

Multiple mating and sequential mate choice in guppies: females trade up

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The trade-up hypothesis outlines a behavioural strategy that females could use to maximize the genetic benefits to their offspring. The hypothesis proposes that females should be more willing to accept a mate when the new male encountered is a superior genetic source to previous mates. We provide a direct test of the trade-up hypothesis using guppies (*Poecilia reticulata*), and evaluate both behavioural and paternity data. Virgin female guppies were presented sequentially with two males of varying attractiveness, and their responsiveness to each male was quantified. Male attractiveness (ornamentation) was scored as the amount of orange coloration on their body. Females were generally less responsive to second-encountered males, yet responsiveness to second males was an increasing function of male ornamentation. These attractive second males also sired a greater proportion of the offspring. There was an overall tendency for last-male advantage in paternity, and this advantage was most exaggerated when the second male was more ornamented than the first. Finally, we found that our estimate of relative sperm number did not account for any significant variation in paternity. Our results suggest that female guppies may use pre-copulatory mechanisms to maximize the genetic quality of their offspring.

Keywords: multiple mating; mate choice; sperm competition; sperm precedence; paternity; guppy

1. INTRODUCTION

The decision-making processes that females use during mate choice have been the focus of much recent research in behavioural ecology (reviewed in Andersson 1994; Jennions & Petrie 1997, 2000). These processes are often studied using 'two-choice' experiments, where a female is simultaneously presented with a pair of males that differ in some trait or combination of traits. Increased association, or mating, with one of the males is used to indicate a female's choice. However, in the wild, females of many species encounter potential mates sequentially and are therefore unable to make simultaneous contrasts among males. Several theoretical studies address the behavioural rules that females could use when encounters with males are sequential; for example, it has been argued that females could assess a male against some attribute of the last male that she encountered (or mated with), or against some internal standard (Janetos 1980; Halliday 1983; Dombrovsky & Perrin 1994; Wiegmann *et al.* 1999). However, few empirical studies have examined female decision making with respect to sequential mate choice in the context of multiple mating.

The trade-up hypothesis describes a behavioural strategy that females could use to maximize the genetic quality of their offspring when males are encountered sequentially (Halliday 1983; Jennions & Petrie 2000). The hypothesis suggests that in non-resource-based mating systems, with some degree of last-male sperm precedence, a female should mate with the first male that she encounters to ensure fertilization of her eggs, but subsequently mate preferentially with males of higher genetic quality. Thus,

virgin females should be relatively indiscriminate, but then become increasingly choosy with each successive mating opportunity. In the first explicit test of the trade-up hypothesis, Gabor & Halliday (1997) found that female newts (*Titurus vulgaris*) that had been singly mated were more likely to mate with a second male when that male had a larger crest than the first. Female newts receive only sperm from males (i.e. no paternal care) and crest size is presumed to be an honest indicator of genetic quality (Green 1991). Therefore, this study suggests that female newts trade up for genetic benefits. Bateman *et al.* (2001) found that female crickets (*Gryllus bimaculatus*) also trade up, for larger males, which may indicate superior genetic quality. A few other studies provide indirect evidence for the trade-up hypothesis, although these studies do not explicitly test the model (Bakker & Milinski 1991; Brooks & Caithness 1995).

Guppies are well suited to tests of the trade-up hypothesis because, in the wild, females often encounter males sequentially (Houde 1997; F. H. Rodd and T. E. Pitcher, personal observation), mate multiply (Kelly *et al.* 1999), and apparently gain only sperm from males (Houde 1997; Pitcher & Evans 2001). In guppies, there is also some degree of last-male sperm precedence (Evans & Magurran 2001) and preferred male phenotypes (e.g. coloration) are known (e.g. Endler & Houde 1995; reviewed in Houde 1997). Guppies are live-bearing fishes with internal fertilization and a promiscuous mating system in which female choice plays an important part in mating outcome (Houde 1988, 1997). In many populations, receptive females show a clear preference for courting males with large areas of orange coloration (e.g. Endler & Houde 1995; Houde 1997). A male's most intense courtship display is the sigmoid display, which is characterized by the male orientating himself in front of a female and vibrating his body

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in an S-shaped posture (Baerends *et al.* 1955; Rodd & Sokolowski 1995). A measure of female sexual responsiveness for a particular male can be estimated by the percentage of sigmoids to which female responds in a positive fashion (e.g. Houde 1988, 1997).

We tested the trade-up hypothesis in guppies (*Poecilia reticulata*) using both behaviour and paternity data. The utility of trading up will clearly be a function of the difference among males in genetic quality, but also of the extent to which the second male's sperm is actually used to fertilize the female's ova: the greater the proportion of sperm from genetically superior males, the greater the genetic benefit to trading up. Therefore, unlike previous studies, we assessed paternity and were able to quantify the actual skew in reproductive success.

2. MATERIAL AND METHODS

We test the trade-up hypothesis by examining the sexual responsiveness of females to sequentially presented males and the subsequent paternity of each male. We first quantified virgin female responsiveness to males that varied in the amount of orange body coloration, then removed the first male and presented a new male matched for body size but with similar, less, or more orange coloration. Based on the trade-up hypothesis, we predicted that the degree to which females are attracted to a second male is an increasing function of the difference in phenotype (i.e. secondary sexual characteristic) between the two males; therefore, a female guppy will be more responsive to a second male if he is more attractive (more orange area) than her first mate, but less responsive if he is less attractive (less orange area). Finally, we infer from the trade-up hypothesis that female responsiveness to the each male is predicted to bias paternity in favour of the more attractive male.

(a) *Experimental fishes*

Females and 'showy' males (males with large amounts of orange coloration) used in this study were descendants of wild-caught fishes from a tributary of the Paria River in the Northern Range, Trinidad (PS 896 886). This river is a low-predation locale where guppies coexist with the gape-limited cyprinodontid fish *Rivulus hartii* and a freshwater prawn (*Macrobrachium crenulatum*). Males from this Paria tributary are highly ornamented, with large areas of the body covered with orange as well as yellow, black and blue. In this population, females prefer males with larger areas of orange coloration (Houde 1987, 1988; Endler & Houde 1995). All males in the second group, the 'plain' males (males with smaller amounts of orange coloration), possessed a small, Y-linked, melanin-based spot that could be used to assign paternity (Hughes *et al.* 1999). All plain males were patrilineally descended from a male from a Trinidadian population on the south slope of the Northern Range (probably from the Guanapo River drainage) and matrilineally descended from females from the Paria tributary.

Guppies for this experiment were obtained by isolating inseminated females in individual 10 l aquaria. Immature guppies were then collected from these tanks and reared to maturity in single-sex sibling groups in 10 l aquaria. Female and male fry were separated before sexual maturity and before males expressed their colour patterns. All females were raised in visual isolation from males, because exposure to males can influence subsequent mate-choice decisions (Breden *et al.* 1995; Rosenqvist & Houde 1997; Hughes *et al.* 1999). All fishes were

sexually mature and four to six months old when used in mate-choice trials.

(b) *Behavioural trials*

Trials ($n = 48$) were conducted in 19 l aquaria containing naturally coloured gravel and an air stone, with tan paper attached to the back and sides to provide a more uniform visual environment. Tanks were illuminated on a 12 L : 12 D (starting at 07.00 EST cycle) with a 20 W 'warm-white' fluorescent bulb placed 30 cm above the water surface. All observations were taken in an otherwise darkened room, during the morning. Dyads of males were initially chosen by eye to have similar body size. The four treatments had four combinations of male dyads (first male/second male): plain/plain ($n = 12$); showy/showy ($n = 12$); showy/plain ($n = 12$); and plain/showy ($n = 12$).

For each female, the experiment began at 10.00 (EST) on day 0 when the virgin female and a male were placed on either side of an opaque Plexiglas partition located in the centre of the experimental tank. The individuals were left for 24 h to acclimate to the tank. The partition was then removed, at which point the male and female could freely interact (day 1). The pair was observed four times for 10 min each at 10.10 (day 1), 11.10 (day 1), 08.00 (day 2) and 09.00 (day 2), and the female's sexual responsiveness was calculated as the total (i.e. summed across all four observation periods) proportion of sigmoids to which female responded positively. Following Houde (1988), we deemed a response positive when the female, at a minimum, oriented towards the male and glided towards him (indicating her willingness to copulate), and/or when the orientation and glide were followed by copulation. This proportional measure of female responsiveness controls for variation in display rate among males and is a good predictor of mating success (Houde 1988, 1997). The first male was removed at 09.10 (day 2) and then, at 10.00 (day 2), a second male was introduced into the aquarium. These fishes were then observed four times for 10 min each at: 10.10 (day 2), 11.10 (day 2), 08.00 (day 3) and 09.00 (day 3), and the female's sexual responsiveness was calculated as the total (i.e. summed across all four observation periods) proportion of sigmoids to which a female responded positively. A female's relative responsiveness to the second male was then calculated as her responsiveness to the second male minus her responsiveness to the first male. Similarly, for mixed dyads consisting of either showy/plain or plain/showy, a female's relative responsiveness to the showy male was calculated as her responsiveness to the showy male minus her responsiveness to the plain male.

At the completion of a trial, both males were anaesthetized in a water bath containing neutralized 0.15 g l^{-1} MS-222 and then photographed using a Nikon CoolPix 950 for colour pattern assessment. A ruler and colour palette (paint chips) were included in each photo to allow uniform comparison among photographs. The fishes were then returned to their individual holding aquaria. Areas of colours and standard length were quantified using NIH Image analysis software (available at <http://rsb.info.nih.gov/niimage/>). The total surface area of each male was measured to calculate the relative areas covered by orange, yellow, blue and black (see Houde 1992; Pitcher & Evans 2001).

(c) *Paternity and sperm number*

To assess paternity (for plain/showy and showy/plain dyads only), each female was isolated in an 8 l aquarium and allowed to produce offspring for up to two broods. The fry were collected immediately after birth and reared until maturity in 4 l

aquaria. Following Hughes *et al.* (1999), paternity of the plain male was calculated as the proportion of male offspring possessing the Y-linked marker; the remaining proportion was assigned to the showy male.

To estimate sperm load, three days after a male had completed his behavioural trials and had been photographed, males from the plain/showy and showy/plain dyads were again anaesthetized and placed on a Petri dish under a dissecting microscope. Following Matthews *et al.* (1997), the gonopodium was swung forward and slight pressure was applied to the side of the abdomen, just anterior to the gonopodium, to release the spermatozeugmata (i.e. sperm bundles). Pressure was applied repeatedly until all sperm bundles were removed. The sperm bundles were then drawn up in a pipette and added to 100 μ l of 0.9% saline solution. To distribute sperm evenly for counting purposes, samples were gently mixed by repeatedly drawing up and expelling the sample from the pipette. Sperm counts were calculated by counting sperm cells in an improved Neubauer chamber haemocytometer under $\times 400$ magnification. The number of sperm in each sample was determined by multiplying the mean of five counts by the sample's dilution factor and initial volume. Sperm counts were expressed as the total number of sperm in the stripped ejaculate. Stripped sperm load has been used as a predictor of ejaculate size during mating in guppies (Pilastro & Bisazza 1999; Evans & Magurran 2001).

We used the sperm load estimates to construct an expected distribution of paternity for each male based on a 'fair raffle' model (Parker *et al.* 1990; see also Cook *et al.* 1997; Evans & Magurran 2001). Under this null model, individual spermatozoa from each male have an equal chance of fertilizing the eggs, and are not preferentially used by the females. Therefore, the proportion of ova fertilized by a male is equal to the proportion of total sperm that is his. In this case, $P_i = S_i / (S_i + S_j)$, where P_i is the paternity of the i th male, and S_i and S_j represent the sperm load of the i th and j th male in a dyad. For example, i and j respectively represent either the first and second male or the showy and plain male in a trial.

(d) Statistical analyses

Because there was no difference in female responsiveness to the plain and showy males in the first behavioural trials, we used an analysis of variance to compare the relative female responsiveness to the second male across the four dyad treatments. Next, we used a geometric mean linear regression (used when both variables are subject to error; Zar 1999) to examine the relationship between relative female responsiveness to the second male and the difference in orange coloration between the two males (second male minus first male) across all four treatments. To examine whether or not population origin of the males (showy versus plain) had a significant effect on female responsiveness, we compared the females from the plain/plain and showy/showy treatments for (i) their responsiveness to first males and (ii) their relative female responsiveness to the second male (second male – first male). To determine whether females are simply more responsive to more ornamented males (i.e. males with more orange), regardless of their previous mate, we included the amount of orange coloration on the second male and the difference in coloration between the first and second male (second minus first) in a multiple linear regression.

Next, to determine whether or not our behavioural measure of female responsiveness was a good indicator of male reproductive success, we assessed the relationship between the second male's paternity (P_2) and relative female responsiveness to the second

male using geometric mean linear regression. To determine whether relative sperm load (based on the raffle model) explained some of the variation in P_2 , while simultaneously controlling for variation in brood size, we developed a randomization test (Manly 1997). The test randomized the expected P_2 by shuffling (with replacement) these values with respect to the observed number of offspring actually fertilized by the second male, taking into account brood size, in each trial. It then calculated the probability of observing the offspring distribution based on the shuffled P_2 values according to the following equation derived from the binomial theorem:

$$\Pr(P_2|S_1, S_2) = \prod \binom{B}{P_2 B} \left(\frac{S_2}{S_1 + S_2} \right)^{P_2 B} \left(\frac{S_1}{S_1 + S_2} \right)^{(1-P_2)B}, \quad (2.1)$$

where B is brood size, all other variables are as defined above and the cumulative product is over all broods.

The shuffling process was repeated 10 000 times and these values were used to calculate the proportion of randomized datasets that had a probability of greater than or equal to the probability calculated from the actual data. The test was used independently for the showy/plain and plain/showy treatments to control for potential order effects.

Finally, to assess the roles of mating order, female responsiveness and relative sperm load in determining paternity, we used a weighted multiple linear regression. The showy male's paternity (P_s), his mating order (first or second), relative female responsiveness for the showy male and relative sperm number (showy male/[showy male + plain male]) were entered as independent variables. $\log_{10}(\text{brood size})$ was used as the weighting factor to account for differences in the quality of the paternity data among families. For example, larger broods are more heavily weighted because they provide more precise estimates of paternity, but with diminishing returns that approximate a log relationship (see Neff & Pitcher 2002).

All means are reported plus or minus one standard error. All female responsiveness data, relative sperm loads and paternity data were arcsine square root transformed prior to statistical analyses to render them normally distributed (Zar 1999). All statistical tests were performed using either SPSS (v. 10), Microsoft EXCEL (v. 2000) or Visual C++.

3. RESULTS

Our experimental design called for two sets of males, one with greater areas of orange than the other; as planned the showy males had significantly greater orange area than the plain males (showy/plain dyads: $17.1\% \pm 0.8\%$ versus $7.5\% \pm 0.7\%$; $t_{11} = 7.72$, $p < 0.001$; plain/showy dyads: $7.8\% \pm 0.7\%$ versus $14.3\% \pm 0.8\%$; $t_{11} = 5.55$, $p < 0.001$) and there was no significant difference in orange area between groups of first and second males in the showy/showy dyads ($17.7\% \pm 1.3\%$ versus $15.5\% \pm 1.1\%$; $t_{11} = 1.76$, $p = 0.11$) and plain/plain dyads ($7.3\% \pm 1.1\%$ versus $6.2\% \pm 0.4\%$; $t_{11} = 0.54$, $p = 0.6$). There were no significant differences in body length or the areas of yellow, black or blue coloration between the two males used within dyads for each of the four treatments (paired t -tests: $p > 0.15$ and d.f. = 11 for each).

Across all four dyad treatments, females were more responsive (the proportion of sigmoids to which a female responded positively) during their first mating opportunity than their second (0.31 ± 0.04 versus 0.19 ± 0.03 ; $t_{47} = 4.03$, $p < 0.001$). During the first mating opportunity

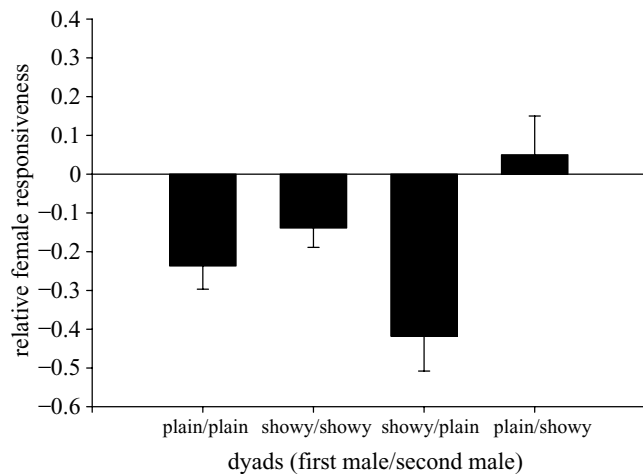


Figure 1. Relative female responsiveness to the second male (responsiveness to second male – responsiveness to first male) across the four treatments. Larger values of relative female responsiveness indicate that the female was, on average, more responsive to the second male than to the first male within the particular treatment.

there was no relationship between female responsiveness and the amount of male orange coloration (on the first day: $r^2 = 0.01$, $F_{1,48} = 0.12$ and $p = 0.73$; the second day: $r^2 = 0.03$, $F_{1,48} = 1.4$ and $p = 0.24$; during the first two days combined: $r^2 = 0.05$, $F_{1,48} = 0.13$ and $p = 0.71$). Thus, virgin females did not initially seem to respond differently to males based on the amount of orange coloration they possessed. During the second mating trial, however, female responsiveness was affected by the difference in orange area between the two males. There was significant variation in relative female responsiveness to the second male among the four treatments (ANOVA $F_{3,48} = 12.1$ and $p < 0.001$; figure 1). Females in the plain/showy dyads were more responsive to the second male than the females in the plain/plain and showy/plain dyads (Tukey tests: $p < 0.01$ each). Females in the showy/showy dyads were also more responsive to the second male than were the females in the showy/plain dyads ($p = 0.03$). None of the other comparisons was significant ($p > 0.1$ for each). When analysed using a linear regression, we found that, across all four treatments, female relative responsiveness to the second male increased as a function of the difference in orange coloration between the two males ($r^2 = 0.26$, $F_{1,48} = 16.1$ and $p < 0.001$; figure 2). When relative female responsiveness was analysed in relation to the difference in orange coloration between the first and second male and the amount of orange coloration on the second male using a multiple linear regression, we found that there was no independent effect of the amount of orange on the second male (difference in orange: $\beta = 0.48$ and $p = 0.005$; orange on the second male: $\beta = 0.18$ and $p = 0.17$). This multiple regression indicates that females did not simply prefer more colourful males, regardless of their previous mate. Finally, we also found that for the showy/showy and plain/plain treatments, female responsiveness to the initial male and relative female responsiveness between the two male dyad combinations did not differ significantly (first male: $t_{22} = 1.6$, $p = 0.13$; second male: $t_{22} = 1.2$, $p = 0.24$),

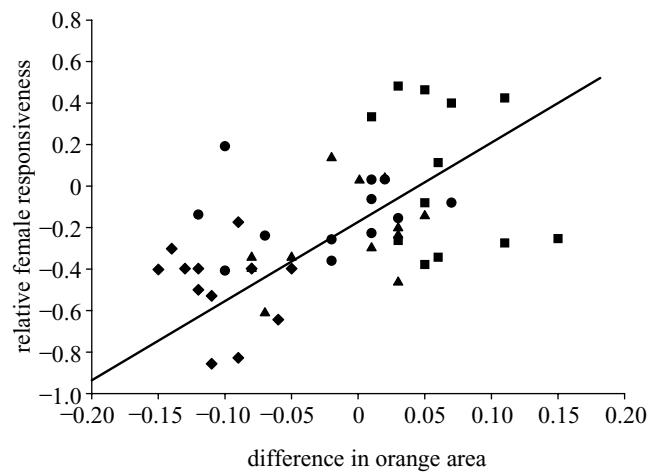


Figure 2. The relationship between relative female responsiveness to the second male (responsiveness to second male – responsiveness to first male) and differences in relative orange coloration between the two males (orange area of second male – orange area of first male). The equation of the line is: female relative responsiveness = $4.1039 \times$ difference in orange area – 0.127. The symbols represent the four treatments: showy/plain (filled diamonds); showy/showy (filled circles); plain/plain (filled triangles); and plain/showy (filled squares).

suggesting that females preferred relatively more colourful second males, regardless of their population origin.

The paternity data confirmed a significant positive relationship between relative female receptivity and second-male paternity ($r^2 = 0.24$, $F_{1,16} = 4.8$ and $p = 0.045$). Thus, our behavioural measure was indicative of male reproductive success. Across the two treatments for which we had paternity data, the number of male offspring produced per female was, on average, 8.5 ± 0.8 (range of 4–16). There was no difference between the showy/plain and plain/showy treatments in the number of offspring scored for paternity (7.6 ± 0.9 versus 9.3 ± 1.3 ; $t_{15} = -1.02$, $p = 0.32$). We also found no indication of a male-biased sex ratio in the broods between the two treatments (proportion of males: 0.48 ± 0.02 versus 0.53 ± 0.03 ; $t_{15} = -1.26$, $p = 0.23$), nor did we find a significant difference in the paternity of second males in first and second broods (0.72 ± 0.08 versus 0.58 ± 0.08 ; paired t -test: $t_{12} = 0.52$, $p = 0.61$).

The number of stripped sperm did not differ between the plain and showy males ($3.5 \pm 0.4 \times 10^6$ versus $4.3 \pm 0.3 \times 10^6$; paired t -test: $t_{16} = 1.57$, $p = 0.14$), nor between first and second males ($4.5 \pm 0.4 \times 10^6$ versus $3.9 \pm 0.5 \times 10^6$; paired t -test: $t_{16} = 1.19$, $p = 0.25$). Furthermore, the randomization test revealed no association between relative sperm load and P_2 within either of the two treatments (showy/plain: $p = 0.43$; plain/showy: $p = 0.39$).

The mean paternity of the second male (P_2) was 0.69 ± 0.07 (range of 0–1; figure 3) for the combined plain/showy and showy/plain treatments. In the plain/showy treatment, the mean P_2 was 0.79 ± 0.08 (range of 0.25–1), whereas in the showy/plain treatment it was only 0.57 ± 0.10 (range of 0–1). However, this difference was not significant ($t_{16} = 1.65$, $p = 0.1$). Across both treatments, the mean paternity of the showy male was larger than the paternity of the plain male, irrespective of mating

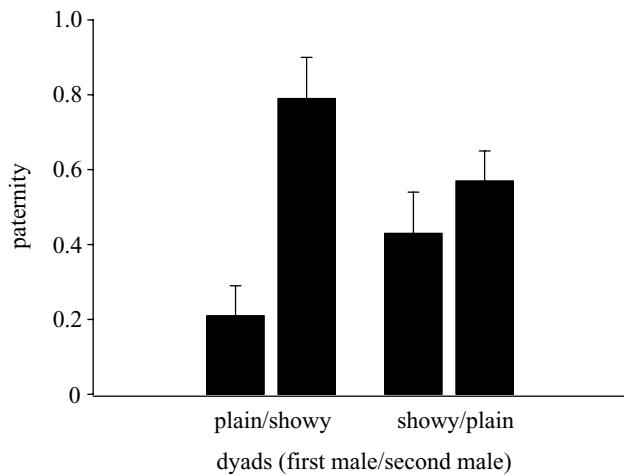


Figure 3. Observed distribution of paternity for the plain/showy and showy/plain dyads (first male/second male). Paternity estimates could not be obtained for seven male dyads because females did not produce broods.

Table 1. Results from a weighted multiple linear regression of showy male paternity.

(Variables tested were mating order of the showy male (first or second), relative female responsiveness for the showy male (responsiveness to showy male – responsiveness to plain male) and relative sperm load of the showy male (showy male/[showy male + plain male]). \log_{10} (brood size) was used as the weighting factor to account for differences in the quality of the paternity data among families. β is the standardized regression coefficient and reflects the direction and magnitude of the effect and p refers to corresponding significance.)

source ^a	β	p
mating order	0.73	0.016
female responsiveness	0.59	0.058
sperm number	-0.34	0.179

^a Overall model: $r^2 = 0.44$, $F_{3,17} = 3.44$, $p = 0.049$.

order (0.63 ± 0.08 versus 0.38 ± 0.08 ; $t_{32} = 2.22$, $p = 0.033$; figure 2). Showy males obtained higher paternity when they mated second as opposed to when they mated first (0.79 ± 0.08 versus 0.43 ± 0.11 ; $t_{15} = 2.48$, $p = 0.03$; figure 3), as did plain males (0.57 ± 0.11 versus 0.20 ± 0.08 ; $t_{15} = 2.37$, $p = 0.032$; figure 3). Overall, the weighted multiple linear regression revealed that mating order of the showy male had a significant effect on his paternity (P_S), relative female responsiveness to the showy male had a nearly significant effect, and relative sperm number had no effect (table 1).

4. DISCUSSION

Our experiment provides support for the trade-up hypothesis. The responsiveness of female guppies to a second male was an increasing function of the degree to which that male was ornamented relative to her first mate. These data are consistent with two previous behavioural tests of the trade-up hypothesis (Gabor & Halliday 1997; Bateman *et al.* 2001). In contrast to these previous studies, we also demonstrate that this bias in responsiveness trans-

lates into paternity: females were more likely to sire offspring with more-attractive second males.

The first prediction of the trade-up hypothesis is that virgin females should be relatively indiscriminate during their first mating opportunity to ensure a sperm supply (Halliday 1983). Our data support this prediction; virgin females did not discriminate between highly ornamented and drab males during their first 2 days of exposure. By contrast, Houde (1987) demonstrated discrimination based on male coloration by virgin female guppies in the laboratory. Females in our study may have been especially responsive and therefore indiscriminate to the first male because of our rearing protocol. Females had been held for four to six months in the absence of males, and hence their estimate of future opportunities to gain sperm may have been especially low. It is unknown how the mate preferences of virgin females in the wild develop since they would usually mature in the presence of at least a few adult males.

The second prediction of the trade-up hypothesis is that females should only be responsive to a potential mate when he is more attractive than her first mate. We found that female guppies were more likely to respond positively to a second male if he was more ornamented than the first male that they mated (figure 1). If the second male had similar or reduced ornamentation compared with the first male, the female significantly reduced her sexual responsiveness. A female's responsiveness should be related to the benefits obtained from the increased quality of the second male, the proportion of offspring that he can be expected to sire and the cost of mating with the male. The cost of mating may be particularly important because it can be substantial for several species (see, for example, Rowe 1994), including guppies (risk of parasite infection: Lyles 1990; Houde & Torio 1992; increased risk of predation: Magurran & Seghers 1994; Godin & Briggs 1996; Magurran 2001; Godin & McDonough 2003). Thus, a female presented with a second male of higher quality than her first mate may still be reluctant to mate when the cost of mating is relatively high (or his relative paternity is likely to be low). We found that females were generally unresponsive to a second male when that male was similar to her first mate in attractiveness, suggesting a possible cost to mating (figure 1). Finally, a more refined test of the trade-up hypothesis might predict that female responsiveness to a second male should increase as he becomes increasingly ornamented relative to the first male; by contrast, female responsiveness should decrease as the second male becomes less ornamented than the first. Our data demonstrate such a continuous response (figure 2).

In the initial formulation of the trade-up hypothesis, Halliday (1983) specified that there must be last-male sperm precedence. However, females may gain some advantage when a second male of higher genetic quality gains at least some paternity, even if he does not gain a majority (Gabor & Halliday 1997). Despite its importance to the trade-up hypothesis, previous studies have not estimated the paternity of the males involved, and therefore the potential benefit of trading up for females. While some degree of last-male sperm precedence has been demonstrated in guppies (Evans & Magurran 2001), we show that second-male paternity is variable and appears to depend on differences in female responsiveness (as a result

of differences in attractiveness) between the first and second male. Showy males clearly exhibited a second-male mating advantage (*ca.* 80%) when females were mated to plain males first (figure 3): by contrast, last-male precedence of drab males was much less pronounced (*ca.* 60%) when females were mated to showy males first. Thus, both mating order and female preference contribute to the outcome of paternity. The observed variation in last male paternity is potentially attributable to

- (i) the frequency of mating with each male;
- (ii) the duration of copulations (which presumably influences the number of sperm deposited);
- (iii) the post-copulatory female manipulation of sperm use (e.g. sperm ejection or sperm choice);
- (iv) other means by which attractive males successfully deposit more sperm (e.g. more sperm per ejaculate; Pitcher & Evans 2001; Pilastro *et al.* 2002); and/or
- (v) higher-quality sperm with greater fertilization ability (see Evans *et al.* 2003).

While it is known, for males from the showy population, that males with more orange coloration possess relatively more sperm (Pitcher & Evans 2001), the actual sperm features that confer a reproductive advantage remain to be quantified and provide an interesting avenue for future research.

Although we provide evidence that female guppies trade up for attractive males, we did not assay offspring fitness. Therefore, we cannot confirm that indirect (i.e. genetic) benefits assumed in the trade-up hypothesis are occurring in this system. The nature of the indirect benefits from mating with attractive males in guppies remains to be fully explored, though some relevant data exist. Previous studies of guppies have demonstrated that preferred males (i) sire more attractive sons (Houde & Endler 1990; Houde 1992, 1994); (ii) sire offspring with higher viability or growth (Nicoletto 1991; Reynolds & Gross 1992; Evans & Magurran 2000; but see Brooks 2000); or (iii) have more or higher quality sperm (Pilastro *et al.* 2002; Pitcher & Evans 2001; Evans *et al.* 2003).

Finally, many recent studies have focused on post-copulatory mechanisms that females may use to bias paternity towards one male over another. Only a few studies have found evidence to support a post-copulatory mechanism (Wilson *et al.* 1997; Tregenza & Wedell 2002), whereas other studies find no evidence of any such mechanism (Stockley 1997; Cunningham & Cheng 1999). The trade-up strategy provides a pre-copulatory mechanism that would allow females to have some control over paternity without evoking post-copulatory mechanisms such as 'sperm choice'. The trade-up hypothesis suggests that multiple paternity in some mating systems may simply be a by-product of female trading-up behaviour and may therefore help to explain the predominance of female multiple mating patterns in nature.

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REFERENCES

- Andersson, M. 1994 *Sexual selection*. Princeton University Press.
- Baerends, G. P., Brouwer, R. & Waterbolk, H. T. 1955 Ethological studies on *Lebistes reticulatus* (Peters). I. An analysis of the male courtship pattern. *Behaviour* **8**, 249–334.
- Bakker, T. C. M. & Milinski, M. 1991 Sequential female choice and the previous male effect in sticklebacks. *Behav. Ecol. Sociobiol.* **29**, 205–210.
- Bateman, P. W., Gilson, L. N. & Ferguson, J. W. H. 2001 Male size and sequential mate preference in the cricket *Gryllus bimaculatus*. *Anim. Behav.* **61**, 631–637.
- Breden, F., Novinger, D. & Schubert, A. 1995 The effect of experience on mate choice in the Trinidadian guppy, *Poecilia reticulata*. *Environ. Biol. Fish.* **42**, 323–328.
- Brooks, R. 2000 Negative genetic correlation between male sexual attractiveness and survival. *Nature* **406**, 329–334.
- Brooks, R. & Caithness, N. 1995 Females use orange as a mate choice cue: a manipulative test. *S. Afr. J. Zool.* **30**, 200–201.
- Cook, P. A., Harvey, I. F. & Parker, G. A. 1997 Predicting variation in sperm precedence. *Phil. Trans. R. Soc. Lond. B* **352**, 771–780. (DOI 10.1098/rstb.1997.0061.)
- Cunningham, E. J. A. & Cheng, K. M. 1999 Biases in sperm use in the mallard: no evidence for selection by females based on sperm genotype. *Proc. R. Soc. Lond. B* **266**, 905–910. (DOI 10.1098/rspb.1999.0722.)
- Dombrowsky, Y. & Perrin, N. 1994 On adaptive search and optimal stopping in sequential mate choice. *Am. Nat.* **144**, 355–361.
- Endler, J. A. & Houde, A. E. 1995 Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution* **49**, 456–468.
- Evans, J. P. & Magurran, A. E. 2000 Multiple benefits of multiple mating in guppies. *Proc. Natl Acad. Sci. USA* **97**, 10 074–10 076.
- Evans, J. P. & Magurran, A. E. 2001 Patterns of sperm precedence and predictors of paternity in the Trinidadian guppy. *Proc. R. Soc. Lond. B* **268**, 719–724. (DOI 10.1098/rspb.2000.1577.)
- Evans, J. P., Zane, L., Francescato, S. & Pilastro, A. 2003 Directional postcopulatory sexual selection revealed by artificial insemination. *Nature* **421**, 360–363.
- Gabor, C. R. & Halliday, T. R. 1997 Sequential mate choice by multiply mating smooth newts: females become more choosy. *Behav. Ecol.* **8**, 162–166.
- Godin, J.-G. & Briggs, S. E. 1996 Female mate choice under predation risk in the guppy. *Anim. Behav.* **51**, 117–130.
- Godin, J.-G. & McDonough, H. E. 2003 Predator preference for brightly colored males in the guppy: a viability cost for a sexually selected trait. *Behav. Ecol.* **14**, 194–200.
- Green, A. J. 1991 Large male crests, an honest indicator of condition, are preferred by female smooth newts *Triturus vulgaris* (Salamandridae) at the spermatophore transfer stage. *Anim. Behav.* **41**, 367–369.
- Halliday, T. R. 1983 The study of mate choice. In *Mate choice* (ed. P. Bateson), pp. 3–32. Cambridge University Press.
- Houde, A. E. 1987 Mate choice based upon naturally occurring colour pattern variation in a guppy population. *Evolution* **41**, 1–10.

- Houde, A. E. 1988 The effects of female choice and male–male competition on the mating success of male guppies. *Anim. Behav.* **36**, 888–896.
- Houde, A. E. 1992 Sex-linked heritability of a sexually selected character in a natural population of *Poecilia reticulata* (Pisces: Poeciliidae) (guppies). *Heredity* **69**, 229–235.
- Houde, A. E. 1994 Effect of artificial selection on male colour patterns on mating preferences of female guppies. *Proc. R. Soc. Lond. B* **256**, 125–130.
- Houde, A. E. 1997 *Sex, colour, and mate choice in guppies*. Princeton University Press.
- Houde, A. E. & Endler, J. A. 1990 Correlated evolution of female mating preferences and male colour patterns in the guppy *Poecilia reticulata*. *Science* **248**, 1405–1408.
- Houde, A. E. & Torio, A. J. 1992 Effect of parasitic infection on male colour pattern and female choice in guppies. *Behav. Ecol.* **3**, 346–351.
- Hughes, K. A., Du, L., Rodd, F. H. & Reznick, D. N. 1999 Familiarity leads to female mate preference for novel males in the guppy, *Poecilia reticulata*. *Anim. Behav.* **58**, 907–916.
- Janetos, A. C. 1980 Strategies of female mate choice: a theoretical analysis. *Behav. Ecol. Sociobiol.* **7**, 107–112.
- Jennions, M. D. & Petrie, M. 1997 Variation in mate choice and mating preferences: a review of causes and consequences. *Biol. Rev. Camb. Phil. Soc.* **72**, 283–327.
- Jennions, M. D. & Petrie, M. 2000 Why do females mate multiply? A review of the genetic benefits. *Biol. Rev. Camb. Phil. Soc.* **75**, 21–64.
- Kelly, C. D., Godin, J.-G. & Wright, J. M. 1999 Geographic variation in multiple paternity within natural populations of the guppy (*Poecilia reticulata*). *Proc. R. Soc. Lond. B* **266**, 2403–2408. (DOI 10.1098/rspb.1999.0938.)
- Lyles, A. M. 1990 Genetic variation and susceptibility to parasites: *Poecilia reticulata* infected with *Gyrodactylus turnbulli*. PhD dissertation, Princeton University, Princeton, NJ, USA.
- Magurran, A. E. 2001 Sexual conflict and evolution in Trinidadian guppies. *Genetica* **112–113**, 463–474.
- Magurran, A. E. & Seghers, B. H. 1994 A cost of sexual harassment in the guppy, *Poecilia reticulata*. *Proc. R. Soc. Lond. B* **258**, 89–92.
- Manly, B. F. J. 1997 *Randomization and Monte Carlo methods in biology*, 2nd edn. London: Chapman & Hall.
- Matthews, I. M., Evans, J. P. & Magurran, A. E. 1997 Male display rate reveals ejaculate characteristics in the Trinidadian guppy, *Poecilia reticulata*. *Proc. R. Soc. Lond. B* **264**, 695–700. (DOI 10.1098/rspb.1997.0099.)
- Neff, B. D. & Pitcher, T. E. 2002 Assessing the statistical power of genetic analyses to detect multiple mating in fish. *J. Fish Biol.* **61**, 739–750.
- Nicoletto, P. F. 1991 The relationship between male ornamentation and swimming performance in the guppy, *Poecilia reticulata*. *Behav. Ecol. Sociobiol.* **28**, 365–370.
- Parker, G. A., Simmons, L. W. & Kirk, H. 1990 Analysing sperm competition data: simple models for predicting mechanisms. *Behav. Ecol. Sociobiol.* **27**, 55–65.
- Pilastro, A. & Bisazza, A. 1999 Insemination efficiency of two alternative male mating tactics in the guppy *Poecilia reticulata*. *Proc. R. Soc. Lond. B* **266**, 1887–1891. (DOI 10.1098/rspb.1999.0862.)
- Pilastro, A., Evans, J. P., Sartorelli, S. & Bisazza, A. 2002 Male phenotype predicts insemination success in guppies. *Proc. R. Soc. Lond. B* **269**, 1325–1330. (DOI 10.1098/rspb.2002.2017.)
- Pitcher, T. E. & Evans, J. P. 2001 Male phenotype and sperm number in the guppy (*Poecilia reticulata*). *Can. J. Zool.* **79**, 1891–1896.
- Reynolds, J. D. & Gross, M. R. 1992 Female mate preference enhances offspring growth and reproduction in a fish, *Poecilia reticulata*. *Proc. R. Soc. Lond. B* **264**, 57–62.
- Rodd, F. H. & Sokolowski, M. B. 1995 Complex origins of variation in the sexual behaviour of male Trinidadian guppies, *Poecilia reticulata*: interactions between social environment, heredity, body size, and age. *Anim. Behav.* **49**, 1139–1159.
- Rosenqvist, G. & Houde, A. 1997 Prior exposure to male phenotypes influences mate choice in the guppy, *Poecilia reticulata*. *Behav. Ecol.* **8**, 194–198.
- Rowe, L. 1994 The costs of mating and mate choice in water striders. *Anim. Behav.* **48**, 1049–1056.
- Stockley, P. 1997 No evidence of sperm selection by female common shrews. *Proc. R. Soc. Lond. B* **264**, 1497–1500. (DOI 10.1098/rspb.1997.0207.)
- Tregenza, T. & Wedell, N. 2002 Polyandrous females avoid costs of inbreeding. *Nature* **415**, 71–73.
- Wiegmann, D. D., Mukhopadhyay, K. & Real, L. A. 1999 Sequential search and the influence of male quality on female mating decisions. *J. Math. Biol.* **39**, 193–216.
- Wilson, N., Tubman, S. C., Eady, P. E. & Robertson, G. W. 1997 Female genotype affects male success in sperm competition. *Proc. R. Soc. Lond. B* **264**, 1491–1495. (DOI 10.1098/rspb.1997.0206.)
- Zar, J. H. 1999 *Biostatistical analysis*. Englewood Cliffs, NJ: Prentice-Hall.

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