THE ROLE OF EXPERIENCE IN MATING PREFERENCES OF THE UNISEXUAL AMAZON MOLLY

by

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Summary

The all-female/bullet5sh Poecilia formosa uses sperm of Poecilia latipinna or P. mexicana for its gynogenetic reproduction. Normally, P. formosa lives in sympathy with only one of these species. Near Tampico, Mexico, one population of P. formosa is living in sympathy with both sperm-donor species. In the present study, using animated videos as stimuli, we examined whether P. formosa from Tampico show a sexual preference for males of one of the two species. We raised P. formosa females with males of P. latipinna or P. mexicana only, and as a control with males of both species simultaneously. We found that previous experience affects mating preferences in P. formosa. Females tend to prefer males of the species they were raised with.

Introduction

The Amazon molly, Poecilia formosa (Girard, 1859), is one of the few uni-sexual vertebrates (Vrijenhoek et al., 1989). As a poeciliid fish with internal fertilisation P. formosa uses sperm from males of P. latipinna (Le

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Sueur, 1821) or *P. mexicana* Steindachner, 1863 for its gynogenetic reproduction. Sperm serve only as a physiological stimulus to trigger embryogenesis (Hubbs & Hubbs, 1932; see Schlupp *et al.*, 1998 for a review). Normally, no genetic material of the sperm-donor male is transmitted to the offspring, exceptions are the formation of triploids and the introgression of microchromosomes (Schartl *et al.*, 1995a; Schlupp *et al.*, 1998). This gene transfer so far is always unidirectional from the sexual to the unisexual species.

Thus, in Amazon mollies, existing sexual preferences — no matter how they arose — must be maintained by mechanisms not involving genetic coupling of trait and preference, because the gene-pools of the two species are effectively separated. Improving the quality of offspring by choosing males with ‘good genes’ (see Kirkpatrick & Ryan, 1991; Andersson, 1994 for reviews) is therefore highly unlikely. As neither the ‘good genes’ model nor the ‘sexy sons’ model (Andersson & Iwasa, 1996) are applicable here, only direct benefits to the choosing Amazon molly female can explain the maintenance of sexual preferences (see also Marler & Ryan, 1997). Direct benefits to the Amazon molly may not be easy to imagine here, but might be e.g. avoidance of disease transmission which was examined in sticklebacks by Milinski & Bakker (1990) or avoidance of male harassment (Schlupp *et al.*, subm.).

Preferences may also be under no selection and simply an old heritage. In this case deleterious mutations should have accumulated over the estimated 100,000 years of existence of *P. formosa* (Darnell & Abramoff, 1968; Avise *et al.*, 1991; Schartl *et al.*, 1995b) and the trait might be degraded. Marler & Ryan (1997) recently reported of a preference for larger males in Amazon mollies that is indistinguishable from that of the sexual species. In the absence of a clear benefit to the choosing Amazon mollies they concluded that phylogenetic inertia best explains the maintenance of this preference in *P. formosa*.

Amazon mollies live sympatrically with at least one of two sexual species, *P. latipinna* or *P. mexicana*, which are also the parental species of the hybrid *P. formosa* (Avise *et al.*, 1991; Schartl *et al.*, 1995b). In Texas, Amazon mollies live in sympathy with *P. latipinna* and in Mexico they usually live in sympathy with *P. mexicana*. Near Tampico, Mexico, at least one population exists where all three species live in syntopy. Since Amazon mollies mate exclusively with males of other species, and two different host species are available besides other species, that are not sperm-donors (Schlupp, pers.
obs.), species discrimination should be well developed in *P. formosa* (Marler et al., 1997). It should be noted though, that both host species represent sufficient sperm donors, thus, theoretically there is no need for Amazon mollies to have a preference for one of them. In other words, one would predict a preference for suitable host males, but not for a specific host species. An especially well suited population to test for species preferences in the Amazon molly is the one living in sympathy with both *P. latipinna* and *P. mexicana* near Tampico, Mexico. In populations with one host only one would always have the absence of a second host as confounding variable (see Landmann et al., in press). A mechanism by which such a preference can be maintained is sexual imprinting.

In the present study, we examine whether *P. formosa* show a preference for males of one of two host species, and whether previous sexual experience has an influence on mate preferences.

In our case three different models of mate choice ontogeny make the following differential predictions: if any kind of learning is involved one would predict a preference for the male type the Amazon mollies were raised with. If the preference is inherited from the single hybridization event that formed *P. formosa*, one would predict a uniform preference for either *P. mexicana* or *P. latipinna*. Given that *P. mexicana* was the maternal ancestor (Avise et al., 1991; Schartl et al., 1995b) a preference for *P. mexicana* males would be predicted, but this is rather speculative. This issue was also discussed by Ryan et al. (1996) in relation to male mating preferences. A third model has been suggested by Rowland (1989), who found preferences for visually stronger stimuli, in our case this would predict a preference for *P. latipinna* that generally have a longer and higher dorsal fin, resulting in a larger body surface area. The two sexual species also have different colour patterns. The second and the third hypothesis can result in identical findings. The null model for all cases would be no preference.

To provide an uniform stimulus, we used video images instead of live males as stimuli in female preference tests. This way some important variables (e.g. activity, motivation and courtship rate) of the stimulus can be controlled during the experiment. Some recent studies in spiders (Clark & Uetz, 1990; McClintock & Uetz, 1996), toads (Roster et al., 1995) and fishes (McKinnon, 1995; Rowland et al., 1995; Rosenthal et al., 1996; Körner & Parzefall, 1997; Landmann et al., in press) showed that video images can be used for studying animal behavior.
Methods

Subjects and housing

The founder fishes of the study population were collected in 1995 near Tampico, Mexico, and were kept under standard conditions in the laboratory in large, randomly outbred populations (for details see Schlupp et al., 1991; Schlüter et al., 1998). Tank sizes ranged from 25 l to 600 l. Fish were maintained at 24-27°C under a 12:12 h light:dark illumination cycle. All fish were fed flake food *ad libitum* twice daily, supplemented with live brine shrimp (*Artemia* nauplii). For our experiments 39 two weeks old, laboratory-born Amazon mollies from a population (M IV/5) of syntopy with both *P. mexicana* and *P. latipinna*, were divided randomly into three groups of thirteen each. One group was raised with two *P. latipinna* males, another group with two *P. mexicana* males, and a third group was raised together with one *P. latipinna* and one *P. mexicana* male. All males were from the M IV/5 population. Thus, all juveniles had identical exposure to males. Ten of the 13 juveniles raised with *P. latipinna* males died before testing. This treatment was repeated four months later, in which again three of the ten additional fish died. Data from these two groups were analysed together. At the time of the experiments, the females were seven to nine months old and sexually mature.

Videorecording and editing procedures

One adult male of *P. latipinna* and one of *P. mexicana* were placed separately in a transparent plastic tank (50 × 25 × 10 cm). A turquoise sheet of paper was attached to the backside of the tank to provide a uniform background. The tank was illuminated overhead by two 500-watt bulbs (3200 K). Recordings were made using a Panasonic AG 450 S-VHS camcorder with a polarisation filter and Sony S-VHS VXSE 240 tapes. The males were recorded while swimming freely across the background from lateral view and while turning to change direction. The males used for filming were never exposed to the test females.

The sequences were imported to an Apple PowerPC 6100/60 AV using ‘Fusion Recorder’ from a Panasonic NV-FS 90 S-VHS recorder. Two sequences were used to create an endless loop with ‘Macro Media Director’. The one showed a straight movement from the left to the right, the other showed the subsequent turn. The sequence with the straight movement included 14 frames; the turn sequence included 54 frames. By using the mirror image of every single frame, we created two additional sequences that showed the straight movement and the turn in the other direction. To reduce the amount of data to be processed by the computer and to delete the water level and the walls of the tank from the videos, a rectangle around the fish, including parts of the original background, was cut out using ‘Adobe Photoshop’. These rectangles were put in front of a virtual background, which was almost identical in colour with the original background for a human viewer. With these four sequences, we were able to create an endless standardised movement pattern of a *P. latipinna* male and a *P. mexicana* male. On the playback monitor the males had a virtual size of about 55 mm (standard length) which is not a supernatural size. The digitised endless loops were exported from a PowerPC to a Pioneer VDR 1000 Laser Disc Recorder, and from the laser disc to Sony VXSE 240 S-VHS cassettes.
Choice test procedures

The experimental tank (60 × 35 × 30 cm) was placed between two Mitsubishi CT-15MS1 monitors, receiving the video signal from a Panasonic NV-FS 200 S-VHS deck and a Panasonic NV-FS 90 S-VHS deck. Identical video players were not available during the test period.

The tank was divided into three equal sections by markings drawn on the front and the backside of the tank. The central section was defined as a neutral zone. The sections near the monitors were defined as preference zones. Visual preferences in an experimental set-up like this are a good indicator for mate choice decisions (Kodric-Brown, 1992).

An adult *P. formosa* female was placed in a transparent Plexiglas cylinder in the neutral zone, then the playback was started. After five minutes of acclimation, the Plexiglas cylinder was carefully removed. The female was observed for five minutes, and the time it spent in each section near the monitors was recorded. Then, the videotapes were switched and the experiment was repeated to detect a potential side bias. We decided *a priori*, that a side bias occurred when a female spent more than 80% of the time in the preference zone on the same side for both trials. Such trials were not included in the analysis. Trials in which the female spent less than 50% of the observation period in the two preference zones were also excluded, because such females were assumed not to be motivated to choose. Each test female was used only once. We used non-parametric tests and all *p*-values are two-tailed.

Results

Amazon mollies raised with *P. latipinna* males

In this experiment, ten females of the two groups raised with *P. latipinna* males were tested. One showed a side bias. The remaining nine females (Fig. 1) spent significantly more time near the *P. latipinna* male (Wilcoxon test: \(z = -2.67, p = 0.0077, N = 9\)).

![Fig. 1](image_url)  
Fig. 1. Time spent near the *P. latipinna* and *P. mexicana* males. Amazon mollies were raised with *P. mexicana* males only. The line in the box represents the median, lower and upper end of the box indicates the 25% and 75% value. The two whiskers are the 10% and the 90% value.
Fig. 2. Time spent near the *P. latipinna* and *P. mexicana* males. Amazon mollies of this group were raised with *P. latipinna* only. The line in the box represents the median, lower and upper end of the box indicates the 25% and 75% value. The two whiskers are the 10% and the 90% value.

**Amazon mollies raised with *P. mexicana* males**

Two of the 13 fish died before testing, two showed a side bias during testing. The remaining nine Amazon mollies showed no significant preference (Fig. 2) but a tendency to remain near the image of the *P. mexicana* male (Wilcoxon test: \( z = -1.60, p = 0.11, N = 9 \)).

**Amazon mollies raised with *P. mexicana* and *P. latipinna* males**

The Amazon mollies raised with both types of host males showed no significant preference (Fig. 3) (Wilcoxon test: \( z = -0.84, p = 0.40, N = 8 \)). Three of them died, one showed a side bias and one spent less than 50% in the preference zones. Four of the tested females individually preferred the *P. latipinna* male, three the *P. mexicana* male, only one was truly undecided and spent about the same amount of time with each male.

**Comparison between experimental groups and control group**

In a comparison between the three groups (Fig. 4), we detected a significant difference in the extent of preference for the two types of males between the group raised with *P. latipinna* and the group raised with *P. mexicana* only (Kruskal-Wallis H-test: \( X^2 = 8.246, p < 0.02, N_1 = 8, N_2 = 9, N_3 = 9 \); Post-hoc Dunn’s test: \( p < 0.05 \)). For this comparison we used the difference between the time near the *P. latipinna* stimulus and the *P. mexicana* stimulus.
Fig. 3. Time spent near the *P. latipinna* and *P. mexicana* males. Amazon mollies of this group were raised with both host species. The line in the box represents the median, lower and upper end of the box indicates the 25% and 75% value. The two whiskers are the 10% and the 90% value.

Fig. 4. Comparison between the three groups. Control: group raised with both host species; mexicana: group raised with *P. mexicana* males; latipinna: group raised with *P. latipinna*. The line in the box represents the median, lower and upper end of the box indicates the 25% and 75% value. The two whiskers are the 10% and the 90% value.

**Discussion**

Depending on the rearing conditions Amazon mollies develop different sexual preferences. From the three postulated hypotheses that might explain the origin of such preferences, two can be rejected. One hypothesis, which predicted a uniform preference for one of the species in *P. formosa* due to a single hybridization, can be rejected, because the three groups show significantly different preferences. A uniform preference for *P. latipinna* because of the greater visual stimulation, as predicted by the third hypothesis did also not occur. The first hypothesis predicted a preference for the known species, which is found significantly in the group raised with *P. latipinna*.
and as a tendency in the group raised with *P. mexicana*, while the females of the third group show variable individual preferences. Thus, our data support the first model, which assumes a learned preference. This is especially striking, as we used a population that in nature occurs with both sperm-donor species and theoretically does not need a species preference. Supporting results were found by Landmann *et al.* (in press), who examined species preferences in populations with only one sperm donor species and were able to find a preference for *P. latipinna* in a population that naturally occurs with *P. latipinna* and was also raised with this species. An exploratory data analysis revealed that the non-significant result of the group raised with *P. mexicana* is due to the preference of a single female preferring *P. latipinna* and would become significant if this female would be excluded. However, this is very speculative, especially given the small sample size.

A mechanism that could account for this preference for familiar types of males in the Amazon molly is sexual imprinting. Imprinting (Lorenz, 1935) implicates the presence of at least one sensible phase and that the process of learning is irreversible (but see Immelmann & Suomi, 1982). Studies examining imprinting have focused mainly on birds (for reviews see Bolhuis, 1991; ten Cate *et al.*, 1993) and mammals (Sambraus & Klee, 1975; Hudson, 1993; Penn & Potts, 1998). In fishes, sexual imprinting has been shown only in a few species including the polymorphic cichlid *Haplochromis burtoni* (Sjölander & Fernö, 1973; Crapon de Caprona, 1982), *Cichlasoma nigrofasciatum* (Weber & Weber, 1976) and the poeciliid *Xiphophorus maculatus* (Fernö & Sjölander, 1973). Interestingly, sexual imprinting could also explain the results of the group raised with both types of males if one assumes, that only one of the two available phenotypes can be learned by imprinting, pointing towards a physiological limit. Sexual misimprinting with the consequence of heterospecific mate choice and thus hybridization has been shown by Grant & Grant (1998) in Darwin’s finches.

Intraspecific influence of previous sexual experience on mate preference has been reported before in guppies (*Poecilia reticulata*). Rowden (1994) found that guppies raised with models of males of three different colours affects female choice, but does not lead to a preference for that colour automatically. One problem with this study was that the colours used may have misrepresented the colours as they are seen by the guppy and thus may not have been adequate stimuli (see e.g. Bennett *et al.*, 1994; Fleishman *et al.*, 1998). In another study Breden *et al.* (1995) showed, that the presence of
colourful males during female development can lead to female preferences different from those of females raised with non-colourful males. This suggested experience to be involved in the shaping of sexual preferences, but the mechanism behind this effect remained unclear. Rosenquist & Houde (1997) found an effect of prior experience on subsequent mate choice if females were raised with two males of different coloration, but not if females had been exposed to high-orange males or low-orange males alone. Together with our report, these studies show that experience clearly influences the ontogeny of mate preferences, but so far no consistent picture emerged. Clearly this requires more research.

Conditioning as another mechanism of learning in male mate choice was shown by Jenkins (1997) and Jenkins & Rowland (1997) in sticklebacks (*Gasterosteus aculeatus*).

The weaker preference for *P. latipinna* males compared to the preference for *P. mexicana* males parallels results of Ryan et al. (1996) who found that *P. latipinna* males discriminate stronger between conspecific females and Amazon mollies than *P. mexicana* males.

The two groups with only one species of males present, showed different mortality during the raising period. 13 of 23 Amazon mollies raised with *P. latipinna* died, whereas only 2 out of 13 Amazon mollies raised with *P. mexicana* died. The presence of a *P. latipinna* male in the third group did not lead to a considerable increase in mortality (3 out of 13). It would be intriguing to assume a host specific effect here, however this is not supported by our general experience breeding Amazon mollies.

Recently, the use of video stimuli has become more common (for a review see Rosenthal, in press). Some of the problems which might occur because of the differences of the visual system of humans and animals are discussed by Fleishman et al. (1998). Our study clearly supports the utility of this technique for studying preferences in mollies.

The present study does not offer a resolution to the question why a seemingly unnecessary preference is maintained in Amazon mollies, nor does it explain how it arose. Marler & Ryan (1997) documented that Amazon mollies show a preference for large males, just as their sexual ancestors. Several studies showed that dispensable genes can be maintained in *P. formosa* (Turner & Steeves, 1989; Schartl et al., 1991; Schlupp et al., 1992). Thus, it is also possible, that the sexual preference reported here is no longer adaptive and not maintained by selection.
The results of our study show that, under some circumstances, Amazon mollies prefer special types of males, although this will decrease the number of potential mates apparently without increasing the number or the fitness of the offspring. The adaptive value of this behavior remains unclear so far and clearly warrants further investigation.

References


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