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Insemination efficiency of two alternative male mating tactics in the guppy (*Poecilia reticulata*)

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In this study we compared the insemination efficiency of two alternative mating tactics (courtship and sneak mating) in the guppy *Poecilia reticulata* by quantifying the number of sperm delivered during a copulation. During a single copulation, guppies delivered between zero and 92% of the sperm available, as determined by mechanically stripping the males' sperm reserve at rest. The absolute number of sperm delivered after courtship was three times larger than that delivered through sneak mating; nonetheless, the variance was large with both tactics and the two distributions largely overlapped. The number of sperm available at rest increased with male size. With both tactics, the number of sperm delivered was positively correlated with the sperm available. Contrary to courtship copulations, in sneak copulations there was no correlation between the number of sperm delivered and male size. However, once the data were standardized for sperm reserve, small males delivered a larger proportion of their available sperm during sneak copulation. The rate of sexual acts (sigmoid and thrust rate) before copulation was not correlated with the number of sperm available. After the occurrence of a copulation in both the courtship and sneak copulation groups, the sexual activity of the male decreased in proportion to the amount of sperm he previously inseminated.

Keywords: alternative tactics; sperm competition; sexual selection; guppy; *Poecilia reticulata*

1. INTRODUCTION

In many animal species competition between males does not end after mating, but continues afterwards when the sperm from two or more males compete for the fertilization of a given set of ova. Because of sperm competition (Parker 1970), male reproductive success depends not just on a male's ability to acquire mates, but also on other factors such as the number and timing of copulations with the mate and the number of sperm he is able to allocate to each mating. As a consequence, species with intense sperm competition are usually characterized by a high gonadal investment and the production of a high sperm count per ejaculate (Birkhead & Möller 1998; Petersen & Warner 1998). Sperm competition has been recognized to affect the evolution of male and female traits and to shape mating systems (Möller 1998). In particular, sperm competition is probably important in the evolution of alternative mating tactics, since it is expected to reduce the variance in male mating success by lowering the mating success of large, dominant males. Alternative tactics determine situations in which small males can also obtain some mating success through sneak copulations by allocating energy to mate search or sperm production instead of growth and mate monopolization (Taborsky 1994).

Poeciliids, a family of live-bearing fish, have been used as a model for the study of sexual selection, in particular of the evolution of secondary sexual characters through female choice. Among these, the guppy *Poecilia reticulata* is one of the most studied species (Houde 1997). Female guppies are sexually receptive on average two to three days per month. During this period they usually mate with two or three males, but since males have very high sexual activity, females receive continued, apparently unwanted mating attempts (Houde 1997). Sperm from copulations are stored in folds lining the ovary and gonoduct, where they remain viable for months (Constantz 1989). Sperm stores are usually the result of mixing of sperm from more than one male, resulting in the multiple paternity of broods (Hildemann & Wagner 1954).

Poeciliid males show two alternative mating tactics.

(i) They can perform a sigmoid display in front of females to persuade them to mate. A receptive female responds by swimming in a tight circle and glides towards a male exposing her abdomen and facilitating insemination. The male then introduces his copulatory organ, the gonopodium, into the female's genital pore. Only post-partum and virgin females are usually receptive to males (Houde 1997).
(ii) If the female is not receptive, males more frequently employ a form of coercive mating: the male approaches the female without any display, swings his gonopodium forward and tries to insert it into the genital pore of the unwilling female. This form of sneak copulation is also termed gonopodial thrusting (Liley 1966).

Male guppies exhibit the two tactics within the same individual. The degree to which each tactic is adopted is influenced by various factors, including female willingness to copulate, the presence of predators, gonopodium

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length, the sex ratio and water turbidity (Houde 1997). While it has been demonstrated that female guppies exert mate choice on the basis of male coloration, morphology and behaviour, the sneaky mating tactic has received much less attention (but see Magurran 1996). Females are known to receive sneaky mating attempts with high frequency (Magurran & Seghers 1994*a*), but the insemination success of the two tactics has never been compared. In previous work performed in the eastern mosquitofish (*Gambusia holbrooki*), which exhibits only the sneak mating tactic, it has been shown that the insemination success of gonopodial thrusting is not trivial and that small males are at an advantage in this form of coercive copulation (Pilastro *et al*. 1997).

In this study we compared the insemination success, i.e. the number of sperm delivered during a single copulation, of male guppies adopting sneaky copulation (gonopodial thrusting) with that of males obtaining copulation with female consent after courtship. It seems reasonable to expect that the number of sperm delivered during a copulation is higher when a female cooperates than with sneak copulation. On the other hand, one may also expect that gonopodial thrusts are sometimes effective in inseminating the females, given the large proportion of time that male guppies invest in gonopodial thrusting attempts (Magurran & Seghers 1994*b*) and the costs associated with sexual activity (e.g. Godin 1995). A second aspect of sperm competition in guppies regards male body size. It has been shown that there is a positive correlation between body size and available sperm in male guppies (Matthews *et al*. 1997). One may therefore expect that large males inseminate larger numbers of sperm. On the other hand, small size has been demonstrated to confer a mating advantage to males in sneak copulation (Bisazza & Pilastro 1997; Pilastro *et al*. 1997). Thus, small males may be able to compensate for their smaller sperm production by a greater ability when using this tactic. We therefore measured the effect of a male's body size on insemination success with each tactic. Finally, a recent paper suggested that males invest in sexual activity in relation to the quantity of sperm they produce, regardless of mating tactic (Matthews *et al*. 1997). Since sexual activity is probably costly to males (Godin 1995), male decisions should be influenced not only by their sperm production, but also by the occurrence of recent copulations. We therefore measured male sexual activity after a copulation in relation to the quantity of sperm delivered and the quantity of sperm still available after copulation to see whether males tune their sexual activity on the basis of the amount of sperm still available and the benefit of remating with the same female.

2. METHODS

The subjects used in this experiment were descendants of fish collected from a feral population near Manaus (Brazil) and maintained unselected in the laboratory. Experimental males and females were stocked together in 150 l aquaria kept at constant temperature (26 °C ± 1) and photoperiod (06.00–21.00 h). To ensure that females had no sperm in their gonoduct from previous matings all females were isolated from males for at least ten days before the test. Males were female deprived for 48 h. The fish were fed to satiation before the beginning of the test.

To compare the two mating tactics, we wanted to exclude the possibility that observed differences, if any, were due to differences in males and not in the tactic *per se*. Test males were therefore randomly assigned to one of the two following experimental conditions. In tests in which we wanted to obtain sneak matings, we used mid-cycle, pregnant females; one female was introduced into an experimental aquarium in which one male had been acclimated for 10 min. In tests in which we wanted to obtain cooperative copulations, we used post-partum females or females deprived of males for at least one month (mean ± s.d. = 56.4 ± 17.2 d). Post-partum females were tested two days after parturition. The test female was introduced into the experimental aquarium and allowed to acclimate for 30 min before the male was introduced. The test males were roughly matched in size before being assigned to one of the two experimental conditions, to have similar averages and variances of male size.

For the experiment we used 150 l aquaria similar to the stock ones. After the beginning of a trial, the test male and female were continuously observed. The number of courtship displays and sneak mating attempts (gonopodial thrusts) were recorded until copulation occurred and for 5 min after copulation. After a successful copulation, the male guppy rapidly jerks his body up and forward; gonopodial contacts between a male and female not followed by jerking never lead to insemination (Liley 1966). Accordingly, copulation was judged to occur when the male touched the female's gonopore and jerked violently at least a few times afterwards. A cooperative copulation was judged to occur when it was preceded by a male sigmoid display and by the typical female gliding approach to the displaying male (Liley 1966). Cooperative copulations were obtained only with post-partum and long-term deprived females. If the male was not able to obtain copulation, the trial was interrupted after 45 min in the case of post-partum or long-term deprived females and after 60 min in the case of mid-cycle females. As a measure of the sexual behaviour of males before copulation we considered only observations in the 5 min preceding copulation. The change in sexual behaviour of the male after copulation was expressed as the ratio between the number of sexual acts (sigmoid displays and gonopodial thrusts) 5 min before and 5 min after copulation.

Ten minutes after a copulation occurred, the inseminated female was captured and anaesthetized with MS222. The female's standard length (SL) was measured to the nearest 0.5 mm and the female was checked for the presence of sperm, following Pilastro *et al*. (1997). Using a glass micropipette, *ca*. 10 μl of physiological solution (0.9% NaCl) was injected into the female's gonoduct. The solution was drained and the operation was repeated five times. The volume drained from the female's gonoduct was diluted into distilled water, sperm were coloured by adding rose bengale dye to the solution and then fixed by adding formalin. The solution was passed through a Millipore filter (0.22 μm pore size) under vacuum. The filter paper was dried, placed on a glass slide and cleared with immersion oil (Shapiro *et al*. 1994). The number of sperm was counted under a light microscope at a magnification of ×400 in an area measuring $0.122\ \text{mm} \times 0.122\ \text{mm}$ ($= 0.01488\ \text{mm}^2$) or $0.244\ \text{mm} \times 0.244\ \text{mm}$ ($= 0.05954\ \text{mm}^2$), according to the sperm concentration. The count was repeated for each of ten separate portions. The mean value of these readings was then multiplied for the total filter area ($1133.5\ \text{mm}^2$). The sperm count was carried out blind of experimental group. To control for the possibility that the sperm collected in the females' gonoducts did not derive from previous copulations, 15 females previously kept with males were

Table 1. *Morphological and behavioural characteristics of males and females used in the two experimental groups*

(Means ± s.d. are given.)

	courtship	*n*	sneak	*n*
male length (SL) (mm)	18.70 ± 2.51	24	19.00 ± 2.23	27
male relative size (SL male/SL female)	0.53 ± 0.08	24	0.58 ± 0.14	27
female length (SL) (mm)	35.30 ± 3.45	24	34.10 ± 6.08	27
courtship displays (min^{-1})[a]	0.93 ± 0.51	24	0.29 ± 0.39	27
thrust attempts (min^{-1})[a]	0.75 ± 0.66	24	1.30 ± 0.89	27
total sexual acts (min^{-1})[a]	1.68 ± 0.93	24	1.59 ± 1.15	27
sperm stripped ($\times 10^6$)	5.37 ± 4.46	20	6.10 ± 3.79	19

[a] Average of the 5 min before copulation.

deprived of males for 12 days and then checked for the presence of sperm. The same procedure was also repeated on a group of eight post-partum females that were also previously deprived of males for at least 12 days. We could not find sperm in the gonoducts in any of these 23 females. Twelve out of 51 males were anaesthetized and measured immediately after the end of the trial. The remaining 39 were isolated from females for two days to allow them to restore their sperm reserve, anaesthetized, measured, the sperm at rest collected following Kuckuck & Greven (1997) and counted as above. All individual fish recovered well after sperm sampling and were put back into their original stock aquaria.

Statistical analyses were performed using the statistical software program SPSS 8.0 (Norušis 1993). The sperm count data were not normally distributed so non-parametric tests were therefore used. Where non-parametric tests were not available, we used parametric tests (partial correlation and multiple regression) and the ranks of the original variables (RANK procedure, SPSS; Norušis 1993).

3. RESULTS

We observed 51 copulations, out of which 27 were sneak copulations while 24 followed the male courting display and were obtained with female cooperation (i.e. they were preceded by a gliding approach by the female). Sneak copulation was observed only with unreceptive females and occurred in only a small fraction (<5%) of the trials. Among the cooperative copulations, 16 were obtained with post-partum females and eight with females deprived of males for at least 30 days. There was no significant difference in the displaying rate of males before copulation (Mann–Whitney *U*-test, $z = 1.29$ and $p = 0.21$) or the number of sperm delivered during copulation ($z = 0.95$ and $p = 0.52$) between trials with post-partum females and trials with male-deprived females. Therefore, the data of the 24 cooperative copulations were pooled for the subsequent analyses. The courtship and sneak groups did not differ in the number of sperm stripped from the male (Mann–Whitney *U*-test, $z = 1.055$ and $p = 0.30$), male and female size ($z = 0.587$ and $p = 0.56$ and $z = 0.03$ and $p = 0.98$, respectively) and male sexual activity (sigmoid displays and thrusts, $z = 1.118$ and $p = 0.27$). However, the courtship rate was approximately three times higher in the courtship group than in the sneak group ($z = 4.70$ and $p < 0.0001$); conversely, the thrusting rate was significantly higher in the sneak group ($z = 2.386$ and $p = 0.017$; table 1).

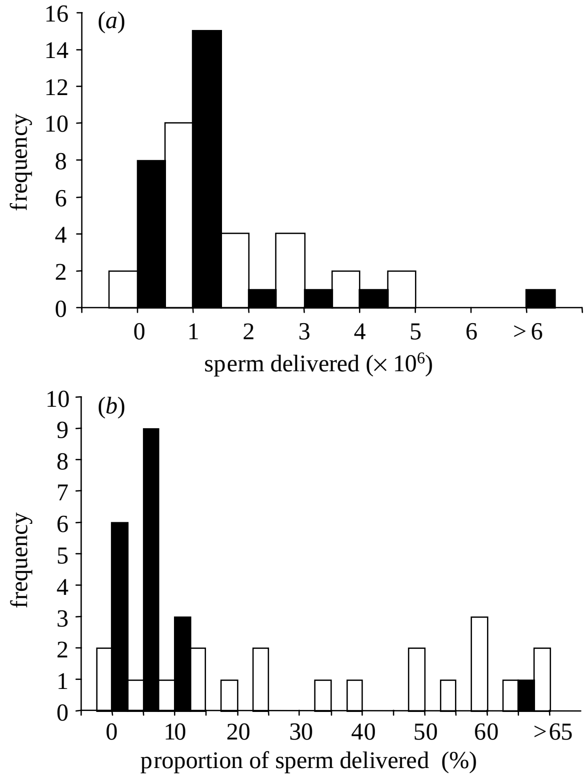

Figure 1. Distribution of the number of sperm delivered by male guppies during a single copulation obtained after courtship with receptive females (open bars) and with gonopodial thrusts with unreceptive females (solid bars). Sperm number is given as (*a*) absolute number and (*b*) in relation to sperm at rest.

On average, copulations with cooperative females resulted in more sperm delivered (median $= 1.22 \times 10^6$, interquartile range $= 0.244$–2.64×10^6 and maximum $= 4.19 \times 10^6$) than sneaky copulations (median $= 0.048 \times 10^6$, interquartile range $= 0$–0.279×10^6 and maximum $= 6.17 \times 10^6$; Mann–Whitney *U*-test, $z = 3.35$ and $p < 0.001$). Nonetheless, the two distributions largely overlapped (figure 1*a*). Two out of 24 males from the courtship group were not able to transfer any sperm. The frequency of unsuccessful copulations was higher in sneaky copulations (eight out of 27), although the difference was only marginally significant ($p = 0.08$, Fisher's exact test).

Assuming that the stripped sperm represent the sperm available for copulation, males delivered a much larger proportion of the sperm they could potentially ejaculate during courtship copulations (median = 34.6%, interquartile range = 12.2–56.9% and maximum = 92.2%) than during sneaky copulations (median = 0.61%, interquartile range = 0–2.6% and maximum = 62.3%; Mann–Whitney *U*-test, $z = 3.92$, $p < 0.0001$, $n_1 = 20$ and $n_2 = 19$; figure 1*b*).

The number of stripped sperm correlated positively with male SL (Spearman's rank correlation, courtship $r = 0.63$, $n = 20$ and $p = 0.003$, sneak $r = 0.51$, $n = 19$ and $p = 0.026$ and pooled $r = 0.58$, $n = 39$ and $p = 0.0001$). Within each mating tactic, the number of sperm delivered was positively correlated with the amount of sperm available, as estimated from stripping (courtship $r = 0.60$, $n = 20$ and $p = 0.005$, sneak $r = 0.69$, $n = 19$ and $p = 0.001$ and pooled $r = 0.33$, $n = 39$ and $p = 0.04$).

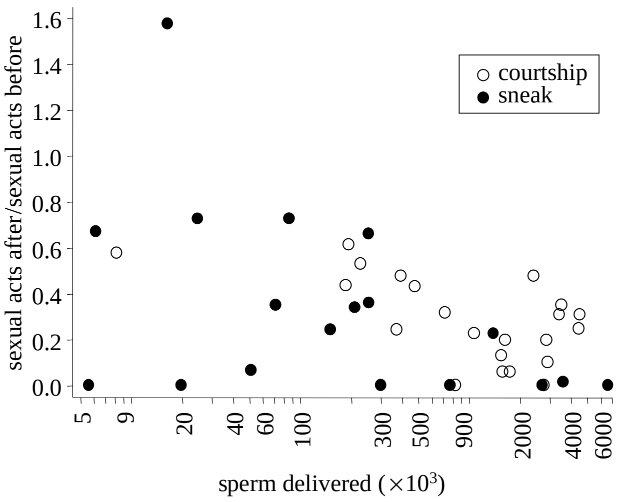

Figure 2. Relative rate of sexual activity after copulation (sigmoid displays and thrusts after copulation/sigmoid displays and thrusts before copulation) in relation to the number of sperm delivered (log scale).

In courtship mating, the number of sperm delivered increased with male size ($r = 0.48$, $n = 20$ and $p = 0.031$). This correlation was entirely due to the greater number of sperm available in larger males, since no residual advantage of large size remained after statistically controlling for the differences in sperm available for copulation (partial $r = 0.17$, $p = 0.49$ and d.f. = 17). In sneak mating, there was no significant correlation between sperm delivered and male size ($r = 0.21$, $n = 19$ and $p = 0.39$). However, once the number of sperm available was controlled for, the number of sperm delivered in this group was significantly and negatively correlated with male relative size (SL male/SL female, partial $r = -0.58$, $p = 0.01$ and d.f. = 16).

The number of stripped sperm did not correlate significantly with courtship or thrust rate before copulation, either within groups or pooling the data from the two groups (all $p > 0.05$), the only exception being the courtship group, where courtship rate was negatively correlated with available sperm (Spearman's rank correlation, $r = -0.61$, $n = 20$ and $p = 0.004$).

Sexual activity decreased significantly after copulation in both the courtship and sneak groups (one-sample t-test, courtship 32.8 (19.8%), $t_{23} = 16.6$ and $p < 0.001$ and sneak 45.5 (41.2%), $t_{26} = 6.88$ and $p < 0.001$). The relative rate of sexual activity after copulation (rate after/rate before ×100) was negatively correlated with the number of sperm delivered (Spearman's rank correlation, courtship $r = -0.55$, $n = 20$ and $p = 0.006$ and sneak $r = -0.66$, $n = 19$ and $p < 0.001$; figure 2). The decrease in sexual activity might depend on the quantity of sperm left after copulation rather than on sperm delivered. In a multiple regression analysis where the estimation of the sperm left after copulation (sperm available—sperm delivered) and the number of sperm delivered were the independent variables, only the latter was significantly and negatively correlated with the observed relative rate of sexual activity after copulation, in both the courtship (sperm left, partial $r = 0.10$ and $p = 0.65$; sperm delivered, partial $r = -0.54$ and $p = 0.02$) and sneak groups (sperm left, partial $r = 0.18$ and $p = 0.38$; sperm delivered, partial $r = -0.76$ and $p = 0.002$).

4. DISCUSSION

In this study we showed that male guppies are able to inseminate a large number of sperm into the gonoducts of unwilling females through gonopodial thrusting. Although on average the number of sperm delivered after a sneaky copulation was approximately one-third of that with cooperative females, in our sample the copulation that transferred the highest number of sperm was a thrust (6.167×10^6 sperm) and three out of the ten copulations with the highest sperm counts ($> 2.5 \times 10^6$) were thrusts. Thus, although less efficient on average in terms of both sperm delivered and frequency of successful copulations over the total number of attempts (Luyten & Liley 1991), sneaky copulations occasionally allowed males to inseminate large sperm numbers into the female gonoduct. Guppy populations with different predation regimes have been shown to differ in their courtship and thrust rates (Houde 1997). It would be interesting to compare the insemination success of the two tactics in populations in which males preferentially adopt one or the other mating tactic.

Considering that females are receptive to males for only two to three days during their breeding cycle, it can be expected that around 90% of sexually mature females are usually unreceptive in a population. In the wild, males perform an average of one mating attempt per minute throughout the day and most attempts are gonopodial thrusts (Magurran & Seghers 1994*a*). Females are therefore exposed to continuous sexual harassment by males for most of their breeding cycle (Magurran & Seghers 1994*a*; Magurran 1996). The results of the present study suggest that the contribution of sperm derived from sneaky copulations to the female sperm store may be more relevant than previously thought. Luyten & Liley (1991) suggested that courting males should have a much higher reproductive success than their sneaky counterpart. However, as pointed out by Magurran & Seghers (1994*b*), the success of the sneaky tactic must be tested in natural conditions, where food is not available *ad libitum* and predators can impose a cost on receptive females or courting males (Magurran & Nowak 1991; Godin 1995). Many authors have regarded gonopodial thrusting as 'the best of a bad job' done by competitively inferior males. This view contrasts with evidence that, in some species, e.g. the swordtail *Xiphophorus nigrensis*, alternative male reproductive tactics have a genetic basis (Zimmerer & Kallman 1989). The results of our study suggest that copulations through gonopodial thrusting may result in the transfer of high sperm numbers, thus potentially undermining female choice. Our results are in agreement with the hypothesis proposed by Magurran (1996) that the forced copulations in guppies may prevent any differentiation process driven by female choice and may be the reason for the low speciation rate observed for this species.

Our results also provide evidence that, on average, males did not transfer all their available sperm during copulations. This opens up the question of whether the male has any control over the quantity of sperm transferred during a single copulation. Since the same male can in principle obtain more than one mating per day with different females, the optimal strategy may be not to use up all their daily sperm production in a single mating. Sperm economy has been shown to be adopted by several fish species with external fertilization and frequent copulations (Petersen & Warner 1998). Whether this also happens in the guppy or in other poeciliids requires further investigation.

In agreement with the results of a previous study (Matthews *et al*. 1997), the number of sperm stripped at rest correlated positively with male size. Large males were therefore at an advantage in sperm competition when copulation occurred with female cooperation. Conversely, with the sneak-tactic male, size has two opposite effects. Since sperm production increases with body size, large males have a larger sperm store available for copulation. On the other hand, small males are able to deliver a larger fraction of their sperm reserve during a single sneak copulation. Whether large males deliver a smaller fraction of their sperm reserve as a result of a sperm allocation strategy or as a consequence of an inferior ability in sneak copulation remains to be investigated. The mating advantage of small males in a sneaky copulation may be a general phenomenon in poeciliids. An insemination advantage of males with a small size has been directly demonstrated in the eastern mosquitofish (Pilastro *et al*. 1997) and, based on purely behavioural observations, in four other species (Farr *et al*. 1986; Bisazza & Pilastro 1997). Males much smaller than the female are probably at an advantage because they are less conspicuous when approaching unwilling females and manoeuvre better during gonopodial thrusts (Pilastro *et al*. 1997).

In contrast to a previous study (Matthews *et al*. 1997), we did not find evidence of a positive correlation between male sexual activity and the number of sperm stripped. Instead, we found a negative association between the number of sperm delivered and subsequent male sexual activity. However, the two studies used different set-ups and fish from different populations and are therefore not directly comparable. There are two possible interpretations of the decrease in mating attempts we observed after copulation. Sexual activity may be tuned to the quantity of remaining sperm or may depend on how successful the copulation has been in terms of the number of sperm delivered. Our results are more in agreement with the second hypothesis, since the decrease in mating attempts after copulation was not associated with the number of sperm left, but only with the sperm delivered. Perceiving how many sperm they have delivered may allow males to decide whether it is more convenient to continue their attempts with the female they have already copulated with or to look for another mate. However, our experiment was not specifically designed to discriminate between these two hypotheses and further research is necessary to clarify this point.

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