RESEARCH PAPER

Mating Preferences of the Gynogenetic Amazon Molly Differ Between Populations Sympatric with Different Host Species

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Abstract

Gynogenetic species rely on sperm from heterospecifics for reproduction but do not receive genetic benefits from mating because none of the paternal genome is incorporated into offspring. The gynogenetic Amazon molly (*Poecilia formosa*) is a species of hybrid origins that are sympatric with one of the two parent species that provide sperm for reproduction, *P. latipinna* or *P. mexicana*. Amazons should not prefer to mate with one species over the other because sperm from both species will trigger embryogenesis, but mating preferences may be present in Amazons through other mechanisms. Amazons may prefer the more familiar species (males found in sympatry), or Amazons may prefer males with the greatest lateral projection area (LPA), a preference that is present in the parent species and may be retained within the Amazon hybrid genome. We tested association preferences of two populations of Amazons sympatric with either *P. mexicana* or *P. latipinna*. We first performed live trials to test whether Amazons preferred one host species over the other and found that neither population of Amazons showed a preference. We then tested whether Amazons preferred sympatric male (familiar) host or the male with the greatest lateral projection area (LPA) using four animated male models that varied in host species and manipulation of LPA. We found Amazons from a population sympatric with *P. latipinna* showed no variation in their association preference across the different models. In contrast, Amazons from a population sympatric with *P. mexicana* (naturally small LPA) spent more time associating with the male models that had smaller LPA, which is more familiar to this population of Amazons. We suggest that Amazons may have population differences in mating preferences, where Amazons sympatric with *P. latipinna* may not show mating preference for host species, but Amazons sympatric with *P. mexicana* may show preferences for more familiar-shaped males.

Introduction

The maintenance of female mating preferences for male traits often involves indirect genetic benefits (Zahavi 1975; Andersson 1994; Rowe & Houle 1996; Jennions & Petrie 2000). Evidence for the 'good genes' hypothesis has been found in many sexually reproducing species (Hamilton & Zuk 1982; Reynolds & Gross 1992; Welch et al. 1998). However, females of unisexual species that rely on sperm from closely related heterospecific males for reproduction such as hybridogens and gynogens also exhibit mating preferences for particular male phenotypes (e.g., hybridogenetic frogs: Roesli & Reyer 2000; Engeler & Reyer 2001; gynogenetic fish: Marler & Ryan 1997; Heubel et al. 2008; Poschadel et al. 2009; Gabor et al. 2011). In the case of these 'sexual parasites', indirect genetic benefits cannot be used to explain the maintenance of mating preferences.

One well-studied unisexual species is the Amazon molly, *Poecilia formosa*. The Amazon molly is a gynogenetic live-bearing species of fish that most likely arose approx. 100 000 yr ago (Schartl et al. 1995; Lampert & Schartl 2008) from a sexually reproducing hybrid of a male sailfin molly, *P. latipinna*, and female Atlantic molly, *P. mexicana* (Hubbs & Hubbs 1932; Avise et al. 1991) which subsequently backcrossed with both parent species until the onset of gynogenesis (Alberici da Barbiano et al. 2013). Gynogens are sexual parasites because these female lineages require sperm from males of closely related species to initiate embryogenesis, but inheritance is strictly maternal (Avise 2008; Lehtonen et al. 2013). Amazon mollies do not receive any indirect genetic benefits from mating with particular host males and similarly, male hosts do not father offspring following mating with Amazon mollies. Amazon mollies occur in sympatry with either of the two parent species throughout their geographic range, and males of both species act as sexual hosts. From a fitness perspective, Amazon mollies should not prefer one male host species to another, because either male's sperm will initiate embryogenesis (Joachim & Schlupp 2012). Yet despite this prediction, Amazon mollies do exhibit preferences for particular male traits (Marler & Ryan 1997; Poschadel et al. 2009; Gabor et al. 2011) and for host species (Landmann et al. 1999).

Amazon mollies may exhibit preferences for particular male phenotypes if mating preferences of the parent species are retained within the Amazon molly's hybrid genome (Marler & Ryan 1997; Dries 2003; Gabor et al. 2011). Due to lack of meiotic recombination and low evolutionary potential (Rasch et al. 1982; Avise 2008), Amazons and other unisexual vertebrates may exhibit traits that are remnants of ancestral species. Similar to females of both parent species, Amazon mollies prefer larger males (Marler & Ryan 1997; Ptacek & Travis 1997; Gabor 1999; Gabor et al. 2011). In addition to body size, other male traits such as the size of the dorsal fin are also used by female mollies when selecting mates (Ptacek 1998; Jordan et al. 2006; Kozak et al. 2008). Male *P. latipinna* morphology is characterized by a large, sail-like dorsal fin but male *P. mexicana* are part of the short fin molly species complex that is characterized by a smaller dorsal fin (Ptacek & Breden 1998). When body size and dorsal fin size are controlled, females of both *P. mexicana* and *P. latipinna* show preferences for males with greater lateral projection area (MacLaren et al. 2004; MacLaren & Rowland 2006). Lateral projection area (LPA) is the total area of a fish when viewed from the side with the fins extended.

Another hypothesis to explain mating preferences exhibited by Amazon mollies is that unisexual females may prefer familiar host males due to sexual imprinting. During sexual imprinting, offspring learn parent phenotypes and later prefer mates that resemble their parents (Immelmann 1975). Oftentimes, offspring imprint on the phenotypes of caretakers rather than genetic parents as illustrated by instances of cross-foster care (Verzijden & ten Cate 2007; Verzijden et al. 2008; Kozak et al. 2011). In the case of sexual parasites, early-life experiences with host males may result in sexual imprinting based on familiar phenotype. Amazon mollies form mixed-species social aggregations (i.e., shoals) with parent species and contain individuals across all age-groups (Schlupp & Ryan 1996). Therefore, juveniles are exposed to adult phenotypes that could influence mating preferences later in life. Amazon mollies prefer male *P. latipinna* when raised with these males but show no significant preference for male *P. mexicana* when raised with *P. mexicana* (Körner et al. 1999; Poschadel et al. 2009). Additionally, Amazon mollies sympatric with *P. latipinna* prefer male *P. latipinna* over male *P. mexicana* (Landmann et al. 1999). However, this result could be due either to Amazon preference for a particular trait of male *P. latipinna*, or familiarity (sexual imprinting) on the sympatric male *P. latipinna*.

We test three hypotheses regarding mating preferences of Amazon mollies: (1) Amazon mollies do not exhibit a preference for one male host species over the other, (2) Amazon mollies exhibit mating preferences for males of the species with a greater LPA, and (3) Amazon mollies exhibit mating preferences for males of the sympatric host species (familiar) In our first experiment, we perform dichotomous-choice association trials with live males to test whether Amazon mollies had a mating preference for either host species (i.e., *P. latipinna*, *P. mexicana*). We conduct our first experiment using Amazon mollies from a population that is sympatric with *P. latipinna* (hereafter referred to as Amazon-*lat*) and Amazon mollies that are from a population sympatric with *P. mexicana* (hereafter referred to as Amazon-*mex*). If familiarity affects Amazon mating preferences, then both populations of Amazon mollies would prefer males of their sympatric host species. However, preference due to familiarity or greater LPA is obscured if the preference for male *P. latipinna* (naturally large LPA) is only seen in the Amazon-*lat* population. In order to gain further resolution on Amazon mating preferences, we perform a second experiment.

In our second experiment, the same two populations of Amazon mollies are given a simultaneous

choice between four animated male models. The models were photographs of male *P. latipinna* or *P. mexicana* printed on transparency paper (following MacLaren et al. 2004; Gumm et al. 2006). Two of the four models had their dorsal fins digitally increased or decreased in size to manipulate LPA. We predict that if familiarity influences association preferences, then Amazon mollies would show differences in preferences between populations that are sympatric with different host species. Amazon-*lat* should prefer male *P. latipinna*, and Amazon-*mex* should prefer male *P. mexicana*. If Amazons have a preference for males with greater LPA, then Amazons from both populations should prefer unaltered male *P. latipinna* and male *P. mexicana* with an artificially enlarged dorsal fin. If Amazon mollies recognize all males as potential hosts, then Amazons from both populations should not exhibit association or mating biases.

Methods

We collected Amazon-*mex* from the Rio Cobe, Mexico (23.97°N, 99.11°W) and Amazon-*lat* from a site in Northern Tamaulipas, Mexico (25.06'43°N, 98.01°W). We maintained Amazon mollies with their sympatric host species in 38-l aquaria (54 × 29 × 33 cm) at a constant temperature (25°C) on 14:10 h light-dark cycle with UV fluorescent lighting. We fed fishes twice daily with fish food (Purina AquaMax 200) and supplemented with live brine shrimp. We isolated Amazon mollies from males for a minimum of 30 d prior to testing to standardize levels of receptivity in females. We only used mature Amazon mollies that were a minimum of 32 mm in standard length (SL) in all trials. Texas State University Institutional Animal Care and Use Committee approved all procedures in this study (IACUC Protocol #0815_0319_19).

Experiment 1: Two-Way Preference Trials of Amazon Mollies Using Live Males

We created a choice chamber from a 57-l aquarium (61 × 30.5 × 30.5 cm) split into five sections. We separated the outer two sections (10 cm) on either side by Plexiglas, perforated with small holes, and demarcated the inner two 'choice' sections with a marker on the outside of the tank, leaving a middle 'no-choice' section in the center. We applied a one-way film to the outside of the chamber to prevent disturbance from observation. We filled the aquarium with treated water to an approx. depth of 20 cm. We illuminated the test tank from above with full spectrum UV fluorescent lighting. We fed all fish prior to

testing. We conducted trials with live males from 0900–1600 h, May–June, 2009. We placed size-matched (±3.0 mm) males of either *P. mexicana* or *P. latipinna* in the outer two sections. We obtained male *P. mexicana* from the Rio Purification, Mexico (24.02'27°N, 98.54'10°W) and male *P. latipinna* from two Central TX populations (29.89°N, 97.82°W) and (28.33°N, 97.24°W). We used males from different populations for both species to control for syntopic biases, this way neither of the two stimuli males would be from the same population as the tested Amazon mollies. In addition, Gabor & Ryan (2001) found no significant difference in male mate preference whether females were from native or foreign populations, suggesting that individuals from different populations do not discriminate between members of other intraspecific populations. We haphazardly chose males of each species from separate aquaria (approx. 10 males in each aquarium). We used only mature males with a fully modified gonopodium and a minimum of 30 mm in SL as subjects. We placed individual female Amazon mollies (Amazon-*mex*: n = 35, Amazon-*lat*: n = 31) in the center section and allowed them to acclimate for 10 min beneath a clear plastic cylinder. After 10 min, we lifted the cylinder and allowed the Amazon molly to move freely in the sections between the males. For the next 10 min, we recorded the total time in either choice section. To control for side bias, we reversed the placement of the males, allowed the female to acclimate for another 10 min, and again we recorded time in either section for 10 min.

Experiment 2: Four-Way Preference Trials of Amazon Mollies Using Model Males

We used models to control for differences in male behavior unrelated to LPA or familiarity. The models are effective stimuli for *Poecilia* spp. based on previous studies (MacLaren et al. 2004; MacLaren & Rowland 2006). We created digital photographs of male *P. mexicana* from the Rio Purification, Mexico (24.02'27°N, 98.54'10°W) where they are sympatric with Amazon mollies and male *P. latipinna* from Northern Tamaulipas, Mexico (25.11°N, 97.56°W) where they are also sympatric with Amazon mollies. In Adobe PhotoShop, we cut fishes images from the background and size-adjusted the images to match actual standard lengths of fish. We used digital photographs of fishes (n = 10) to create sets (n = 5) of four models. Within a set of models, there were two LPA-modified and two un-modified males. LPA-modified males had their dorsal fins transformed to match the size and shape of

the unmodified male of the opposite species of that set. For example, modified *P. mexicana* males had their dorsal fins enlarged and manipulated to match the size and shape of the unmodified male *P. latipinna* of their set, and vice versa (Fig. 1). We printed color images of the fish using a Hewlett Packard 7350 printer (Hewlett Packard, Palo Alto, CA, USA) onto transparencies. We placed white paper in the shape of the body between two mirror images before gluing all pieces together. The resulting two-dimensional model was two-sided with an opaque body and transparent fins.

We created a choice chamber aquarium out of glass with four arms to assess preference among four model males (Fig. 1a,b). We connected each arm (12.7 × 25.4 × 25.4 cm) to a center 'no-choice' section (25.4 × 25.4 × 25.4 cm). We filled the aquarium to an approx. depth of 15 cm with treated water (approx. 20-l). We placed models outside the test tank at the end of each arm and illuminated the models using conical metal lamps with four Spot Grow (60W) light bulbs to provide uniform lighting. We placed white paper dividers between lights to prevent subject females from viewing non-uniform areas outside the choice chamber, such as the observation area. We used a system of four pulleys connected to a motorized fan to animate the models in a 23-cm-diameter circular path. A variable resistor controlled the speed (approx. 7 rotations per min) of rotation. We uniformly spread a pebble substrate on the bottom of the test tank as well as outside the aquarium beneath the rotating models in a rough rectangle (25 × 24 cm). We used the pebble substrate underneath the models to create the illusion of a continuous medium between the test tank and area where models were located. We presented models on a background made of four large gray sections of poster board.

We conducted trials with male models from 0900–1600 h, June–July, 2009. We fed all Amazon mollies prior to testing. We randomly chose one of the five sets of models and then each of the four models of that set was randomly assigned to an arm of the choice chamber. We haphazardly selected an Amazon molly (Amazon-*mex*: n = 33, Amazon-*lat*: n = 27) from the same stock aquarium of Amazon mollies used for experiment 1 so that females from both
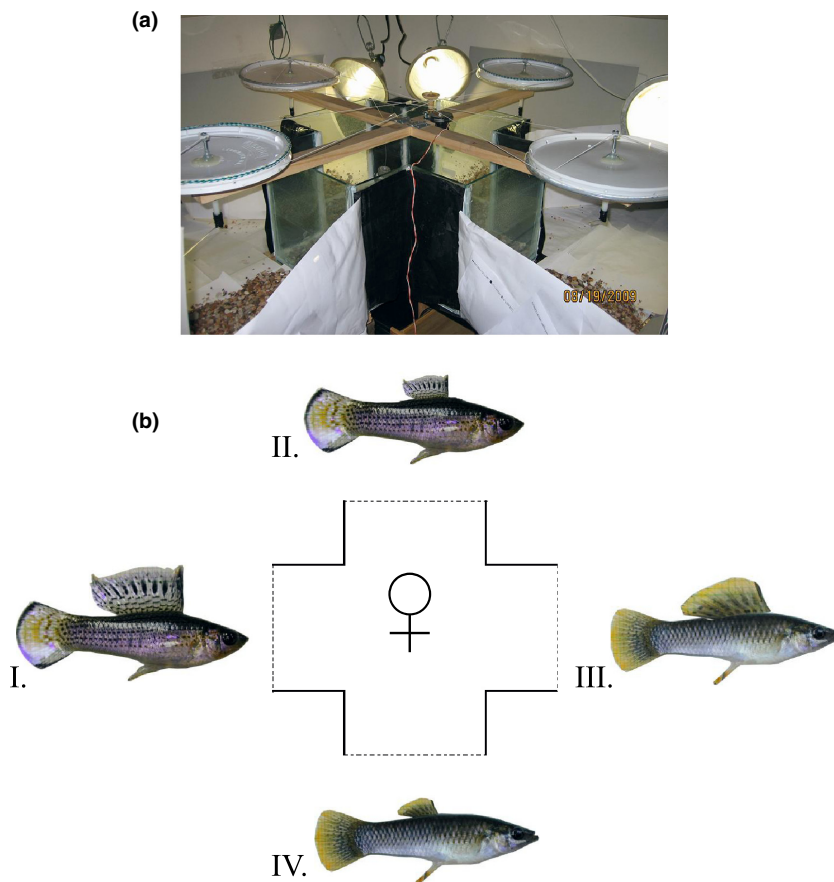
Fig. 1: (a) Photograph of 4-way experimental set-up and (b) diagram for a 4-way choice design in experiment 2 with models: I. *P. latipinna*, II. *P. latipinna* with modified small fin, III. *P. mexicana* with modified large fin, and IV. *P. mexicana*. Solid lines represent opaque walls and dashed lines represent transparent walls. Position of stimuli was randomized in each trial. Stimuli not scaled to size.

experiments had the same prior experience. We placed her beneath a transparent cylinder in the center of the choice chamber. We allowed fish to acclimate for 10 min while we animated the four models. After 10 min, we gently lifted the cylinder from the chamber using fishing line attached to the top of the cylinder. We then observed the Amazon molly for 10 min. We recorded the total time spent in each arm of the choice chamber on four stopwatches. We made all observations from above the tank at an angle.

Statistical Analysis

For experiment 1, within each source population of Amazon mollies, we analyzed the time that Amazon mollies spent in association with male *P. mexicana* or male *P. latipinna* using paired *t*-tests. We used total association time of both trials to calculate preferences. We eliminated males that exhibited side biases (i.e., total association time for one side of tank = 0) from further analyses. There was no significant difference in SL (standard length) between Amazon-*mex* and Amazon-*lat* (two-sample *t*-test: $t_{61} = -0.310$, p = 0.757; mean ± SE of SL (mm): Amazon-*mex* = 43.43 ± 0.615, Amazon-*lat* = 43.78 ± 0.984) and there was no significant difference in SL between male *P. mexicana* and male *P. latipinna* (two-sample *t*-test: $t_{65} = -0.134$, p = 0.894; mean ± SE of SL (mm): *P. mexicana* = 41.08 ± 0.603, *P. latipinna* = 41.06 ± 0.623). Therefore we did not include these variables in further analyses.

For experiment 2, we used a linear mixed effects model to test differences in Amazon association preferences for different male stimuli. Each Amazon population was analyzed separately. We used LPA and familiarity as fixed effects and individual Amazons as the random effect. We categorized each of the four male stimuli according to the degree of familiarity for each Amazon population with the sympatric male species being most familiar, the two modified males being intermediate in familiarity and the allopatric male species being least familiar. For example, for Amazon-*lat* which are sympatric with *P. latipinna*, the male *P. latipinna* stimulus was classified as most familiar, the modified male *P. latipinna* and the modified male *P. mexicana* stimuli were intermediate in familiarity, and the male *P. mexicana* stimulus least familiar. We categorized LPA as a binomial trait such that the *P. latipinna* and the modified *P. mexicana* stimuli considered large LPA males, and *P. mexicana* and modified *P. latipinna* was considered small LPA males. If Amazon mollies spent their total association time in one arm, we considered these females as biased and

eliminated them from further analyses. There was no significant effect of model set used ($F_{4,177} = 0.036$, p = 0.998) and stimuli position ($F_{20,177} = 0.018$, p = 1.00) on Amazon preferences and therefore, these factors were not used as factors in any of our analyses. There was also no significant difference in SL between Amazon-*mex* and Amazon-*lat* populations (two-sample *t*-test: $t_{58} = 0.492$, p = 0.625; mean ± SE of SL (mm): Amazon-*mex* = 44.69 ± 0.693, Amazon-*lat* = 45.22 ± 0.833) and therefore, Amazon SL was not used as a factor in our analyses. Our results did not change when using strength of preference (time spent with a stimulus/total time spent with all stimuli) or association time as our response variable and therefore, we will report all analyses using association time. We conducted all analyses in R version 2.14.1 (R Core Development Team).

Results

Experiment 1

Both populations of Amazon mollies showed no significant association preference for either live male *P. mexicana* or male *P. latipinna* (Fig. 2; paired *t*-tests: Amazon-*mex*: $t_{34} = 0.602$, p = 0.551; Amazon-*lat*: $t_{30} = 0.279$, p = 0.782).

Experiment 2

Amazon-*lat* and Amazon-*mex* populations showed different association preferences for familiarity or LPA using models. Amazon-*lat* showed no variation in the amount of time associating with the different models (Fig. 3b; Familiarity: $F_{1,78} = 0.42$, p = 0.521; LPA: $F_{1,78} = 2.92$, p = 0.091; Familiarity x LPA:
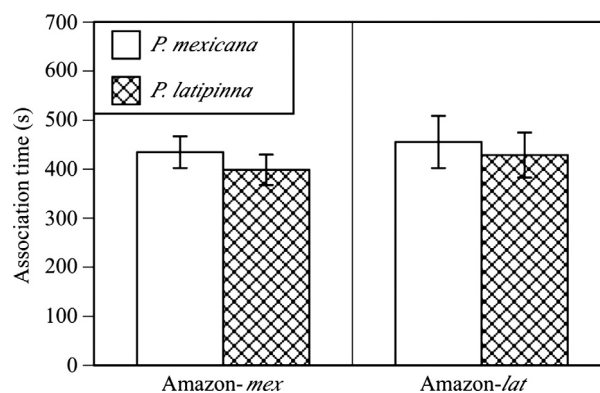
Fig. 2: Mean association time (s) ±SE of Amazon-*mex* and Amazon-*lat* populations for experiment 1.
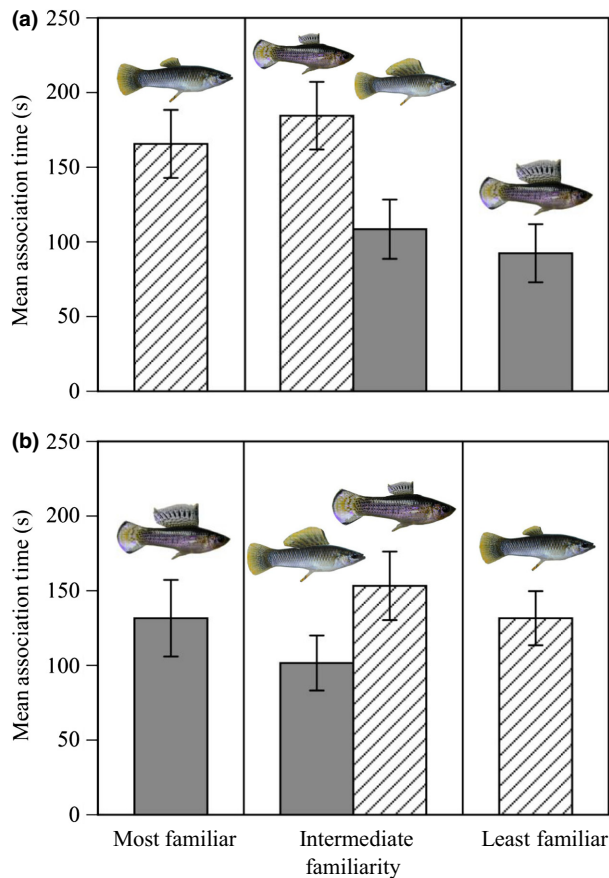
Fig. 3: Mean association time (s) ±SE of: (a) Amazon-*mex* population and the (b) Amazon-*lat* population with male stimulus models: *P. latipinna*, *P. mexicana* with modified fin (large), *P. latipinna* with modified fin (small), and *P. mexicana*. Gray bars indicate male stimuli with large LPA and diagonally lined bars indicate male stimuli with small LPA.

$F_{1,78} = 0.41$, p = 0.523). In contrast to the Amazon-*lat* population, Amazon-*mex* showed significant differences in association time across the four male stimuli. Both familiarity and LPA significantly affect choice (Familiarity: $F_{1,96} = 5.94$, p = 0.017; LPA: $F_{1,96} = 6.40$, p = 0.013), but there was no interaction between the two factors ($F_{1,96} = 0.678$, p = 0.413). Amazon-*mex* spent more time associating with the modified *P. latipinna* and the unmodified *P. mexicana* (Fig. 3a), which are stimuli that are categorized as having small LPA. Amazon-*mex* also spent the least amount of time associating with unmodified *P. latipinna*, which is the most unfamiliar male of the four male models (Fig. 3a).

Discussion

Females from neither Amazon-*mex* nor Amazon-*lat* populations preferred to associate with one live host male species (*P. mexicana* or *P. latipinna*) over the other. This result supports the hypothesis that sexual parasites should not exhibit host mate preferences because either species will initiate embryogenesis. The hypothesis is further supported by the results of experiment 2 where Amazon-*lat* also demonstrated no preferences for different male model stimuli. However, in contrast to these results, we also found that Amazon-*mex* population showed a significant preference for the familiar-shaped males when standardized stimuli were used. The results of experiment 2 indicate that Amazon mollies may show population-level variation in mating preferences for sympatric host species.

Contradicting results from other studies on mating preferences of Amazon mollies for male host species suggests that there may be high among population variation in mating preferences. Amazon mollies exhibit no preferences when presented with male *P. mexicana* and male *P. latipunctata*, another sympatric host species (Joachim & Schlupp 2012). However, in another study, Amazon mollies have shown preferences for male *P. latipinna* (Landmann et al. 1999) and male *P. latipinna* only if raised with *P. latipinna* (Körner et al. 1999), or for a male trait that is found in the sympatrically occurring species (Poschadel et al. 2009). Differences in mating preferences between Amazon-*mex* and Amazon-*lat* populations in our second experiment also support population-level variation among Amazon mating preferences. Testing multiple Amazon-*mex* and Amazon-*lat* populations could reveal whether population variation in Amazon mating preferences for host species is affected by sympatry with a specific host.

If there are population differences in mating preferences of Amazon mollies, then we should have seen results in our live trials that were congruent with results from experiment 2. Differences in the results of our two experiments could be attributed to the use of live males and male models. Using live males instead of male models does not account for the behavior of live males during mating trials, which may influence preferences of Amazon mollies. Male *P. latipinna* exhibit courtship behavior whereas male *P. mexicana* do not (Ptacek & Travis 1996; Ptacek 1998). In addition, our live male trials allowed for the exchange of chemical cues, which may influence behavior (Farr & Travis 1986). If Amazon mollies base familiarity on these chemical cues, then males from different populations should be as unfamiliar to Amazons as males from an allopatric species. However, it is unlikely that chemical cues played a role in assessing familiarity of males because Amazon-*mex* appeared to

prefer familiar male models using some aspect of the fin- to-body size ratio (results of this study for experiment 2). Therefore, familiarity can be assessed using visual phenotypes alone rather than chemical cues.

Differences in male discrimination against heterospecifics could contribute to differences in mating preferences between Amazon-*mex* and Amazon-*lat* populations. Male *P. latipinna* prefer conspecific females over Amazon mollies (Ryan et al. 1996; Gabor & Ryan 2001; Gabor & Grober 2010), which suggests that Amazon mollies sympatric with *P. latipinna*, such as the population tested in this study, would not show discrimination for any male because they may lose out on already-reduced mating opportunities. However, preference for conspecific females is weaker in *P. mexicana* (Ryan et al. 1996) or completely lacking in one population (Gabor et al. 2012), which may allow for these Amazon mollies to show greater discrimination in host males. Greater discrimination may be beneficial for Amazon mollies if mating with certain male phenotypes carry fitness benefits or costs. For example, some male phenotypes may be more likely to transmit diseases or some males may deliver less sperm to heterospecifics (Aspbury & Gabor 2004). Amazon mollies also exhibit preference for larger males (Marler & Ryan 1997; Gabor et al. 2011), which suggest that there may be a benefit to mating with particular males. An alternative hypothesis is that female preferences for male traits were retained within the hybrid genome of Amazon mollies (Marler & Ryan 1997; Dries 2003).

Association preferences between the four male models could only be evaluated visually by Amazons and therefore, preferences were likely to be based on stark morphological differences between the two species. Although the phenotypes of male *P. latipinna* and male *P. mexicana* noticeably differ in pattern and coloration, the silhouettes of the two small-finned, preferred models are similar (Fig. 1b). Our results suggest that the Amazon-*mex* population demonstrated a preference for a familiar male body shape rather than the familiar markings in experiment 2 because Amazon-*mex* showed a significant preference for the modified (small-finned) *P. latipinna* and familiar, un-modified *P. mexicana* (naturally small-finned). If Amazons based their preferences on familiar markings, then Amazon-*mex* would have preferred *P. mexicana* regardless of fin size. Our results on familiar body shape contrasts those found on familiar markings in a previous study by Poschadel et al. (2009) that showed Amazon mollies sympatric with *P. latipinna* preferred males with black vertical stripes, which is present in *P. latipinna* males, while Amazon mollies sympatric

with *P. mexicana* exhibit a preference for non-striped males. Similar results to ours were found by MacLaren & Daniska (2008) in *Xiphophorus helleri*, where females preferred males that had a fin- to-body size ratio that matched the males found in their own population rather than an enlarged dorsal fin (greater LPA). However, Amazon-*lat* did not show a preference for any male model, which did not fit these predictions.

Although female *P. mexicana* and *P. latipinna* associate with model males with greater LPA (MacLaren et al. 2004; MacLaren & Rowland 2006), Amazon mollies in our study exhibit no such preference. MacLaren & Rowland (2006) suggest that female preferences for greater LPA in many poeciliid species (MacLaren & Daniska 2008; MacLaren et al. 2011; MacLaren & Fontaine 2012) is due to an ancestral preference for larger males. Any trait that increases a male's apparent size will be preferred through this pre-existing bias. However, if the preference for greater LPA was present in the ancestral species, then Amazon mollies should also exhibit this preference for greater LPA. Amazon mollies would not lose this preference because as an asexual species, they are not subject to sexual selection that may operate in sexual species. Our results contradict the hypothesis of a pre-existing bias for greater LPA in poeciliids.

In conclusion, we found that Amazon mollies did not show preferences for male host species, but our trials using male models suggests that Amazon-*mex* may show preferences based on familiar-shaped males. Further investigation may be warranted for between-population variation in the preferences of Amazon mollies for familiar (sympatric) males. Familiarity due to sexual imprinting may explain why the Amazon-*mex* population has a preference for male body shape. Future studies can manipulate the social environment in which Amazon mollies from different populations are raised to determine the role of sexual imprinting on the preferences of Amazon mollies. Understanding variation in preferences of Amazon mollies for certain male hosts may also provide information on selection for males with greater discrimination against heterospecifics. Although Amazon mollies have been described as having low evolutionary potential due to their asexual reproduction, variation in Amazon preferences could also lead to selection of different lineages if preferences are heritable and beneficial. Amazon mollies may exhibit some level of recombination (mostly likely mitotic rather than meiotic recombination) within its genome (Alberici da Barbiano et al. 2013), which would provide the standing genetic variation necessary for selection.

Moreover, greater levels of genetic variation in an asexual species may account for the population-level variation as proposed here.

Acknowledgements

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