Male display rate reveals ejaculate characteristics in the Trinidadian guppy *Poecilia reticulata*

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SUMMARY

Previous work (Reynolds & Gross 1992) has demonstrated that mate choice enables female guppies, *Poecilia reticulata*, to obtain heritable benefits ('good genes') for their offspring. It is not yet known whether males also signal their functional fertility, that is, their viability as fathers, to female guppies. Our study tested the hypothesis that a male's behaviour reveals characteristics of his ejaculate. We uncovered a strong correlation between display rate and sperm number in male guppies derived from two wild Trinidadian populations. This result indicates that information on the functional fertility of potential mates is available to females. However, our investigation also found a significant relationship between the frequency of sneaky mating and sperm number. Female guppies in wild populations, particularly those experiencing a high predation regime, are constantly subjected to sneaky mating attempts. As the males responsible for these sneaky matings have highest reserves of sperm they may have the capacity to undermine female choice. The implications of these findings for sperm competition are discussed.

1. INTRODUCTION

It has recently been proposed that male phenotype reveals functional fertility. This means that females who choose mates on the basis of key phenotypic characteristics gain information about their viability as potential fathers (Sheldon 1994). Evidence for this hypothesis is provided by Mjelstad (1991), who found a strong correlation between display rate and the proportion of motile sperm in the ejaculate in the capercaillie (*Tetrao urogallus*). Nitchuk & Evans (1978) also showed that male sharp-tailed grouse, *Tympanuchus phasianellus*, with central lek positions had higher testicular sperm counts.

Some of the most compelling evidence for female choice has been provided by studies of the guppy, *Poecilia reticulata*. Guppies are small Poeciliid fish that occur in Trinidad and north-eastern South America. They are livebearers with internal fertilization. Females are fertile for restricted periods (Liley 1966), during which they show preferences for males on the basis of colour patterns (Houde 1987), morphology (Endler & Houde 1995) and courtship rate (Farr 1980; Nicoletto 1993). It is not yet known if female choice selects for males of greater fertility.

Males perform a sigmoid display to persuade receptive females to mate. During the display the male moves in front of the female, extends his fins, arches his body into a sigmoid shape and vibrates around his vertical axis (Liley 1966; Luyten & Liley 1985). The male may hold this position for several seconds before making a mating attempt. A receptive female will cooperate by swimming in a tight circle, thus exposing the genital pore and facilitating insemination (Kadow 1954; Liley 1966).

However, males also employ sneaky mating. This is attempted without male display or female cooperation. The male approaches the female from behind, swings his gonopodium forward and tries to insert the tip into the female's genital pore. This behaviour is also known as a gonopodial thrust (Baerends *et al.* 1955; Liley 1966). Although the extent to which sneaky mating undermines female choice is unknown, we predict that males that engage most frequently in this behaviour have higher sperm counts (Harvey & Bradbury 1991).

Males of many species of fish, for example coho salmon (*Oncorhynchus kisutch*) (Gross 1985), Atlantic salmon (*Salmo salar*) (Gage *et al.* 1995) and bluegill sunfish (*Lepomis macrochirus*) (Dominey 1980; Gross 1982), specialize in one mating strategy. Male guppies, in contrast, can adopt either strategy, although the degree to which each method is used has been shown to depend on a number of factors, including predation pressure (Endler 1987), gonopodium length (Reynolds *et al.* 1993), female receptivity (Liley 1966), male density (Rodd & Sokolowski 1995) and water turbidity (Luyten & Liley 1991).

The reproductive behaviour of the guppy means that they are prime candidates for sperm competition. During the receptive phase females will mate with a number of males (Houde 1987). Outside this period they experience a high incidence of sneaky mating attempts. Magurran & Seghers (1994) found that wild female guppies were subjected to an average of one sneaky mating per minute. There is also evidence that multiple paternity occurs (Winge 1937; Hildemann & Wagner 1954; Haskins *et al.* 1961).

Our study tested Sheldon's (1994) phenotype-linked fertility insurance hypothesis by investigating the possibility that the male guppy's behaviour indicates

ejaculate characteristics. We also tested the prediction that the males who engage most actively in sneaky mating, are those with the greatest number of sperm.

2. METHODS

(a) *The study populations*

Male guppies used in this experiment were descendants of wild-caught stocks from the Upper Tunapuna and Lower Tacarigua. The Upper Tunapuna is a low predation river where guppies coexist with *Rivulus hartii*, a minor predator, and the Lower Tacarigua is a dangerous locality for guppies as a variety of predators, including the pike cichlid, *Crenicichla alta*, are found there. The female guppies in this experiment were also descended from Trinidad populations.

(b) *Mating behaviour*

Male mating behaviour was observed over a period of six weeks. The same five females, which were mixed-strain stock to exclude the possibility of biasing the behaviour of males from either population, were used throughout the trials. Observations were made in a 59 × 29 × 35 cm deep aquarium with water 27 cm deep. The aquarium contained a small clump (6 cm high) of Java moss, *Vesicularia dubyana*. Temperature was maintained between 24.5 °C and 25.5 °C and illumination was provided by an 18 W bulb on a 12 h light/dark cycle. The fish were fed to satiation with Tetramin[®] before behavioural observations were carried out.

In each trial, five males from one population were introduced to a tank containing the five females. The guppies were allowed to settle overnight before observations commenced. The number of sigmoid displays and sneaky matings performed by each male were recorded over a 15 min period. This was repeated five times for each male. A male was judged to have successfully delivered a thrust to a female if his gonopodium made contact with a female's genital region. A total of 80 different males, 40 from each population, were observed.

(c) *Ejaculate features*

Each of the 80 males was then isolated overnight prior to analysis of his ejaculate features. The male was humanely killed and any excess water removed from the body surface. The fish was then placed on a Petri dish under a low-power dissection microscope. The gonopodium was swung forward and gentle pressure was applied to the side of the abdomen, at the base of the gonopodium. This action released the ejaculate, in the form of a number of spermatozeugmata, which were then stroked down the gonopodium and on to the Petri dish. This was repeated three times for each fish to ensure that all available sperm had been removed. Occasionally some of the spermatozeugmata became attached to the surface of the male and these were removed and added to the stripped ejaculate.

Once ejaculate volume had been measured with capillary tubes, a known volume of distilled water was added and the spermatozeugmata were left to break down for 20 min. To aid the breakdown of sperm bundles and to distribute sperm evenly, the sample was repeatedly drawn up and expelled from a Gilson pipette.

Sperm were then counted twice for each male using an 'improved Neubauer chamber' haemocytometer. The number of sperm in each stripped ejaculate was determined by multiplying mean sperm count by the sample's dilution factor and initial volume. Mean sperm lengths for each male

were calculated as an average of 15 individual sperm lengths.

The wet weight, wet weight – gut, total and standard length, and gonopodium length of each male were recorded. The testes were then removed and were dried (24 h at 60 °C) with the male's body to give dry weight and testes dry weight for each fish. Gonosomatic index (GSI) was determined by calculating dry testes mass as a percentage of dry body mass (de Vlaming *et al.* 1982).

3. RESULTS

Fish showed consistent courtship behaviour over the five 15 min observations (Kendall coefficient for Tunapuna thrusts $W = 0.56$, $\chi^2 = 108.4$, $p < 0.001$ and sigmoids $W = 0.726$, $\chi^2 = 141.57$, $p < 0.001$; Tacarigua thrusts $W = 0.658$, $\chi^2 = 128.3$, $p < 0.001$ and sigmoids $W = 0.776$, $\chi^2 = 151.3$, $p < 0.001$). Given this consistency we selected the median (out of the five) thrust and sigmoid frequencies for each fish, and used these values in all subsequent statistical analyses.

Tacarigua males attempted significantly more sneaky matings per unit time than Tunapuna males ($t_{78} = 2.0$, $p = 0.048$; figure 1). The number of sigmoids performed did not significantly differ between the populations ($t_{78} = 0.02$, $p = 0.98$; figure 1).

The number of gonopodial thrusts performed per unit time was positively correlated with the number of sperm per ejaculate in Tunapuna ($r = 0.34$, $n = 40$, $p < 0.05$; figure 2) and Tacarigua ($r = 0.59$, $n = 40$, $p < 0.01$; figure 2) males. Both populations also showed

Figure 1. Sigmoid rate (a) and thrust rate (b) per 15 min for $n = 40$ males from each population. Error bars indicate the 95% confidence interval.

Figure 2. The relationship between sperm numbers per ejaculate and sigmoid and thrust rate for Tacarigua (open circles) and Tunapuna (closed circles) males. Sperm numbers correlate with both sigmoids and thrusts for both populations. * = $p < 0.05$; ** = $p < 0.01$. Significance levels were calculated using a Bonferroni correction, see text for details.

a highly significant positive correlation between sigmoid rate and numbers of sperm per ejaculate (Tunapuna $r = 0.73$, $n = 40$, $p < 0.01$; Tacarigua $r = 0.61$, $n = 40$, $p < 0.01$; figure 2). The number of thrusts and sigmoids correlated with mean sperm length in Tunapuna males ($r = 0.35$ and 0.40 , $n = 40$, $p < 0.05$), but not in Tacarigua males ($r = 0.29$ and 0.26 , $n = 40$, $p > 0.05$). Interestingly, sperm numbers per ejaculate correlated with mean sperm lengths in both Tunapuna ($r = 0.49$, $n = 40$, $p < 0.05$; figure 3) and Tacarigua males ($r = 0.52$, $n = 40$, $p < 0.01$; figure 3). There was no correlation between the GSI and either thrusts ($r = 0.17$, $n = 40$, $p > 0.05$) or sigmoids ($r = 0.003$, $n = 40$, $p > 0.05$) in Tunapuna males, but there were correlations between GSI and both thrusts and sigmoids in Tacarigua males (respective r values = 0.38 and 0.49 , $n = 40$, $p < 0.05$). Reynolds *et al.* (1993) found a significant correlation between gonopodium length and number of thrusts. However, this was not found to be the case in either of the populations used in this study (Tunapuna $r = 0.163$, $n = 40$, $p > 0.05$; Tacarigua $r = 0.031$, $n = 40$, $p > 0.05$). Males from Tacarigua showed a positive correlation between number of sigmoids and number of thrusts ($r = 0.416$, $n = 40$, $p < 0.05$), but no correlation was found for the Tunapuna males ($r = 0.312$, $n = 40$, $p > 0.05$). The significance levels of all correlations were calculated

using a Bonferroni adjustment (0.05 and $0.01/13$ (Sokal & Rohlf 1981)), where 13 corresponds to the number of correlations performed per population.

Although Tacarigua males were significantly smaller (measured as dry weight of fish) than Tunapuna males ($t_{77} = 2.7$, $p = 0.009$), the gonosomatic indices were virtually identical in both populations ($t_{77} = 0.01$, $p = 0.99$). Total ejaculate volumes, numbers of sperm per ejaculate and sperm length did not differ significantly between the populations ($t_{77} = 1.1$, 0.86 and 0.04 , respectively, $p > 0.05$).

4. DISCUSSION

Our results indicate that, for both populations, the number of sperm per stripped ejaculate is highly correlated with sigmoid rate (figure 2). This has implications for male and female guppies. First, it is in the interest of a male to advertise the fact that he has a high sperm count, and not to waste energy chasing and displaying to females if he has no ejaculate ready for use. C. Bozynski and N. R. Liley (personal communication) have shown that recently stripped males spend less time following females and attempt fewer sexual acts than males whose sperm reserves are still intact. If spermatogenesis is constant then males may simply be more ardent in their mating attempts when

Figure 3. Sperm number per ejaculate against sperm lengths for males from Tacarigua (open circles) and Tunapuna (closed circles). $^* = p < 0.05$; $^{**} = p < 0.01$. Significance levels were calculated using a Bonferroni correction, see text for details.

they have greater reserves of sperm available. Second, because the behaviour of a male conveys information on his sperm availability, females may be able to assess ejaculate features. Our data, therefore, support Sheldon's (1994) phenotype-linked fertility insurance hypothesis that female preferences arise because the morphological and behavioural features that females find attractive covary with ejaculate features.

It has long been accepted that female guppies exert choice and base their mating preferences upon individually variable male coloration and morphology (Endler 1983; Kodric-Brown 1985; Houde 1987; Endler & Houde 1995), but there is also experimental evidence that female guppies choose males on the basis of their behaviour (Farr 1980). Nicoletto (1993) found that females responded to males that had higher display rates. Given the strong correlation between sigmoid frequency and sperm number, females that select males on the basis of courtship rate will be choosing males with higher sperm counts. This means that in addition to gaining indirect benefits, such as those proposed by the 'good genes' model of sexual selection (Reynolds & Gross 1992), female guppies can

obtain direct benefits as a consequence of mate choice. On the other hand, if female guppies in the wild prove to have either copious stores of sperm or plentiful opportunities to obtain sperm (i.e. they are not sperm-limited), then they have little to gain in terms of reproductive success by selecting males with the greatest sperm reserves. Females are known to face high levels of sneaky mating (Magurran & Seghers 1994) and also to mate multiply during their receptive period (Houde 1987). Females should, therefore, have no problem in obtaining sufficient sperm to fertilize their offspring. However, no work has been carried out in the wild to establish if sperm are, in fact, this abundant. If sperm are not limited, the relationship between sperm reserves and male behaviour will be male-driven rather than female-driven, and females might be expected to weight reliable male signals such as coloration more highly when choosing a mate. Further work on the relationship between male coloration and behaviour, ejaculate characteristics and female choice is needed to distinguish between these two possibilities.

Birkhead & Fletcher (1995) have argued that consistent differences between males in ejaculate features are required if females are to choose between males on the basis of differences in their ability to fertilize eggs. In our study, each male was only sampled once and therefore no information was collected on the consistency of ejaculate features within males. However, this does not preclude it from providing evidence for Sheldon's (1994) hypothesis. Because sperm were stripped from males soon after the behavioural observations, we can be confident of the relationship between sperm number and courtship rate. We would expect any subsequent change in ejaculate characteristics to be accompanied by corresponding changes in behaviour, so that at any one time the display rate of the male reveals his current sperm count.

As noted in §1, females solicit matings for only a few days during each reproductive cycle. At other times they face continual sexual harassment from males. The relative success rate of sneaky mating is still unknown (Magurran 1996), but the high frequency of this behaviour in wild populations of guppies suggests that it could occasionally undermine female choice. This hypothesis is supported by our results, which indicate that the males that engage most actively in sneaky mating have the highest sperm counts.

Observations in the wild indicate that risky sites, such as the Tacarigua, are characterized by high levels of courtship activity, particularly sneaky matings (Magurran & Seghers 1994). This relationship between predation regime and male courtship behaviour was also evident in our study (figure 1). Interestingly, the relationship between sneaky mating frequency and sperm number is also strongest in fish descended from the high predation population (Tacarigua) (figure 2). We predict, therefore, that sneaky mating will account for proportionally more fertilizations in high predation localities than in low predation ones. There may also be a higher incidence of multiple paternity in such localities.

During their period of receptivity, it is known that

females may choose to mate with several males (Houde 1987). Furthermore, the high frequencies of sneaky mating attempts observed in the wild (Magurran & Seghers 1994) will introduce sperm from yet more males into the female reproductive tract. Sperm competition will be an inevitable consequence of these behaviours.

There are two ways a male can improve his chances during sperm competition. If sperm competition follows the rules of a lottery (Parker 1990*a*), the greater the number of sperm a male has competing for fertilizations, the greater his chance of success. Another option is to produce longer sperm that will be able to swim faster and therefore reach the egg before slower, shorter sperm (Parker 1990*b*; Briskie & Montgomerie 1992; Gage 1994). Our results found an interesting correlation within each population between sperm length and the number of sperm per ejaculate (figure 3), indicating that male guppies are using both strategies to maximize reproductive success. This result is contradictory to Parker's theory (1982) that a trade-off exists between sperm size and sperm number.

There is, however, evidence that sperm competition in the guppy may not be as straightforward as the lottery model. Winge (1937) reports that if a male is allowed to mate with a receptive non-virgin female, then this second male will achieve paternity of the next brood. The timing and sequence of matings may, therefore, have a crucial role to play in sperm competition in this species. Much research remains to be done.

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