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Male mate choice and sperm allocation in a sexual/asexual mating complex of *Poecilia* (Poeciliidae, Teleostei)

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Male mate choice is critical for understanding the evolution and maintenance of sexual/asexual mating complexes involving sperm-dependent, gynogenetic species. Amazon mollies (*Poecilia formosa*) require sperm to trigger embryogenesis, but the males (e.g. *Poecilia mexicana*) do not contribute genes. Males benefit from mating with Amazon mollies, because such matings make males more attractive to conspecific females, but they might control the cost of such matings by providing less sperm to Amazon mollies. We examined this at the behavioural and sperm levels. *P. mexicana* males preferred to mate with, and transferred more sperm to conspecific females. However, if males mated with *P. formosa*, sperm was readily transferred. This underscores the importance of male choice in this system.

Keywords: Amazon molly; asexuality; gynogenesis; sperm expenditure; mating preference

1. INTRODUCTION

The evolutionary significance of sexual reproduction is still one of the major problems in evolutionary biology (West *et al.* 1999). Mating systems including an asexual all-female and a related sexual species have recently provided insights into the evolutionary dynamics of asexuality versus sexuality (Schlupp *et al.* 1994, 2002; Vrijenhoek 1994; Schartl *et al.* 1995). Particularly interesting is how such systems can be stable. Asexual all-female vertebrates often depend on sperm of heterospecific males (gynogenesis; Beukeboom & Vrijenhoek 1998). Sperm serves only to trigger embryogenesis, but male genes are not used, so males have little to gain from these matings. In such systems male mate choice may be the key to stability and has been the focus of empirical research (Schlupp *et al.* 1991, 1994; Loyning & Kirkendall 1996; Ryan *et al.* 1996; Schlupp & Ryan 1997; Gabor & Ryan 2001).

In the Amazon molly (*Poecilia formosa*), males of three species provide sperm (*P. mexicana*, *P. latipinna*, *P. latipunctata*; Schlupp *et al.* 2002). The sexual males from these species prefer conspecific females, yet under certain circumstances they do mate with Amazon mollies (Schlupp *et al.* 1991; Ryan *et al.* 1996). Males gain indirect benefits of mating with heterospecific females when conspecific females copy the mate choice by Amazons (Schlupp *et al.* 1994; Heubel *et al.* submitted). This necessitates that males interact visibly with Amazons, but it does not require actual sperm transfer. Recently, Aspbury & Gabor (2004) reported that *P. latipinna* males prime more sperm after visual exposure to a conspecific as compared with a *P. formosa* female. This indicates that males can adjust their investment into sperm, but leaves open the important question of whether males actually transfer less sperm to Amazon mollies.

The present study tested for male choice at sperm level and measured the amount of sperm *P. mexicana* males provided to sexual and asexual females.

2. METHODS

(a) *Study organisms*

Poecilia mexicana and *P. formosa* were collected near Tampico, Tamaulipas, Mexico. Fishes were maintained as mixed-sex single-species laboratory stocks in 100–200 l aquaria at 25 °C under an artificial 16 : 8 h illumination cycle. They were fed twice a day *ad libitum*. To obtain virgin females, we isolated offspring after birth and removed males from the tanks before sexual maturity. Male mollies were identified by a transformed anal fin, the gonopodium. Males were randomly taken from the stock tanks (standard length, mean ± s.d.: 30.73 ± 4.89; range: 24.5–40.0). Prior to the tests, the males were isolated in 25 l aquaria for 24 h. All fishes were measured to the nearest 0.5 mm after the test.

(b) *Choice tests*

The test tank (49×24×24 cm) was filled with water of 24–25 °C. The bottom was covered with bright gravel. Three sides were covered with black plastic foil. Illumination was provided by a 60 W incandescent bulb placed 30 cm above the tank, in addition to diffuse daylight. The observer sat approximately 2.5 m from the tank. To initiate a trial, two virgin females of each species, matched for size, were introduced into the test tank (*P. mexicana*: 38.22 ± 4.55 mm; *P. formosa*: 37.68 ± 3.59 mm). A male was introduced into a clear Plexiglas cylinder (8.5 cm diameter) in the centre. After 15 min for acclimation, the cylinder was gently removed, and all three fishes could interact. We scored nipping frequency and number of copulation attempts for each female during 30 min. Nipping typically, but not always, precedes copulations (Parzefall 1969). All fishes were used only once in the experiments.

(c) *Sperm counts*

(i) *Females*

Following the observations, the fishes were immediately anaesthetized using MS222. Fishes were then placed in a Petri dish with the head and gills embedded in wet paper. Sperm was transferred into Eppendorf tubes containing 100 µl 0.9 M NaCl solution. Sperm was retrieved from the females under a stereo-microscope by introducing a small flexible plastic micropipette into their genital opening. The pipette contained an aliquot of the 100 µl NaCl solution. The solution was gently injected to flush the female genital tract. To retrieve the sperm-containing solution we removed all of the solution directly from the female genital opening and the genital tract. This procedure was repeated three times (Evans *et al.* 2003). All of the solution was transferred into the original Eppendorf tube and combined to 100 µl. This solution was gently shaken and an aliquot was used to count sperm.

(ii) *Males*

To measure the amount of sperm that males retained, the gonopodium was swung forwards three times to stimulate sperm release. Then, the body sides were massaged from the dorsal to the base of the gonopodium using cotton tips and a pair of tweezers. Sperm was removed at the base of the gonopodium. It was transferred into a tube with 100 µl NaCl solution using a micropipette. Since very small amounts of sperm were not easily visible, the base of the gonopodium was rinsed with a drop of the 100 µl NaCl solution and this was transferred into the tube as well.

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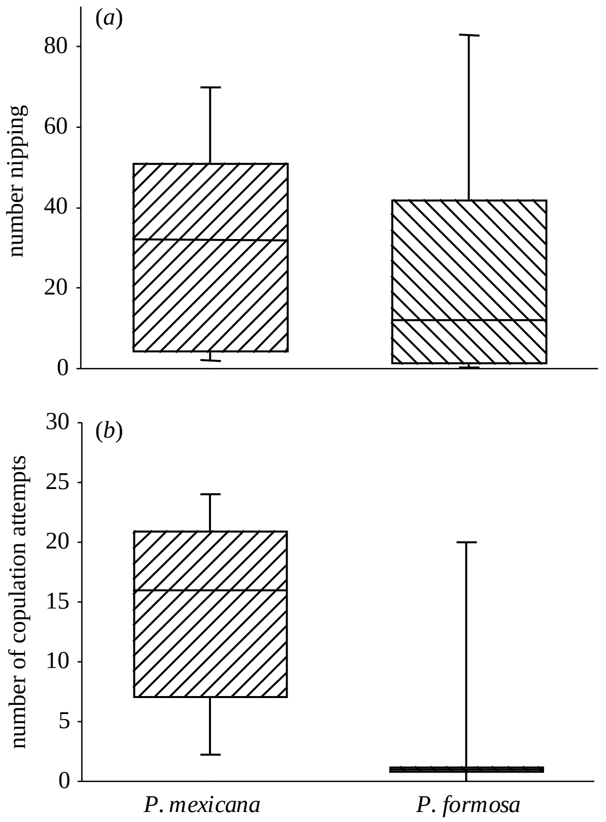

Figure 1. (*a*) The frequency of the sexual behaviours nipping and (*b*) copulation attempts by *P. mexicana* males. A male could interact with a conspecific (left) or an Amazon female (right). Boxplots show the median (middle line), the interquartile range (box) and the 5% and 95% values (whiskers).

After these treatments, all fishes were immediately transferred to aerated aquaria. No mortality was associated with this treatment.

(iii) *Counting sperm*

Two drops of the sperm-containing 100 µl solution were transferred to a Thoma counting chamber and covered by a slide. Sperm was equally distributed. The total number of spermatozoa was counted in six randomly chosen fields and the average value for one field was calculated. To increase accuracy, in case of very low sperm counts, up to three more randomly chosen fields were counted. Sperm touching the upper and on the right line of a field were counted for the sample, those touching the lower and left line were omitted. From the resulting data for the known volume of the counting chamber, the total amount of sperm (dissolved in 100 µl) was calculated. Since our data were not normally distributed, we used non-parametric statistics throughout. All *p* values are two-tailed.

3. RESULTS

(a) *Male mating behaviour*

The males showed nipping in 17 of the 20 trials. They showed significantly more nipping with conspecific females than with *P. formosa* females (Wilcoxon signed-rank test: $T=31$, $n=17$, $p=0.031$; figure 1*a*) and copulated more often with the conspecific females ($T=2$, $n=8$, $p=0.025$; figure 1*b*). Copulations were observed in eight cases.

(b) *Sperm allocation*

In seven cases we retrieved sperm from both females. Significantly more sperm was recovered from *P. mexicana* females (Wilcoxon signed-rank test: $T=3$, $n=7$, $p=0.036$; figure 2). In one trial, four copulations were observed, but no sperm was retrieved from this *P. mexicana* female.

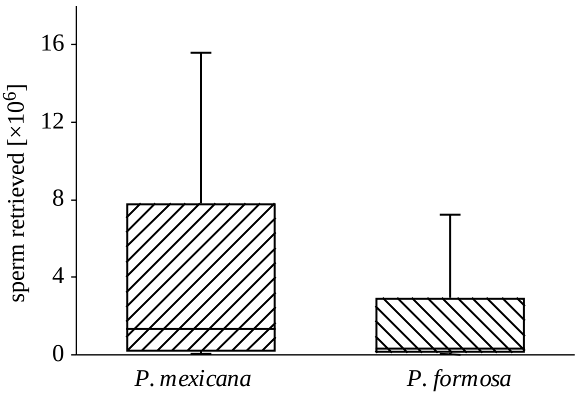

Figure 2. The total amount of sperm retrieved from conspecific (*P. mexicana*, left) and Amazon molly females (right). For explanation of boxplots see figure 1.

(c) *Male size and available sperm*

The total amount of sperm retrieved from a male was positively correlated with male body size (Spearman rank-correlation: $r_s=0.44$, $n=20$, $p=0.049$). Males transferred, on average (mean ± s.d.), 13.8 ± 26.6% of their total sperm to the two females combined.

4. DISCUSSION

Poecilia mexicana males could mate with either a conspecific female or a female of the ‘sexual parasite’, *P. formosa*. Males preferred to mate with conspecific females and transferred less sperm to the asexual females. In our study, the focus male interacted with two females. We measured the outcome of these interactions, but cannot say with certainty which mechanism lead to the outcome. In such a situation, access to females reflects both male preferences (Ryan *et al.* 1996), and female–female interactions (Schlupp *et al.* 1991; Foran & Ryan 1994). Potentially, differences in sperm retrieval reflect morphological differences between the two species, but given that *P. mexicana* is the maternal ancestor of the hybrid *P. formosa*, major differences between females of the two species appear unlikely. Our subjective impression was that sperm recovery was equally easy for the two species.

Several males did not copulate with any female. This was not anticipated and may reflect ongoing conflicts over access to the sexual female. However, our experimental design has the advantage of allowing the study of a situation that prevails in nature, mimicking the typically occurring mixed groups. Not all copulations actually led to sperm transfer. Consequently, we did not calculate sperm transfer per copulation.

Our prediction was that *P. mexicana* males would show some apparent copulations with Amazons to increase their attractiveness by heterospecific mate copying (Schlupp *et al.* 1994; Heubel *et al.* submitted), but they would benefit from transferring less sperm during these copulations. For example, in the hemiclonal water frog (*Rana esculenta*), ‘host’ females reduce the number of eggs laid with inappropriate males (Reyer *et al.* 1999). Evans *et al.* (2003) demonstrated that *Gambusia holbrooki* males can adjust sperm expenditure in response to sperm competition, providing a potential mechanism. We found that the level of sperm transferred to Amazon mollies agrees with the level of male mating preference. Hence, our results suggest that mate choice happens at the level of pre-copulatory mating preferences. This is in agreement with another study (Aspbury & Gabor 2004), in which differential priming of sperm by *P. latipinna* males as a function of visual exposure to a conspecific or a heterospecific female was reported.

Availability of sperm increased with increasing male body size (e.g. Evans *et al.* 2003 for *G. holbrooki*). Increased sperm availability in large males might contribute to the maintenance of a preference for larger males in *P. formosa*. Male size did not influence the number of offspring when one *P. latipinna* male and one Amazon molly female were housed together (Marler & Ryan 1997). However, under more natural conditions, i.e. in the presence of females of the 'host species', copulations with large males might provide sufficient sperm more readily.

In conclusion, *P. mexicana* males are choosy about the kind of females they mate with. However, if they mate, sperm is readily transferred to either kind of female. In mollies, sperm appears not to be limited under natural conditions (Balsano *et al.* 1989), and the cost of occasional sperm transfer to a heterospecific female may be relatively low. Our study underscores the central role of pre-copulatory male mate choice in this mating system.

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