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Possible role of female discrimination against 'redundant' males in the evolution of colour pattern polymorphism in guppies

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Multiple paternity of offspring can result from active preferences on the part of females or sexual harassment by males. We examined sexual responses of female guppies to a previous mate versus a novel male (experiment 1) or to a male with a colour pattern similar to that of the previous mate versus a novel male (experiment 2). Females showed significantly more sexual responses to courtship by novel males than to previous mates in experiment 1 or to males that resembled previous mates in experiment 2. These results suggest that females discriminate actively against previous mates, and extend this discrimination to males with similar colour patterns to previous mates. This could lead to negative frequency-dependent sexual selection against common colour patterns (a 'redundant male effect'), which could contribute to the maintenance of the extraordinarily high levels of genetic polymorphism in guppy colour patterns.

Keywords: guppy; *Poecilia reticulata*; multiple mating; maintenance of polymorphism; mate choice

1. INTRODUCTION

Studies of multiple mating have rarely tested the hypothesis that females actively prefer novel mates when remating (but see Archer & Elgar 1999; Zeh *et al.* 1998). Alternatively, polyandrous mating may be the non-adaptive result of forced matings or cases in which females accept 'superfluous matings' to avoid costs of sexual harassment by males (Arnqvist 1989; Watson *et al.* 1998).

In this study, we examined the sexual responses of female guppies one day after an initial mating order to test two hypotheses:

(i) that remating females prefer a new male over a previous mate; and
(ii) that this preference could lead to discrimination against males similar in appearance to the previous mate.

Female guppies commonly mate multiply (Kelly *et al.* 1999), even though a single insemination is sufficient to allow a female guppy to produce offspring for several months (Winge 1937). Only if females are actively seeking additional matings with different males would we predict behavioural discrimination between novel and original mates. Discrimination against males that appear similar to previous mates could result in a reproductive disadvantage to males with common colour patterns, a 'redundant male effect' analogous to the 'rare male effect' proposed for *Drosophila* (Ehrman & Petit 1968; Spiess & Spiess 1969).

The highly polymorphic colour patterns of male guppies are heritable with X and Y linkage (Haskins *et al.* 1961; Endler 1978; Houde 1992; Brooks & Endler 2001). Paternally related males may resemble one another so closely that they are indistinguishable to human observers. Mate choice by female guppies is based on male colour pattern, notably orange spots (Houde & Endler 1990; Endler & Houde 1995). The maintenance of Y-linked genetic polymorphism in guppy colour patterns remains paradoxical in the face of natural and sexual selection. A 'redundant male' mating disadvantage could lead to negative frequency-dependent sexual selection, one probable explanation for the polymorphism in colour patterns (Charlesworth & Hughes 2000).

2. MATERIAL AND METHODS

We used four-to-six-month-old descendants of wild individuals taken from the Paria River of Trinidad. Groups of juveniles (usually broods of full- and half-sibs) were isolated from stock tanks and separated by sex before reaching sexual maturity; males were housed with mature females, and females were kept separately with only other virgin females. Experimental fish were drawn at random from these groups of males and females, except in experiment 2 in which pairs of similar appearing males were taken from the same stock aquarium. Virgin females, when placed with males, usually mate within minutes, or at most within hours (A. E. Houde, unpublished data), but continue to respond sexually to males' courtship for 2–3 days (Liley 1966; Houde 1997). Fish were kept on a 12 L : 12 D cycle and fed twice daily with commercial flake food and brine shrimp nauplii.

All experimental trials were conducted in 38 l aquaria separated into three sections (11 cm, 27 cm and 11 cm wide, respectively) by perforated opaque plastic dividers, covered on three sides with brown paper and containing sand-coloured gravel. The observer sat 1 m from the open side of the tank. A 40 W broad spectrum fluorescent light was suspended 20 cm above each tank.

In experiment 1, experimental groups included a virgin female, the male with which she was paired initially, and a second, 'novel' male, which differed in colour pattern from the initial male. For experiment 2, pairs of males with similar colour patterns ('twins'; most probably full-sibs) were selected and housed separately with three non-virgin females. 'Twins' were matched for nearly all colour pattern elements. In experiment 2, one 'twin' was used in the initial trial, while the other 'twin' was used in the second trial.

In both experiments, a male and a virgin female were placed in the middle section of the tank for 24 h, during which we assumed, based on previous work (A. E. Houde, unpublished data), that the initial mating occurred. In experiment 1, the male was removed from the middle section the following morning ('day 2') and arbitrarily placed in the right or left (determined by coin flip) end section, out of view of the female. A novel male was added to the other end section, and all three fish were left to acclimate for 10 min. The male in the left end section was then added to the middle section with the female and observed continuously for 20 min, beginning when both fish resumed swimming. Following this trial, the male was returned to his end section, and after another 10 min, the fish in the right end section was added and observed continuously with the female for 20 min. The female was introduced to the novel male first in nine out of the 17 day 2 trials used in the analysis of experiment 1. During day 2 trials, if copulation occurred, the male was removed, and the addition of the second male was delayed for 30 min because female guppies may be unresponsive for up to 30 min after copulation (Houde 1997). All of these trials took place from 08.50–09.50.

In experiment 2, virgin females were placed with one 'twin' for the initial 24 h mating trial and then with the other 'twin', and a randomly selected, unrelated, 'novel' male, for the mating trial. This experiment was otherwise identical to the preceding experiment, with the exception that the original male was replaced with his 'twin' after the initial mating. In experiment 2, females were introduced to the novel male first in nine out of the 20 day 2 trials. This experiment was conducted in the four week period following experiment 1 using entirely different fish.

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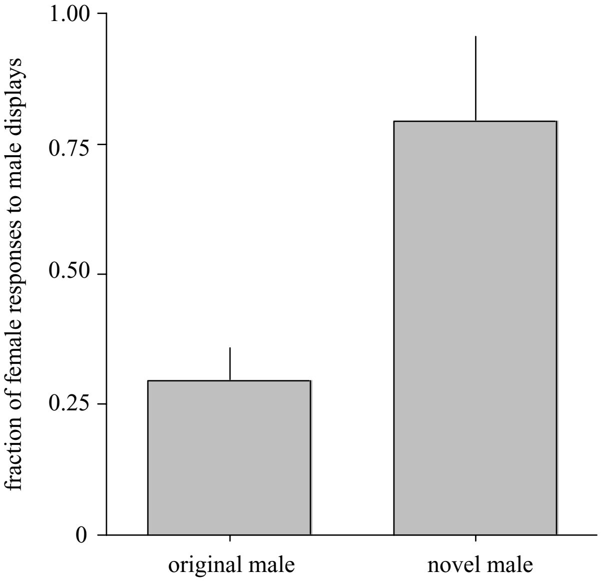

Figure 1. The fraction of sexual responses by females to male courtship displays in day 2 trials for original and novel males. The fraction of responses is a measure of male sexual attractiveness to females. Medians and interquartile ranges are shown.

During trials, we recorded whether or not the females showed an unambiguous sexual response to each 'sigmoid' courtship display performed by males. We scored a sexual response if the female oriented towards the displaying male and approached with a characteristic 'glide' response (Liley 1966). We used the fraction of male displays that elicited a female response as a measure of male attractiveness; this measure predicts male mating success (Houde 1997).

We used non-parametric Wilcoxon tests for paired comparisons and Spearman rank correlations. For each male, the fraction of response by the female was calculated by dividing the total number of his courtship displays receiving a sexual response by the female by the total number of displays he performed. Trials in which one of the males did not display or the female responsed to neither display were discarded.

After each trial, males were photographed digitally. Body area (not including tail) and areas of orange spots were measured using NIH Image v. 1.62 software. The relative area of orange was calculated as the total area of orange divided by the body area. Results from experiment 1 and experiment 2 were not compared statistically because they were conducted sequentially and therefore any differences could be temporally confounded.

3. RESULTS

No statistically significant difference was observed between the number of displays performed by novel versus original males in experiment 1 (Wilcoxon $z = -1.190$, $p = 0.234$, $n = 17$) or experiment 2 (Wilcoxon $z = -1.220$, $p = 0.223$, $n = 20$), indicating that each female received a comparable number of displays and therefore had equal opportunities to respond to either male. No statistically significant difference was observed between the relative orange coloration of novel and original males in experiment 1 (Wilcoxon $z = -1.046$, $p = 0.295$, $n = 20$), nor between the novel and twin males (Wilcoxon $z = 1.008$, $p = 0.313$, $n = 20$), nor the original and twin males (Wilcoxon $z = -0.112$, $p = 0.911$, $n = 20$) in experiment 2.

In experiment 1, females responded significantly more often to the novel male than to the original male in day 2 observations (Wilcoxon $z = 3.527$, $p < 0.001$, $n = 17$; figure 1). In 16 out of the 17 trials in which both males exhibited courtship displays, the novel male received a higher fraction of female responses. Copulation occurred in seven out of the 17 trials in experiment 1; the female mated with the novel male in all seven cases. Similarly, females in experiment 2 responded significantly more strongly to the novel male than to the original male's 'twin' (Wilcoxon $z = 3.375$, $p = 0.001$, $n = 20$; figure 2) and females responded more frequently to the novel male in 17 out of 20 trials. Copulation occurred in seven out of the 20 experiment 2 trials; the female mated with the novel male in three trials, the 'twin' in three trials, and both males in one trial.

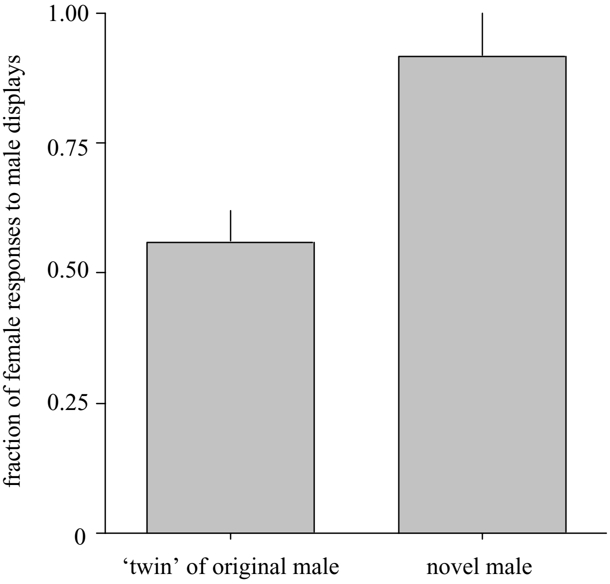

Figure 2. The fraction of sexual responses by females to male courtship displays in day 2 trials for 'twins' with colour patterns similar to the original male and the novel males. Medians and interquartile ranges are shown.

In both experiments, the difference in orange coloration between the novel and original male was positively but non-significantly related to the difference in response rate (experiment 1: Spearman $r_s = 0.356$, $n = 17$, $p = 0.18$; experiment 2: Spearman $r_s = 0.344$, $n = 20$, $p = 0.14$). Thus, there appears to be only a slight, if any, additional effect of orange coloration on females' discrimination in favour of novel males.

4. DISCUSSION

Our results indicate that female guppies discriminate in favour of novel mates in the context of remating. The implications of this study are twofold:

(i) that multiple mating in guppies is likely to be the result of active selection of novel mates by remating females (experiment 1), suggesting that multiple mating may reflect adaptive behaviour on the part of guppy females rather than a non-adaptive or maladaptive consequence of sexual harassment by males; and

(ii) that this discrimination against previous mates extends to males with similar colour patterns (experiment 2).

Although our results do not include direct mating success data, they are the first, to our knowledge, to directly show active behavioural discrimination in favour of novel males by female guppies after an initial mating and the first, to our knowledge, in any species to suggest that this discrimination may extend to similar appearing males. The fact that this discrimination extends to 'twins' of original mates may provide a behavioural mechanism for negative frequency-dependent selection and the maintenance of paradoxically high levels of colour pattern polymorphism in guppy populations. Work is underway to determine if this behavioural preference of females leads to increased mating success of novel males.

In a similar experiment, Pitcher *et al.* (2003) showed increased responsiveness and sperm utilization by females when presented with males that had more attractive colour patterns than previous mates, supporting the 'trade up' hypothesis for the evolution of multiple mating (Jennions & Petrie 2000). We did not note a significant effect of relative degree of orange coloration on female response to the second male, but our experimental design did not provide a great deal of power to detect such an effect.

Our results are consistent with those of Farr (1977), who found increased mating success of males with uncommon colour patterns and Hughes *et al.* (1999), who found increased mating success of males with colour patterns unfamiliar to females. In addition, our study provides direct behavioural evidence for female preference for novel males in the specific context of multiple mating. There may be a general preference on the part of guppy females for novel males which could explain our results as well as those of these previous studies, or the preference for novel males that we found may be specific to the context of multiple mating.

We also found that this discrimination extended to males with colour patterns similar to those of the initial mate ('twins'). This could result from females' failure to recognize the twin as a different fish from the original mate, or it could represent an adaptive mechanism for avoiding relatives of males a female has already mated with or rejected. These are not mutually exclusive and each could be based on a visual similarity of twins or similarity in other phenotypic traits owing to common descent.

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