reyer, 2001); gynogenetic fish (Marler and Ryan, 1997; Heubel *et al.*, 2008; Poschadel *et al.*, 2009)]. Gynogenesis is a form of unisexual reproduction where females produce diploid eggs, but they require sperm from males of closely related species to initiate embryonic development; however, the male genome is not incorporated into

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the offspring (Dawley, 1989). Gynogens are all-female species of hybrid origin, and sexually parasitize males of the parental species. Given the hybrid origin of gynogenetic lineages, it has been hypothesized that they inherit traits, including mating preferences, from their sexual ancestors at the time of hybridization, and that these traits are then frozen in time (Vrijenhoek, 1979). Because gynogens are of hybrid origin, comparisons between gynogens and their parental species can reveal patterns of inheritance of mating preference (Marler and Ryan, 1997). In addition, because male genes are not incorporated into the diploid gynogen's offspring (but see Schartl *et al.*, 1995a; Lamatsch *et al.*, 2004), the maintenance of any observed mating preference cannot be based on genetic benefits to the offspring.

One unresolved question is why mating preferences of gynogens persist, especially in older lineages – more than 100,000 years for *Poeciliopsis* sp. (Quattro *et al.*, 1992) and 120,000 for *Poecilia formosa* (Stöck *et al.*, 2010). Exhibiting a preference for males with preferred traits could be costly if the gynogens compete with females of the sexual species for access to preferred males. Losses of female preferences have been demonstrated in sexually reproducing species (Wiens, 2001; Morris *et al.*, 2005). There are several possible mechanisms that could lead to the loss of female mating preferences in gynogens, including fixation of new mutations (Hill, 1982), adaptation from standing genetic variation inherited at hybridization (Barrett and Schluter, 2008), or mitotic recombination (Mandegar and Otto, 2007). Even in the absence of costs of preferences, hypotheses to explain benefits of female mating preferences based on direct benefits or good genes or genetic correlations do not apply to gynogenetic lineages, because the male genes are not incorporated into the offspring, and there is no paternal care or provisioning. The maintenance of mating preferences in gynogens could be due to other direct benefits not related to paternal care (e.g. females produce more offspring when mating with males with the preferred trait), or sensory biases, such that a mating preference persists as a correlated response of selection on female sensory systems in other (non-mating) contexts (reviewed in Fuller *et al.*, 2005).

Some studies of the evolution and maintenance of female preferences have focused on evaluating the mean population preference, while ignoring the importance of the potential for variation both within and between females (Widemo and Saether, 1999; but see Wagner *et al.*, 1995; Hankinson and Morris, 2003; Ritchie *et al.*, 2005; Schielzeth *et al.*, 2010). Even in cases where the average population-level female preference is for a particular male trait, there may be variation in the expression of this preference across females, or within individuals across time. One approach to understanding patterns of variation in the expression of mating preferences is to estimate the consistency of female preferences across time (Cummings and Mollaghan, 2006). Consistency of female preferences can be estimated by the coefficient of variation in the strength of a female preference for a male trait, measured more than once. Consistency is a measure of variation in an individual's choices that is independent of population level variation, because the coefficient of variation takes into account both the variation within females as well as the mean of each female's response. Therefore, comparisons can be made between multiple females even if the females differ in their means (Sokal and Rohlf, 1995). This attribute makes it possible to use the coefficient of variation to compare the consistency of individual mating choices when individuals differ in their mean responses to stimuli. Measuring the consistency of female mating preference can shed light on the nature of variation within females. However, it is also important to understand the relative degrees of variation in mating preferences both within *and* between females. Analyses of preference data collected on multiple females across time can be used to assess the potential for heritable variation in mating preferences. One measure that sets the upper limit to herit-

ability is repeatability (r) of the preference (Boake, 1989). Repeatability allows for comparisons of heritability of mating preferences across species. Measures of r relate variation within individuals to the variation between individuals, and are often used as an upper limit of the heritability of a trait because the numerator of the measure includes environmentally based variance between individuals in addition to the genetic variance (Lessels and Boag, 1987; Boake, 1989).

The unisexual–bisexual species complex of livebearing fish composed of the gynogenetic Amazon molly, *Poecilia formosa*, and its parental species, sailfin mollies (*P. latipinna*) and Atlantic mollies (*P. mexicana*), provides an interesting system in which to examine consistency and repeatability of mating preferences. Amazon mollies are hypothesized to have originated via hybridization approximately 120,000 years ago, with *P. latipinna* as the putative paternal species and *P. mexicana limantouri* as the maternal species (Schartl *et al.*, 1995b; Stöck *et al.*, 2010). In addition, Amazon mollies have been purported to have a monophyletic origin (Tiedemann *et al.*, 2005; Stöck *et al.*, 2010). *Poecilia latipinna* are distributed in coastal lagoons across much of the Gulf of Mexico into Northern Mexico and along the Atlantic coast from Florida to South Carolina. *Poecilia mexicana limantouri* ranges along the Atlantic slope near the Rio Grande drainage in Nuevo Leon, Mexico south to the lower Rio Tamesi near Tampico. *Poecilia formosa* are sympatric with their parental species from southeastern Texas through northeastern Mexico near Tampico. *Poecilia latipinna* and *P. formosa* have been introduced in a few populations in central Texas (Brown, 1953).

Female *P. latipinna* (Marler and Ryan, 1997; Ptacek and Travis, 1997; Gabor, 1999; MacLaren, 2006; Huebel *et al.*, 2008) and female *P. mexicana* (Marler and Ryan, 1997; Plath *et al.*, 2004, 2008; MacLaren and Rowland, 2006a; Heubel *et al.*, 2008) prefer to associate with larger conspecific males. The unisexual, Amazon mollies also prefer larger males of their sexual hosts (Marler and Ryan, 1997; Heubel *et al.*, 2008) but repeatability in association preference for large size has not been examined for females of these three species. Male *P. latipinna* show continuous distribution of male size but there is a genetic basis to male size. Smaller males mature at a younger age than do larger males, and smaller males attempt more sneaky copulations, whereas medium and larger males are more likely to court females before mating (Travis and Woodward, 1989). Male *P. mexicana* do not show the same genetic basis to size as do *P. latipinna*. Males of both species do not grow much once they reach maturation. Unlike *P. latipinna*, *P. mexicana* do show dominance hierarchies (Farr, 1989).

It is not known to what degree any preference is heritable within the parental and Amazon molly lineages. Because Amazon mollies reproduce clonally, a heritable preference will be passed on from the female to her offspring. In this study, we estimate the consistency of preferences expressed for males varying in size for individuals of each of the three female species in this unisexual–bisexual species complex of mollies. We examined preferences of *P. latipinna* and Amazon mollies for large male *P. latipinna* and female *P. mexicana limantouri* preference for large male *P. mexicana* (for short). We related measures of consistency to individual preferences. We then examined the repeatability of molly mating preference of each species for larger male mollies.

METHODS AND MATERIALS

We used Amazon mollies collected from a population sympatric with *P. latipinna* in Tamaulipas, Mexico (25.30°N, 97.86°W; 2005, 2006) and female *P. latipinna* collected from a population sympatric with Amazon mollies in Tamaulipas, México (25.07°N, 97.02°W;

2008) with male *P. latipinna* collected from an introduced population sympatric with Amazon mollies in Hays County, Texas, USA (29.89°N, 97.82°W; 2006, 2008). We tested female and male *P. mexicana* collected from a sympatric population with Amazon mollies in Tamaulipas, Mexico (24.04°N, 98.90°W; 2008). We maintained fishes at 6 ppt salinity on a 14:10 light/dark cycle at about 25–27°C and fed ISO flake food supplemented with brine shrimp twice daily. We maintained males in mixed sex tanks. We separated females from males for at least 30 days before testing. We isolated females for 48 h before trials.

We tested the association preferences of individual Amazon mollies ($n = 28$), female *P. latipinna* ($n = 16$), and female *P. mexicana* ($n = 26$) across 2 days for paired males that differed in standard length ($SL \geq 10$ mm) with a 2-day interval between tests. Experiments were conducted from 10.00 to 16.00 h from April to June 2008 and May to August 2009. We only used each pair of males for one female across both days of testing. We used a standard dichotomous test aquarium design for measuring female association preference in poeciliid fishes (see Houde, 1997). MacLaren and Rowland (2006b) found that female *P. latipinna* preference was much lower when males were presented sequentially compared with dichotomous tests.

We split the test aquarium (57 L; 61 × 30.5 × 30.5 cm) into five sections. We separated the outer two sections (10 cm) by Plexiglas dividers that permitted both visual and chemical cues. Sections adjacent to the Plexiglas were preference zones (10 cm). At the beginning of each trial we placed one smaller male in one end compartment, and one larger male was placed in the other. We placed a female in the middle section (20 cm) of the aquarium under a clear plastic cylinder and all fish were allowed to acclimate for 10 min, after which time the cylinder was carefully removed and a 10-min trial began. We recorded association time when the female's snout entered a preference zone. We returned all fish to holding tanks (18.9-L tanks) between tests. We randomized side placement of males for each trial with the limitation that half the females were tested with the larger male on the right side of the tank for the first trial and on the left side of the tank for the second trial. We tested the remaining females with the reverse placement of males.

Statistical analyses

We used paired *t*-tests to determine whether females preferred to associate with larger than smaller males on each of the 2 days, for all three species. To examine the strength of preference, we calculated the time spent associating with the larger male minus time spent associating with the smaller male per 10-min treatment. We calculated repeatability from the mean square among groups and the mean square between groups (error) from a one-way analysis of variance (ANOVA) on strength of preference across 2 days of testing, with individual females as the main effect (Lessels and Boag, 1987). We calculated the standard error (S.E.) as the square root of the sampling variance of the intraclass correlation (Becker, 1984). We confirmed the assumption that the variance in strength of preference across females was equal for both days of testing using Levene's test. We tested for a change in overall responsiveness across the days of testing by comparing responsiveness ($\{ \text{time with larger male (s)} + \text{time with smaller male (s)} \} / 600 \text{ s}$) between days using ANOVA. We also examined the relationship between the strength of preference on Day 1 and the strength of preference on Day 2 using simple linear regression.

To estimate consistency, we calculated the coefficient of variation (CV) in the strength of preference scores across the 2 days of testing for each female of all species (Cummings and Mollaghan, 2006; Gabor and Aspbury, 2008). We examined the relationship between the coefficient of

variation and the average strength of preference using simple linear regression for each species. We compared the strength of preference for large males across the three species and the 2 days of testing using a repeated-measures ANOVA on strength of preference. We also examined if any of the three species showed more variation in the strength of preference across the 2 days of testing by comparing the coefficient of variation across females using Welch's ANOVA (Sokal and Rohlf, 1995).

RESULTS

Amazon mollies

On each of the two days of testing, Amazon mollies preferred to associate with the larger male (paired *t*-tests, *n* = 28: Day 1: *t* = 4.791, *P* = 0.0001; Day 2: *t* = 2.732, *P* = 0.011; Fig. 1). Individual Amazon mollies did not differ significantly from one another in their strength of preference (ANOVA: $F_{27,28} = 1.202$, *P* = 0.316), and repeatability of strength of preference was not different from 0 ($r \pm \text{s.e.} = 0.092 \pm 0.270$). We confirmed the assumption

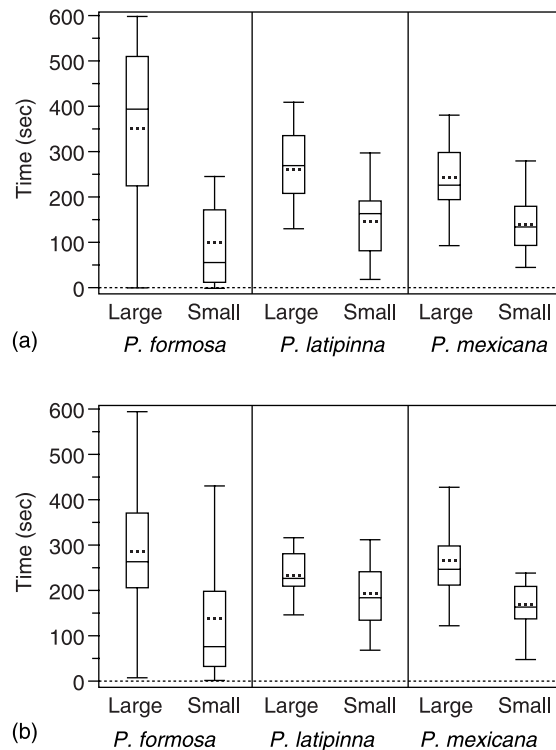


Fig. 1. Boxplots showing the distribution of time (s) that females spent associating with larger or smaller males across Day 1 of testing (a) for *P. formosa* (*n* = 28), *P. latipinna* (*n* = 16), and *P. mexicana* (*n* = 26); and across Day 2 of testing (b) for *P. formosa* (*n* = 28), *P. latipinna* (*n* = 16), and *P. mexicana* (*n* = 26). Solid lines show median, dashed line shows mean, box shows upper and lower quartile, and whiskers show 10th and 90th percentiles.

of the analysis that the variances among females were equal across each day of testing (Levene test: $F_{1,54} = 0.153$, $P = 0.697$).

Responsiveness of Amazon mollies was high across the 2 days of testing, and did not differ between days (mean \pm s.e. responsiveness: Day 1: 0.761 ± 0.037 ; Day 2: 0.707 ± 0.033 ; ANOVA: $F_{1,54} = 1.197$, $P = 0.279$). There was also no significant difference in the strength of preference across the 2 days of testing (ANOVA: $F_{1,54} = 1.820$, $P = 0.183$). There was no significant relationship between strength of preference on Day 1 and that on Day 2 (simple linear regression: $r^2 = 0.012$, $F_{1,26} = 0.318$, $P = 0.578$; Fig. 2a).

There was no significant relationship between the average strength of preference of an Amazon molly and the CV in preference (simple linear regression: $r^2 = 0.044$, $F_{1,26} = 1.206$, $P = 0.282$). There was no relationship between female standard length and strength of preference on either day of testing (simple linear regression: Day 1: $r^2 = 0.011$, $F_{1,26} = 0.265$, $P = 0.611$; Day 2: $r^2 = 0.0006$, $F_{1,26} = 0.016$, $P = 0.902$). Female mean size (range) was 45.0 ± 3.4 mm (39.2–51.7 mm).

Poecilia latipinna

On the first day of testing, female *P. latipinna* preferred to associate with the larger male, but not on the second day (paired *t*-tests, $n = 16$: Day 1: $t = 3.885$, $P = 0.0007$; Day 2: $t = 1.747$, $P = 0.101$; Fig. 1). Individual *P. latipinna* did differ significantly from one another in their strength of preference (ANOVA: $F_{15,16} = 5.676$, $P = 0.001$), and repeatability of strength of preference was greater than 0 ($r \pm$ s.e. = 0.700 ± 0.186). We confirmed the assumption of the analysis that the variances among females were equal across each day of testing (Levene test: $F_{1,30} = 0.001$, $P = 0.971$).

Female *P. latipinna* responsiveness was high across the 2 days of testing, and did not differ between days (mean \pm s.e. responsiveness: Day 1: 0.691 ± 0.041 ; Day 2: 0.721 ± 0.033 ; ANOVA: $F_{1,30} = 0.329$, $P = 0.571$). There was no significant difference in the strength of preference across the 2 days of testing (ANOVA: $F_{1,30} = 2.156$, $P = 0.152$). There was a significant relationship between strength of preference on Day 1 and that on Day 2 (simple linear regression: $r^2 = 0.648$, $F_{1,14} = 25.379$, $P = 0.0002$; Fig. 2b).

There was no significant relationship between *P. latipinna* average strength of preference and the CV in preference (simple linear regression: $r^2 = 0.044$, $F_{1,14} = 0.650$, $P = 0.434$). There was no relationship between female standard length and strength of preference on either day of testing (simple linear regression: Day 1: $r^2 = 0.004$, $F_{1,14} = 0.06$, $P = 0.810$; Day 2: $r^2 = 0.151$, $F_{1,13} = 2.14$, $P = 0.169$). Female mean size (range) was 40.41 ± 1.15 mm (32.0–48.9 mm).

Poecilia mexicana limantouri

On each of the 2 days of testing, female *P. mexicana* preferred to associate with the larger male (paired *t*-tests, $n = 26$: Day 1: $t = 4.643$, $P = 0.0001$; Day 2: $t = 4.005$, $P = 0.0005$; Fig. 1). Individual *P. mexicana* did not differ significantly from one another in their strength of preference (ANOVA: $F_{25,26} = 0.936$, $P = 0.564$), and repeatability of strength of preference was not different than 0 ($r \pm$ s.e. = -0.033 ± 0.288). We confirmed the assumption of the analysis that the variances among females were equal across each day of testing (Levene test: $F_{1,50} = 0.066$; $P = 0.799$).

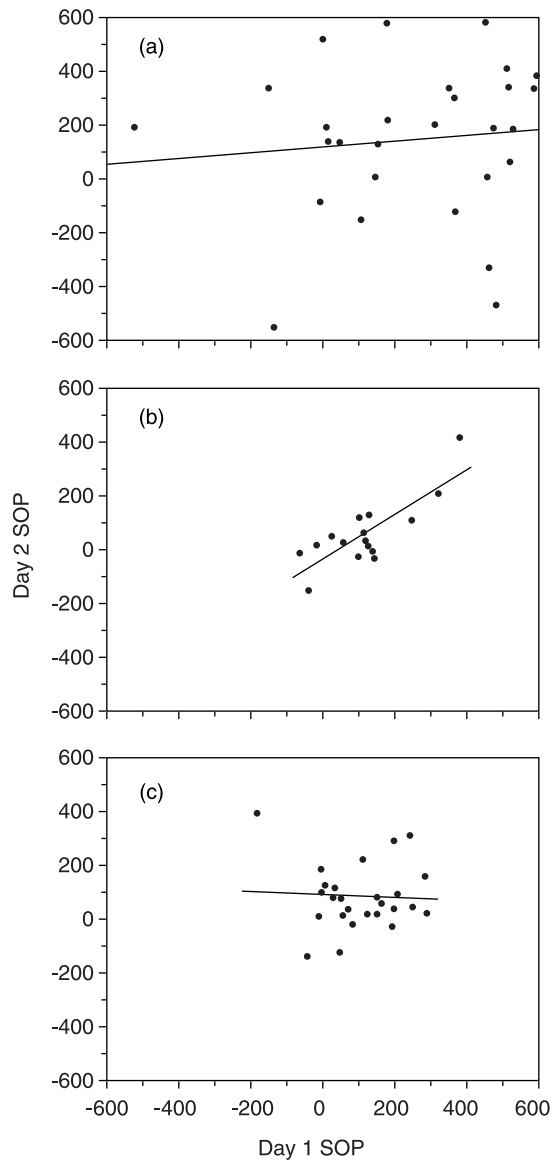


Fig. 2. Relationship between strength of preference (SOP: time with larger male/total time with both males) on Day 1 and Day 2 for (a) *P. formosa* ($n = 28$), (b) *P. latipinna* ($n = 16$), and (c) *P. mexicana* ($n = 26$).

Female *P. mexicana* responsiveness was high across the 2 days of testing, but differed between days (mean \pm s.e. responsiveness: Day 1: 0.640 ± 0.020 ; Day 2: 0.717 ± 0.017 ; ANOVA: $F_{1,50} = 8.236$, $P = 0.006$). There was, however, no significant difference in the strength of preference across the 2 days of testing (ANOVA: $F_{1,50} = 0.056$, $P = 0.814$). There was also no significant relationship between the strength of preference on Day 1 and that on Day 2 (simple linear regression: $r^2 = 0.003$, $F_{1,24} = 0.064$, $P = 0.803$; Fig. 2c).

There was no significant relationship between female *P. mexicana* average strength of preference and the CV in preference (simple linear regression: $r^2 = 0.025$, $F_{1,24} = 1.643$, $P = 0.212$). There was no relationship between female standard length and strength of preference on either day of testing (simple linear regression: Day 1: $r^2 = 0.0008$, $F_{1,24} = 0.020$, $P = 0.889$; Day 2: $r^2 = 0.0004$, $F_{1,24} = 0.11$, $P = 0.919$). Female mean size (range) was 43.62 ± 0.956 mm (34.86–55.02 mm).

Species comparisons

There was a significant effect of female species, but not of day, and no significant interaction between female species and day on the strength of preference (Table 1). *Post-hoc* paired comparisons revealed that Amazon mollies have a significantly greater strength of preference for large males than do female *P. latipinna* (paired *t*-test: $t = 2.335$, $P = 0.021$) and female *P. mexicana* (paired *t*-test: $t = 2.336$, $P = 0.020$). Furthermore, individual Amazon mollies show significantly more variation in their strength of preference across days than either *P. latipinna* or *P. mexicana* (Welch's ANOVA on CV of strength of preference: $F_{2,43.7} = 4.415$, $P = 0.018$).

DISCUSSION

Our results are in line with previous research that showed a female preference for larger males that is found in both the parental species as well as the hybrid lineage (Marler and Ryan, 1997). This preference is exhibited by the unisexual Amazon mollies, even though males provide no genetic contribution to the offspring, as well as none of the more obvious direct benefits (e.g. paternal care). Furthermore, Amazon mollies have on average a stronger strength of preference than females of either of their parental species, but show no repeatability in their mating preference for larger males as might be predicted given that they are a clonal species (irrespective of the parental species).

Despite a mean preference for larger males on both days of testing, several lines of evidence suggest that individual Amazon mollies are not consistent in their preferences. First, if females were consistent across the days of testing we would expect a positive relationship between the strength of preference on Day 1 and that on Day 2, which we did not find. Second, Amazon mollies show lower average consistency in strength of preference

Table 1. Results of repeated-measures ANOVA on arcsine transformed female strength of preference (= time spent associating with larger male/total time associating with larger and smaller males combined)

Source	Numerator d.f.	Denominator d.f.	<i>F</i>	<i>P</i>
<i>Between individuals</i>				
Species	2	67	3.976	0.023
<i>Within individuals</i>				
Day	1	67	3.169	0.080
Species × Day	2	67	0.862	0.427

Note: Significant *P*-values are in bold.

than either of their parental species. Finally, our estimate of repeatability indicates that approximately 9% of the variation observed in the strength of preference for large males is due to relative variation among females, whereas 81% of the variation was within females. One hypothesis for the lack of consistency is that Amazon mollies may be first choosing one male and then the other male, as this might increase their chance of finding a male that will mate with them.

Like Amazon mollies, female *P. mexicana*, the putative maternal ancestor of the gynogenetic Amazon mollies, also show low repeatability in their mating preferences, and also show a population mean preference for larger males. This lack of repeatability could be due to females having consistent and unanimous preferences for large males (Widemo and Saether, 1999). Female *P. mexicana* on average have a very low consistency in strength of preference, and there is no relationship between the consistency and the average strength of preference.

Unlike the other two species, female *P. latipinna* do show repeatable preferences for larger males. Our estimate of repeatability indicates that 70% of the variation in strength of preference is attributed to variation among the females, and 30% of the variation is within females. This result suggests that females are consistent in their preferences, but that females are not unanimous in their preferences – some females in this population consistently prefer larger males, and some consistently prefer smaller males. This is supported by the observation that there is a significant positive relationship between the strength of preference on Day 1 and that on Day 2 for female *P. latipinna*. Despite the population mean preference for large males on Day 1, some females do prefer smaller males. We do not think age plays a part in this result because we did not find a significant relationship between female size and average strength of preference. It is not clear why females might prefer smaller males, given that females are harassed more by smaller males than larger males (Schlupp *et al.*, 2001). However, Heubel and Plath (2008) found that *P. mexicana* and *P. formosa* fed less in the presence of a large male, suggesting benefits to associating with smaller males.

The strength of the gynogenetic Amazon molly preference for large males is greater than the mean strength of preference for larger males exhibited by females of the parental species. This result is counter-intuitive, if Amazon mollies face a cost of competition with female *P. latipinna* and *P. mexicana* for access to preferred males, given their shared population level mean preference for large males [*P. latipinna* (Schlupp *et al.*, 1994; Ptacek and Travis, 1997; Gabor, 1999; Gabor and Page, 2003; MacLaren *et al.*, 2004; MacLaren, 2006; this paper); *P. mexicana* (Marler and Ryan, 1997; Plath *et al.*, 2004, 2008; MacLaren and Rowland, 2006a; Heubel *et al.*, 2008; this paper)]. Furthermore, larger male *P. latipinna* are more sperm limited than smaller males (Aspbury and Gabor, 2004).

A preference for larger males could result from direct benefits, such as higher fecundity, protection from predators, reduced costs of finding a mate, or reduced costs of mating based on this preference (Andersson, 1994). Marler and Ryan (1997) found no evidence for direct fecundity benefits to Amazon mollies that mated with larger males, but tests of other direct benefits have not been performed. At the proximate level, preference for large male size could be an incidental by-product of a preference for increased lateral projection area, as has been found in the parental species of Amazon mollies [*P. latipinna* (MacLaren *et al.*, 2004) and *P. mexicana* (MacLaren and Rowland, 2006a)]. Similarly, the observed preference in Amazon mollies could be a result of a sensory bias. A female mating preference that is a result of a sensory bias is an ancestral by-product of natural selection on the sensory systems (Ryan, 1990) and could result in consistent preferences in individual females, as well as many females possessing the trait (if the sensory bias represents an ancestral trait). Such consistency at the

individual level as well as at the population level leads to low levels of repeatability, given low variation both within and between females (Widemo and Saether, 1999). Our results do not refute the sensory bias hypothesis given that 50% of the Amazon mollies spent an average of $\geq 75\%$ of association time with the larger male (and 86% of the females spent an average of $\geq 50\%$ of association time with the larger male). The consistent and nearly unanimous choice of Amazon mollies for larger males could be due either to similar genetics (fixed differences in clonal species) or environmental effects (Boake, 1989).

In this experiment, we tested three species of mollies each from a single population. One possibility is that there is population-level variation in molly association preferences across different populations. Across-population variation in mating preferences has been found in both female (reviewed by Gumm and Thaker, 2009) and male mollies for conspecifics versus heterospecifics (Gabor and Ryan, 2001; Gumm and Gabor, 2005; Gabor and Aspbury, 2008; Heubel *et al.*, 2008; Heubel and Schlupp, 2008; Aspbury *et al.*, 2010). However, Gabor and Aspbury (2008) found no difference in repeatability by male *P. latipinna* across multiple populations indicating that repeatability, unlike mate preferences, remains consistent across populations. Furthermore, our result of all three species of mollies preferring larger males coincides with those from prior studies using numerous different populations [*P. latipinna* (Marler and Ryan, 1997; Ptacek and Travis, 1997; Gabor, 1999; MacLaren, 2006; Heubel *et al.*, 2008); *P. mexicana* (Marler and Ryan, 1997; Plath *et al.*, 2004; MacLaren and Rowland, 2006a; Heubel *et al.*, 2008; Plath *et al.*, 2008); *P. formosa* (Marler and Ryan, 1997; Heubel *et al.*, 2008)]. In addition, for Amazon mollies, the monophyletic origin (Teidemann *et al.*, 2005; Stöck *et al.*, 2010) supports the hypothesis that variation across populations is not expected. Thus we argue that these results are likely a species-level outcome, not a population-level effect.

The finding that Amazon mollies prefer larger males could have implications for the evolution of male size in populations of *P. latipinna* sympatric with Amazon mollies. Gynogens are of hybrid origin, and sexually parasitize males of the parental species. This parasitic interaction is similar to host–parasite dynamics, as gynogen mating preferences may create frequency-dependent selection on the males of the parental species. In sympatric populations, conspecific female preferences for larger male size (Marler and Ryan, 1997; Heubel *et al.*, 2008; this study) should result in directional selection for larger male size. However, if selection favours males that do not engage in costly matings with Amazon mollies (Gabor and Ryan, 2001; Aspbury and Gabor, 2004; Robinson *et al.*, 2008), we would predict selection for smaller male size in sympatry but this may be balanced by mate choice copying (see Schlupp *et al.*, 1994). This trade-off could favour males of intermediate sizes, balancing the benefits of attracting conspecific females with the costs of attracting heterospecific females. This prediction has not been tested.

It is possible that Amazon mollies inherited their mating preferences from the maternal species (*P. mexicana*) and that this preference has not evolved in either species since the time of origin of Amazon mollies (120,000 years). While it is impossible to examine the ancestral mating preferences of these species, we hypothesize that the observed preferences are not derived in Amazon mollies. First, it has been found that the mating preferences of both parental species are a by-product of preference for a larger lateral projection area (MacLaren *et al.*, 2004; MacLaren and Rowland, 2006a) and that both males and females of *P. latipinna* show a generalized preference for large individuals of both sexes, suggesting that the preference is not solely driven by mate choice (Gabor, 1999). Second, random mutations that have arisen in Amazon mollies since their hybrid origin are unlikely to have generated enough genetic variation for selection to change any preference traits inherited at hybridization (Vrijenhoek, 1979; Tiedemann *et al.*, 2005; Stöck *et al.*, 2010). Further explorations of the hypothesis that the

preferences observed in Amazon mollies are ancestral with the preference found in their maternal parental species are needed.

In conclusion, this unisexual–bisexual complex provides a novel system for examining the evolution of mating preferences because the parental species of Amazon mollies persist and can also be tested. We found that Amazon mollies have consistent preferences for larger males, but repeatability of the preference is low. They are more similar in the consistency and repeatability of their preference to their maternal parental species (*P. mexicana*) than they are to their paternal parental species (*P. latipinna*). The observed preferences may be a result of a sensory bias for large or a correlated preference for a larger lateral projection area. The preferences of Amazon mollies for larger males could have important implications for testing hypotheses related to the predicted direction of evolution of male size.

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