Male permissiveness in a unisexual–bisexual mating complex promotes maintenance of a vertebrate unisexual sperm-dependent species

Laura Alberici da Barbiano [a,*], **Lorena Rangel** [b], **Andrea S. Aspbury** [a] **and Caitlin R. Gabor** [a]

[a] Department of Biology, Texas State University — San Marcos,
San Marcos, TX 78666-4615, USA
[b] Department of Botany and Plant Pathology, Oregon State University,
Corvallis, OR 97331, USA
[*] Corresponding author's e-mail address: la1122@txstate.edu

Abstract
Unisexual–bisexual mating complexes are found when an all female sperm-dependent (gynogenetic or hybridogenetic) species relies on heterospecific males for reproduction. Mistakes in species recognition or discrimination on the part of the males are fundamental for the persistence of unisexual–bisexual mating systems, but should be selected against because mating with heterospecific females does not lead to fitness benefits for the males. Here, we focused on the *Poecilia latipinna–P. formosa–P. mexicana* mating complex, where *P. formosa* is a gynogenetic species of hybrid origin and *P. latipinna* and *P. mexicana* are its parent species and sexual hosts (sperm donors). We examined male mating permissiveness (the propensity to express recognition errors) by presenting males of both sperm donor species with conspecific and heterospecific females in a no choice design. Whereas in prior studies males generally discriminated between heterospecific and conspecific females, we found no evidence for a greater conspecific recognition vs. recognition of *P. formosa* in males of either sperm donor species. This permissive behavior by males could be explained by the close relatedness between *P. latipinna* and *P. mexicana* to *P. formosa*.

1. Introduction

Species recognition and mate quality recognition are the two main processes involved in mate choice (Ryan & Rand, 1993). However, these processes

may interfere with each other when signals produced by heterospecifics overlap with mate signals of high quality conspecifics (Pfennig, 1998). Conflict between species recognition and mate quality recognition may result in mis-mating with heterospecifics, which in turn will lower the fitness of the individuals that engage in heterospecific mating. Recognition is operationally defined to occur when an individual shows a behavioural response to a stimulus. Preference (or discrimination) is said to occur when an individual responds more (or less) strongly to one stimulus over another (Ryan & Rand, 1993). The key difference between the two behavioural responses is that preference and discrimination imply a comparison between stimuli, whereas recognition can occur even if only one stimulus is presented at one time (Ryan & Rand, 1993).

During mate choice, one of the sexes is predicted to be more permissive than the other, depending on the costs of erroneous responses for either sex. Here we define permissiveness as having a higher propensity to engage in recognition errors. In túngara frogs (*Physalaemus pustulosus*) males show a higher degree of permissiveness to calls projected by males on a speaker than by females (Bernal et al., 2007). However, the differences in permissiveness in this system are task-specific rather than only sex-specific. For example male calling is more permissive than female or male phonotaxis (Bernal et al., 2007). In the túngara frog system, female and male permissiveness in approaching an incorrect signal (heterospecific mate or heterospecific chorus) is a costly behaviour. By responding to an incorrect signal, females could risk mating with heterospecific males, and waste a significant amount of their reproductive effort, and males could risk spending a significant amount of energy chorusing with heterospecific individuals to a heterospecific female audience. For a male, joining an incorrect chorus is a more costly mistake than responding to a neighbouring male (Bernal et al., 2009).

Male permissiveness in species recognition is essential for the maintenance of unisexual–bisexual mating complexes. These systems are found when an all-female (unisexual) species relies on sperm from a host species for reproduction (Dawley, 1989). In vertebrates, all known unisexual species are of hybrid origin (reviewed by Avise, 2008) and in most cases use their parent species as hosts (for exceptions see Dawley, 1989; Niemeitz, 2002; Choleva et al., 2008). The sperm-dependent unisexual species is commonly referred to as a sexual parasite and must live in sympatry with the host species because it cannot reproduce by itself; hence mating mistakes on the

part of males of the host species are fundamental for the maintenance of the parasite, but should be selected against due to the costs to males associated with mating with heterospecifics (Coyne & Orr, 2004). If males evolve a strong discrimination against mating with the sexual parasites, the unisexual species would go extinct. However, Heubel et al. (2009) proposed that males that are too discriminating might also suffer a reduction in fitness, as males that are too choosy face the risk of rejecting a conspecific female. This situation is analogous to Reeve's (1989) optimal acceptance threshold model. If a male's threshold is too restrictive, the male runs a high risk of erroneously rejecting desirable recipients (conspecific females), but if a male's threshold is too permissive he runs a the risk of erroneously accepting undesirable recipients (heterospecific females) (Reeve, 1989).

Poecilia formosa (Amazon molly) is a gynogenetic, unisexual species that arose from a hybridization event between a female *P. mexicana* (Atlantic molly) and a male *P. latipinna* (sailfin molly) or an extinct ancestor (Avise et al., 1991; Schartl et al., 1995). *Poecilia formosa* lives in sympatry with at least one of the two parent species throughout its range from the Tampico region in Mexico, to the South East of the USA. Male *P. latipinna* vary in their degree of discrimination towards heterospecifics (Gabor & Ryan, 2001; Gumm & Gabor, 2005; Gabor & Aspbury, 2008; Gabor et al., 2010) and recognition of conspecifics (Aspbury et al., 2010a) between populations, as well as their degree of discrimination towards heterospecifics between seasons (Heubel & Schlupp, 2008). Male *P. mexicana* have a weaker strength of preference for conspecific females when presented together with *P. formosa* than the strength of preference of male *P. latipinna* when tested using the same experimental design (Ryan et al., 1996), but transfer more sperm to conspecifics than heterospecifics (Schlupp & Plath, 2005). Male *P. latipinna*, however, lose their preference for conspecifics when heterospecific females are larger than conspecific females (Gumm & Gabor, 2005), suggesting a conflict between species recognition and mate quality recognition cues. Yet, males from one population of *P. latipinna* sympatric to *P. formosa* have shown consistent preferences over time for conspecific mid-size females, even if the heterospecific stimuli presented in the experiments were larger (Gabor et al., 2010; Alberici da Barbiano et al., 2011).

Dichotomous choice tests have generally been used to examine mate choice by males of the two parent species of *P. formosa*. However, because

signals from both a conspecific and a heterospecific are presented simultaneously to males, most previous studies on male mate choice in this system have examined species discrimination by males. Aspbury et al. (2010) examined species recognition (measured as the sperm priming response and as association time) in male *P. latipinna* when males were given access to chemical cues of females. Male *P. latipinna* from populations both sympatric and allopatric to *P. formosa*, did not differ in their sperm priming response when exposed to either conspecific or heterospecific stimuli. However, males from allopatric populations to *P. formosa* (herein referred to as allopatry) preferred to associate with conspecific chemical cues over no cues, whereas males from populations sympatric to *P. formosa* (herein referred to as sympatry) preferred to associate with no cues than with conspecific chemical cues (Aspbury et al., 2010). These results not only suggest that levels of male permissiveness differ between populations, but also that males might be less or more permissive, depending on the context and type of interaction.

In the present study we tested the hypothesis that males of the parent species of the gynogenetic *P. formosa* (*P. latipinna* and *P. mexicana*) differ in their species recognition. We predict that male *P. latipinna* will be less permissive than male *P. mexicana* because Ryan et al. (1996) found that male *P. latipinna* have a stronger preference for conspecifics than male *P. mexicana* during discrimination tests.

2. Material and methods

We collected fish from two populations in Mexico in 2009: *P. latipinna* sympatric to *P. formosa*: 25°07′N, 98°02′W; *P. mexicana* sympatric to *P. formosa*: 24°04′N, 98°90′W. Only *P. formosa* from the population sympatric to *P. latipinna* were used for testing because we did not have enough *P. formosa* from the other population. We maintained fishes in the lab on a 14:10 light dark cycle using bulbs that simulate natural UV light: 40 W Coralife Day-Max Aquarium daylight, 40 W Coralife Actinic 03 Blue, 40 W Coralife 10 000 K high intensity purified super daylight, and 40 W fluorescent. We isolated females and males for 30 days in all-female and all-male tanks. We fed fishes ISO flake food twice a day supplemented with brine shrimp.

All trials were conducted in a 53-l aquarium (61 × 30.5 × 30.5 cm) divided in four sections marked on the glass of the tank. The outer two sections (10 cm) on either side were separated by Plexiglas dividers with

holes that allowed for access to both visual and chemical cues. The outer sections separated the treatment individuals from the test individuals. The next sections, just inside the Plexiglas, were the association zones (10 cm) (Gabor & Ryan, 2001). A no-choice set-up was chosen because using this design Aspbury et al. (2010) found that these fishes do not always prefer to associate with cues over no cues.

We tested the association preference of 15 males from each population in random order in each of three treatments: (1) female *P. latipinna* vs. no fish, (2) female *P. mexicana* vs. no fish, and (3) female *P. formosa* vs. no fish. We randomly placed females on the left or right stimuli sections and no stimulus on the opposite side. We sized matched stimulus fish (± 2 mm) across the three treatments for each male. We placed the test individual in the middle section of the testing aquarium under a plastic cylinder for a 10-min acclimation period. After acclimation, we removed the cylinder and recorded the subject's behaviour for 10 min. We recorded association time when the subject entered the association zone near the stimulus. There was a 5-min interval between each treatment and the subject was allowed to acclimate again for 10 min.

We defined stimulus association time as time spent with the stimulus minus time not spent associating with the stimulus. If subjects spent more time associating with the stimulus than exploring the tank or the region near the empty chamber, then positive values of stimulus association time were recorded. We performed a χ^2 analysis to determine if the subjects spent more time associating with the stimuli than would be expected if the fish spent the same amount of time in each section. We examined the stimulus association time across species and stimuli using a cross-over repeated measures ANOVA. Significant within and/or between subject effects were further analyzed using Tukey's HSD post-hoc tests. All analyses were two-tailed, and α set to 0.05. We conducted all analyses using R (r-project).

3. Results

Male subjects spent more time associating with the female stimulus than predicted by chance (male *P. mexicana*: female *P. mexicana* $\chi^2_{2,15} = 9.97$, $p = 0.01$; *P. formosa* $\chi^2_{2,15} = 7.59$, $p = 0.02$; female *P. latipinna* $\chi^2_{2,15} = 11.57$, $p = 0.005$; male *P. latipinna*: female *P. mexicana* $\chi^2_{2,15} = 7.55$, $p = 0.02$; *P. formosa* $\chi^2_{2,15} = 8.85$, $p = 0.02$; female *P. latipinna* $\chi^2_{2,15} = 8.55$,

Table 1.

Repeated measures ANOVA with cross-over design on relative association time with main effects subject (ID), subject species (*P. mexicana* or *P. latpinna*), stimulus treatment (*P. mexicana*, *P. latipinna* or *P. formosa*), the order of the treatments for each subject and the interaction between subject species and the stimulus species.

Effect	df	*F*	*p*
Subject	28	1.97	0.02[*]
Subject species	2	2.96	0.09
Stimulus species	2	2.02	0.12
Treatment order	2	1.03	0.31
Subject species × stimulus species	2	3.30	0.04[*]

[*] Significant difference from chance ($p < 0.05$).

$p = 0.02$; corrected $\alpha = 0.025$), suggesting that males of both parent species recognized all three stimuli.

Stimulus association time was not different between subject species, stimulus species, or treatment order, but it was significant between subjects (Table 1). There was a significant interaction between subject species and stimulus species (cross-over repeated measures ANOVA, $F_{2,54} = 3.30$ $p = 0.04$; Table 1). Male *P. latipinna* associated longer with female *P. latipinna* than did male *P. mexicana* (Tukey's HSD, $p = 0.05$; Figure 1), and male *P. mexicana* associated more with female *P. latipinna* than with *P. formosa* (Tukey's HSD, $p = 0.03$; Figure 1). We also verified that there was no effect of sequence order by which each stimulus was presented to males of either species (Wilcoxon rank-sum tests: (subject species-stimulus species): *P. latipinna–P. formosa* $W_{14,4} = 3.8$, $p = 0.4$; *P. latipinna–P. latipinna* $W_{14,4} = 4.5$, $p = 0.3$; *P. latipinna–P. mexicana* $W_{14,4} = 6.6$, $p = 0.2$; *P. mexicana–P. formosa* $W_{14,5} = 7.2$, $p = 0.2$; *P. mexicana–P. latipinna* $W_{14,5} = 7.9$, $p = 0.1$; *P. mexicana–P. mexicana* $W_{14,5} = 5.2$, $p = 0.4$).

4. Discussion

Several studies using a dichotomous choice design have shown that male *P. latipinna* prefer conspecific females over gynogenetic females (Ryan et al., 1996; Schlupp & Ryan, 1997; Gabor & Ryan, 2001; Gumm et al., 2006; Aspbury et al., 2010b), whereas male *P. mexicana* do not (Balsano et al., 1981; Ryan et al., 1996; Gabor et al., 2012). These results led us to hypothesize
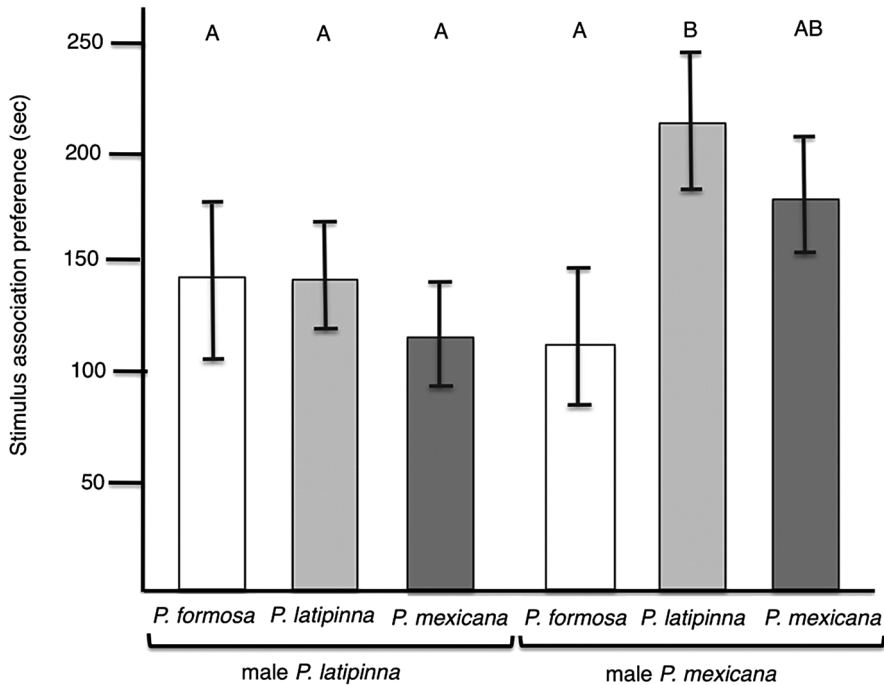
Figure 1. Stimulus association preference (time associating with the stimulus minus time not associating) for the two subject species (*P. mexicana* and *P. latipinna*) across treatments (*P. formosa* = light grey, *P. latipinna* = grey and *P. mexicana* = dark grey). Different letters indicate significant differences using Tukey's HSD post-hoc comparisons.

that male *P. latipinna* are less permissive than male *P. mexicana*. However, the results of the present study do not support this hypothesis. Male *P. latipinna* and *P. mexicana* recognized all three stimuli and associated similarly with conspecific females and *P. formosa*.

Whereas male *P. mexicana* did not spend significantly different amounts of time with conspecific females and *P. formosa*, the same males spent significantly more time associating with female *P. latipinna* than female *P. formosa*. On the other hand, male *P. latipinna* did not associate differently between any of the stimuli. Male *P. mexicana* might have associated more with female *P. latipinna* due to the greater lateral projection area presented by the larger fin of these females (more fin rays) compared to either *P. formosa* or *P. mexicana*. This bias has been found in female *P. mexicana*, and is likely an outcome of a pre-existing sensory bias (McLaren & Rowland, 2009), which might be inherited by male as well as female *P. mexicana*. This

hypothesis could be consistent with the interpretation that male *P. mexicana* do recognize *P. latipinna* as a different species, but spend more time associating with them because of a bias. However, it is also possible that male *P. mexicana* do not recognize female *P. latipinna* as heterospecifics, instead, their association preference towards these females might be due to a novelty effect (Hughes et al., 1999). The male *P. mexicana* that we tested are allopatric to *P. latipinna*; therefore, they had never been in the presence of female *P. latipinna* before testing. Male *P. mexicana* did not associate differently between conspecific females and *P. formosa*. It is possible that male *P. mexicana* recognize *P. formosa* as a different species, but do not associate differently between them. Alternatively, given that *P. formosa* is a hybrid between a female *P. mexicana* and a male *P. latipinna* (Avise et al., 1991), there might be ancestral traits inherited by *P. formosa* from its maternal progenitor that attract male *P. mexicana*.

The presence or absence of other cues (e.g., chemical, tactile, visual), or multiple cues, plays a role in a male's ability to discriminate against heterospecifics or recognize conspecifics. When males were tested for physiological responses to species recognition, Aspbury & Gabor (2004) found that males primed more sperm for conspecifics than heterospecifics if presented with both visual and chemical cues. When presented only with chemical cues, males did not prime sperm differently for the two stimuli (Aspbury et al., 2010a), suggesting that chemical cues alone are not sufficient for conspecific recognition in this species but visual cues are required. However, both visual and chemical cues were provided to the males in the present study, and yet male *P. latipinna* did not associate significantly more with conspecific females than any other female. It is possible, however, that there were different physiological responses between male *P. latipinna* and *P. mexicana*, yet we did not record those in the present study.

The recorded association of males with female *P. formosa* suggests that perhaps this behavior may not be a target of selection. Heubel et al. (2009) suggest that incomplete mate discrimination by males is beneficial to not just the sperm-dependent unisexual females, but also to the males that they sexually parasitize, because it increases the likelihood of not missing conspecific mates. A recent study testing male *P. latipinna* in a mesocosm setting, found that at least 30% of *P. formosa* are fertilized in a population regardless of the frequency of gynogenetic individuals in the population (Alberici da Barbiano et al., 2011), however the insemination of *P. formosa* did not affect the

insemination of female *P. latipinna* and, therefore, did not negatively affect the fitness of females of the host species. Nevertheless, it is still unknown whether individual variation in males' species recognition and discrimination leads to individual variation in fitness in males. Overall the present results, together with results from previous studies, suggest that perhaps selection is acting mostly on the species discrimination ability of males rather than species recognition mechanisms (at least for *P. latipinna*). However, we do not know if species discrimination and species recognition are two distinct mechanisms acted upon separately by selection or if they are correlated traits. During mate choice, individuals could make decisions using both discrimination and recognition, neither discrimination or recognition, or only one process. Phelps et al. (2006) suggest that animals use a single assessment mechanism, and they provide evidence that in túngara frogs, species recognition and mate choice likely stem from a shared perceptual process. If this is the case with the *P. formosa–P. latipinna–P. mexicana* mating complex, then permissive behaviour may be maintained due to the additional costs of possibly rejecting a conspecific.

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