Uncoupling the links between male mating tactics and female attractiveness

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Because not all females are equally attractive, and because mating reduces the chances of getting further copulations, males should prefer better-quality mates. In this paper, we use the Trinidadian guppy (*Poecilia reticulata*) to explore the effects of two non-correlated measures of female quality—size and reproductive status—on male mating decisions. All male guppies employ two alternative mating tactics. We found that large females, particularly those from a high predation site, were the target of most sneaky mating attempts. The response persisted in fish raised under standard conditions over several generations in the laboratory. In addition, non-pregnant females received more courtship displays. We conclude that males can discriminate among females and that they uncouple their mating tactics to track different axes of quality.

1. INTRODUCTION

Recent research has revealed that neither gender is bound by the stereotypical sex roles of classic sexual-selection theory (Knight 2002). Thus, although males are often cast as the promiscuous sex, females can also gain direct and indirect benefits through multiple mating (Jennions & Petrie 2000). Equally, because a male expends time and sperm during copulation, mating with one female reduces the chances of obtaining other partners. Differences in female quality mean that it pays for males to be choosy (Berglund & Rosenqvist 2001) as it is not only females that potentially reap fitness benefits from mate choice (Andersson 1994). Nonetheless, the ability of males to simultaneously evaluate independent metrics of female quality has not hitherto, to our knowledge, been investigated.

Males are predicted to be choosy when their investment in parental care is relatively important or when their potential reproductive rate is lower; in these cases females are the competing sex (Johnstone *et al.* 1996; Berglund & Rosenqvist 2001). However, even in systems in which male investment is low, males show some preferences for traits that are reliable indicators of female quality, such as size (Sargent *et al.* 1986; Kraak & Bakker 1998; Wong & Jennions 2003) or coloration (Amundsen & Forsgren 2003). A key component of female quality is the amount of energy available for offspring production. In ectotherms growth is typically indeterminate, resulting in considerable variation in adult female size, which in turn is a good indicator of reproductive potential (Reeve & Fairbairn 1999). The use, by males of female size as a surrogate of fecundity, has been particularly well documented in fishes (Côte & Hunt 1989; Kraak & Bakker 1998). Size, however, is not everything; female reproductive status will also affect a male's chances of successful insemination.

The guppy (*Poecilia reticulata*), an ovoviviparous freshwater fish native to Trinidad, has a promiscuous mating system (Houde 1997) in which all males employ two mating tactics: they may either solicit consensual matings with receptive females by displaying, or attempt sneaky matings with unresponsive females. The existence of these two tactics allows us to test the hypothesis that males can identify more than one axis of female quality, and that they will adjust their mating behaviour accordingly.

In this paper, therefore, we test the prediction that male mating behaviour is mediated by both size and female receptivity. We compare two populations of wild guppies, upper and lower Aripo, previously shown to differ in their propensity to make risk-sensitive adjustments to courtship (Magurran & Seghers 1990). Our expectation is that lower Aripo males, which are more flexible in their use of sneak matings, and where fecundity rises more steeply with body size (Bronikowski *et al.* 2002) will show most discrimination between large and small females. We follow this up by asking whether lower Aripo guppies raised in the laboratory replicate the choices of their wild counterparts.

2. METHODS

Mating behaviour observations were carried out using wild individuals from two sections of the Aripo River in Trinidad. The upper Aripo (10°41.1481′ N, 61°13.9564′ W) is a low predation locality whereas the lower Aripo (10°39.0369′ N, 61°13.4046′ W) is a typical high predation site. Fish (46 females and 27 males in each collection point) were captured in March 2003 using seine nets and transported to the University of West Indies, Trinidad. Once in the aquarium, fish were kept in tanks (120 cm × 45 cm × 45 cm) that were continuously filtered and aerated. Average (± s.d.) water temperature during the experiments was 24.1 (±1.7) °C.

We observed male behaviour in three identical tanks (45 cm × 30 cm × 30 cm) furnished with natural river gravel. One female and four males were randomly taken from stock tanks and placed in the observation tank. We used this sex ratio because it is within the range found in the wild and at the same time it was expected to produce high levels of mating activity. Guppies were allowed to become accustomed to the observation tanks until they resumed normal foraging and courtship behaviour; this took from 30 min to 3 h. We then recorded the total number of sigmoid displays and gonopodial thrusts received by the female in 15 min. A thrust was computed only when the gonopodium made physical contact with the female's genital region.

After each observation the males were transferred back to the stock tank avoiding repetition of the same males in two consecutive trials. Females were humanely killed by an overdose of benzocaine, measured using a digital calliper (to the nearest 0.01 mm) and dissected to determine the number of embryos and their developmental stage, according to Haynes (1995). For statistical analyses we grouped the females with immature, early yolked and mature eggs (stages 1–3) as 'non-pregnant'. Females with embryos at different stages of embryonic development (stages 4–11) were grouped as 'pregnant'. All the wild fish used were part of a different study that necessitated genetic analyses so were going to be killed anyway.

A second series of observations was completed using descendants of lower Aripo guppies reared under laboratory conditions for several generations. We selected 44 females across a wide range of sizes. The number of males available in the aquarium was enough to avoid repeating them. The observation tank (59 cm × 29 cm × 35 cm) contained gravel, a clump of Java moss for cover and was equipped with a filtering and aerating system. Fish were allowed to settle in the observation tank for *ca.* 1 h. The protocol for the observations was otherwise the same as the one described above. Average (± s.d.) water temperature in the observation tank was 24.8 (±1.2) °C.

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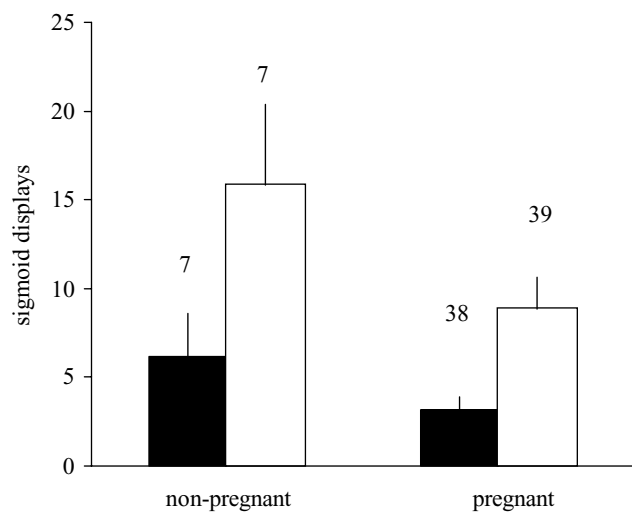DOI 10.1098/rsbl.2004.0207

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Figure 1. Average (+ s.e.) number of courtship displays performed by males to pregnant and non-pregnant females. Numbers indicate sample sizes for each group. Open bars, upper Aripo fish; filled bars, lower Aripo fish.

3. RESULTS

Variability in the number of courtship displays performed by wild male guppies was considerable for both upper (0 to 47 displays in 15 min) and lower (0 to 26) Aripo fish. However, female size did not explain this variability in any of the two groups (linear regressions: $r^2 < 0.020$, $p > 0.478$). A two-way ANOVA revealed between-population differences in a number of displays ($F_{1,87} = 9.59$, $p = 0.003$) and a weak but significant effect of female reproductive status ($F_{1,87} = 4.05$, $p = 0.047$), but no population × status interaction ($F_{1,87} = 0.64$, $p = 0.424$; figure 1). Bigger females received more gonopodial thrusts (figure 2a), although the positive trend was significant for lower Aripo fish ($r^2 = 0.279$, $p < 0.001$) but not for upper Aripo fish ($r^2 = 0.065$, $p = 0.089$), and the slopes were different (ANCOVA: $F_{1,87} = 7.35$, $p = 0.008$; figure 2a).

Fecundity increased with female size for both upper ($r^2 = 0.390$, $p < 0.002$) and lower ($r^2 = 0.629$, $p < 0.001$) Aripo females. As anticipated, the slopes of the regression lines were different (ANCOVA: $F_{1,82} = 7.19$, $p < 0.009$), being steeper for lower Aripo females.

Male mating behaviour of aquarium-reared lower Aripo fish paralleled that observed in wild fish. The total number of sigmoid displays in 15 min periods ranged from 1 to 38. Again, the amount of this variance explained by female size was not significant ($r^2 = 0.016$, $p = 0.409$). The number of gonopodial thrusts was also variable (0 to 20), but here almost half of this variability was explained by female size ($r^2 = 0.458$, $p < 0.001$; figure 2b).

4. DISCUSSION

As expected, both female size and reproductive status played an important role in explaining variability in male behaviour and males adjusted their courtship effort in a manner expected to confer fitness benefits. Importantly, the two male tactics tracked different axes of female quality. There is already evidence that male guppies do not choose partners at random (Kelley *et al.* 1999; Dosen & Montgomerie 2004). Furthermore, preferences for large
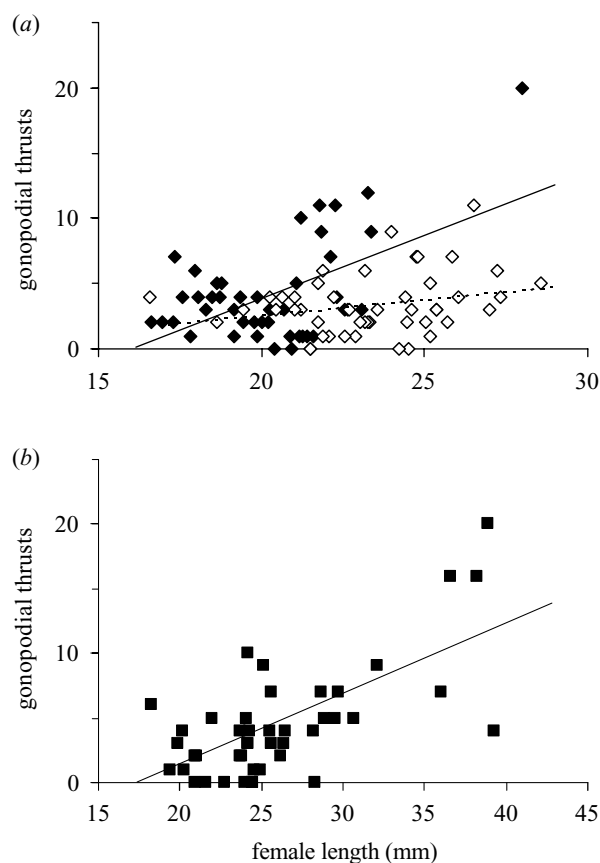
Figure 2. Effects of female size on the number of sneaky matings performed (a) by wild males from upper (open symbols) and lower (filled symbols) Aripo; and (b) by laboratory-reared lower Aripo males.

females have been detected in some studies (e.g. Baerends *et al.* 1955; Abrahams 1993) though not in others (see Houde (1997) and below for a discussion). Our results extend these findings by demonstrating that males can evaluate non-correlated fitness cues.

Displaying is an expensive behaviour (Magurran & Seghers 1990; Magurran & Nowak 1991) and leads to a copulation only when the female is virgin and for short periods after giving birth. Male guppies can accurately identify a receptive female using several cues including responsiveness to courtship (Houde 1997). They gain no benefit from displaying more to an attractive female once it is evident that she is not receptive. Our results are consistent with this idea, because more courtship was directed to non-pregnant females (figure 1). Indeed, there was no relationship between the number of displays and female size in any of the groups. This result is in contrast to Abrahams (1993) and Baerends *et al.* (1955) who found that larger females were courted more. However, fish in our experiment could freely interact; males had the opportunity to use both mating tactics and females could swim away. We suggest that these differences in design account for the different outcomes.

Sneaky mating, on an attempt per attempt basis, is comparatively inexpensive in terms of time and energy and reduces the risk of being detected by predators (Magurran & Nowak 1991). Because gonopodial thrusts are an efficient method of sperm transfer (Evans *et al.*

2003) this tactic has the potential to achieve paternity, irrespective of female reproductive status. Our prediction that gonopodial thrust rate would be related to female size was most strongly supported in lower Aripo fish, irrespective of whether they were wild-caught or laboratory reared. This implies either a direct evolved response to variation in predation risk, or an indirect response to risk, manifested through fecundity differences.

An alternative and more parsimonious explanation for the positive relationship between gonopodial thrusts and female size is that it is easier to hit a bigger target. Our experimental protocol counted only forced mating attempts when they resulted in genital contact. It is possible that males did not prefer to sneak to bigger females: they just succeeded more frequently with them. Thus, the relative size of males and females could be the variable influencing male behaviour or the ease with which males can transfer sperm (Bisazza & Pilastro 1997; Pilastro *et al.* 1997). However, the fact that the upper and lower Aripo females were from the same size range, and the relationship was particularly strong in the latter makes it unlikely that sexual size dimorphism is the only explanation for the results.

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