

Female mating preferences for colourful males in a population of guppies subject to high predation

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Female guppies *Poecilia reticulata* descended from the Tacarigua population in Trinidad subject to high predation exhibited strong preferences for males with relatively high levels of carotenoid colouration. The study, which controlled for differences in male courtship, revealed that mate choice in this population is based on the expression of orange pigmentation, irrespective of differences in male motivation.

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Female guppies *Poecilia reticulata* Peters base their mating preferences on male body colouration and the intensity of courtship display (Liley, 1966; Houde, 1997). The extent to which these traits influence female mating preferences varies between populations (Endler & Houde, 1995), and in particular between those differing in predation intensity. For example, when females from populations subject to high predation (high predation populations) are exposed to a predator they tend to exhibit reduced preferences for showy males in comparison to fish at low risk of predation (Godin & Briggs, 1996; Gong, 1997). This effect is likely to be especially pronounced if associating with highly conspicuous males makes females themselves more vulnerable to predators (Pocklington & Dill, 1995; Gong & Gibson, 1996). In contrast, females from populations subject to low predation (low predation populations) typically exhibit strong sexual preferences for males with high levels of orange (carotenoid) pigmentation (Breden & Stoner, 1987; Houde & Endler, 1990; Endler & Houde, 1995). Although female choice for male colouration has been documented in numerous low predation populations (Houde, 1997; Kodric-Brown & Nicoletto, 1997; Jirotkul, 1999; Lopez, 1999; Kodric-Brown & Nicoletto, 2001; Pitcher *et al.*, 2003), surprisingly few studies have reported the mating preferences of females from high predation populations (*e.g.* for UV cues, Smith *et al.*, 2002 and display rates, Stoner & Breden, 1988; Gong, 1997). Those that have reported

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female mating preferences for relatively colourful males did not disentangle male courtship behaviour from the expression of orange colouration (Houde & Torio, 1992; Godin & Briggs, 1996; Houde & Hankes, 1997). The present study experimentally controls for differences in male courtship behaviour and examines the preferences of females descended from a high-predation population for males with relatively high levels of orange pigmentation.

The study focused on first generation descendents from the Tacarigua River in Trinidad, where guppies coexist with a variety of predators, including the pike cichlid *Crenicichla Eigenmann* (Magurran & Seghers, 1994). An additional reason for studying pre-mating female preferences in this population is that it has been the subject of several recent studies examining the outcome, in terms of relative paternity share, of post-copulatory sexual selection (Evans & Magurran, 2000, 2001; Evans *et al.*, 2003). Post-copulatory sexual selection comprises sperm competition, where the sperm from different males compete for fertilization (Parker, 1998) and cryptic female choice, where females bias sperm utilization in favour of particular males (Thornhill, 1983). In particular, two of these studies revealed that post-copulatory sexual selection selects for colour traits known to influence precopulatory female mating decisions in other populations. For example, Evans *et al.* (2003) found that when sperm from a pair of males were artificially inseminated in equal numbers into a naïve virgin female, individuals with relatively high levels of carotenoid pigmentation gained greater parentage than their less ornamented rivals. A subsequent study (Pilastro *et al.*, 2004) revealed that females exhibit 'cryptic' preferences for particular males by manipulating the number of sperm transferred at copulation in favour of those with relatively high levels of carotenoid colouration (where the females' perception of relative male colouration was manipulated). While both studies confirm that post-copulatory sexual selection can select for particular phenotypic traits, neither explicitly demonstrated that females from this population choose those same traits during pre-insemination episodes of sexual selection. A simple mate choice experiment was therefore performed on this population to determine whether sexually receptive female guppies prefer to associate with males exhibiting relatively high levels of carotenoid colouration.

Virgin females, which are almost invariably sexually receptive (Houde, 1997), were used for the preference tests. These females were reared in single sex tanks (150l) and used when 6 months old. Test males, on the other hand, were maintained in mixed sex tanks (150l, *c.* five fish per litre with 1:1 sex ratio) and used for the choice trials when they were fully sexually mature (*c.* 4–5 months old). Males were selected (initially by eye) with either relatively high ($n = 14$) or low ($n = 14$) levels of carotenoid colouration (selected on the basis of the size and number of carotenoid spots) but were otherwise approximately matched for size (mean \pm s.e. standard length, L_S , colourful males 17.4 ± 0.4 mm and drab males 16.7 ± 0.5 mm; *t*-test, d.f. = 26, $P = 0.29$). The choice tests followed the methods described by Bisazza & Pilastro (2000). Specifically, one male from each group (hereafter termed colourful or drab respectively) was selected at random and individually placed in a compartment on either side of a central choice chamber ($60 \times 40 \times 25$ cm, filled to 20 cm) containing a sexually naïve but receptive virgin female (Fig. 1). The two male compartments (each measuring $40 \times 10 \times 25$ cm, filled to 20 cm) were

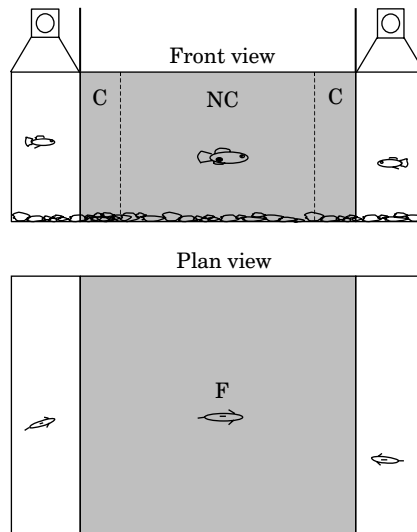


FIG. 1. Experimental design, depicting front and plan views of the dichotomous choice tank. C, choice zones; NC, no-choice zone; F, choosing female. Male compartments were illuminated to facilitate unidirectional visual access into both adjacent compartments.

individually illuminated with a fluorescent solar tube (18 W, True Lite[®]) while the central female compartment remained shaded. This resulted in unidirectional visual access into the side compartments; females could see into both male compartments but males were unable to see into the central female chamber. This made it possible to examine female preferences on the basis of colour pattern variation, while potential differences in sexual motivation (*e.g.* courtship) among males were controlled. Houde & Hankes (1997) did not control for interactions among their test males and they were able to interact visually with the female. Furthermore, the present design prevented males from seeing one another, thus avoiding the possible confounding influence of male-male interactions on female association patterns (Houde, 1997). The female's central chamber was divided into three sections using an indelible pen: a central 'no-choice' zone flanked by two 6 cm-wide 'choice zones' adjacent to the two compartment tanks. Opaque dividers, which could be raised and lowered from a remote location (behind a black cloth blind), prevented visual access by the female into the two side tanks during an initial acclimatisation period (30 min).

Following the settlement period the opaque dividers were raised, allowing the female visual access into the two adjacent compartments. As a measure of female preference for either male, side association data were collected during a 30 min observation period. Specifically, the female's position (in or out of either of the two choice zones) was recorded every 5 s throughout the 30 min period, providing 360 point samples per test female (Houde, 1997). For each point sample, the investigator recorded the female's position every 5 s using a timer set to 'bleep' every 5 s. To eliminate possible side bias in the preference measures, colourful and drab males were alternated between the left and right sides of the central choice chamber on consecutive trials (*i.e.* in half of the trials

colourful males were placed on the left side of the central chamber and in half they were placed on the right). A preference index was calculated as the number of point samples in which the female was within 6 cm of the colourful male, divided by the total number of samples in which she was in both choice zones. Hence, this index included only the periods in which females were closely associating with either male. The results revealed that females ($n = 14$) associated more frequently with the colourful males than they did with the drab ones [mean \pm s.e. proportion of samples in which females were close to the colourful male = 0.73 ± 0.05 ; one-sample t -test after arcsine square root transformation for an expected (transformed) value for no preference: d.f. = 13, $P = 0.001$]. Following each trial, both males were anaesthetized (MS-222) and photographed using a digital camera. Image analysis software (UTHSCSA Image Tool, available at <http://www.ddsdx.uthscsa.edu/dig/download.html>) was used to measure each male's colour patterns, following the methods in Pitcher & Evans (2001). These measures confirmed that the colourful group had a significantly greater area of carotenoid spots than their drab counterparts (e.g. difference in the proportion of area covered with carotenoid spots; t -test following arcsine square root transformation, d.f. = 26, $P < 0.0001$; Table I).

These results confirm that female guppies from a high-predation population exhibit strong preferences for males with high levels of body colouration, complementing numerous studies performed on low predation (notably from the Paria River) populations (Houde, 1987). It would be interesting to determine whether the strength of female preferences in this and other high-predation populations is dependent on the level of risk, and thus whether females exhibit a 'risk-sensitive' mate choice strategy. The experiment was designed to investigate the influence of male colouration, and particularly the carotenoid component of these patterns, on female sexual preferences. To this end, the potentially confounding influence of differential sexual motivation among males was avoided by preventing behavioural interactions between males and females. Thus, although it was possible to test whether females preferred to associate with males that differed solely in body colouration (or morphological traits correlated with colouration), it was not possible to score 'acceptance' behaviours that would have confirmed that females were indeed sexually motivated (e.g. the glide response, described by Liley, 1966); Houde & Torio (1992) and Houde & Hankes (1997) used female responses to courting males to assess female mating preferences. Virgin female guppies, however, often respond spontaneously towards males even when they are not engaged in courtship and there is ample evidence indicating that such responses are sexually motivated

TABLE I. Mean \pm s.e. trait values (carotenoids, total colouration and standard length) for the two experimental male groups (colourful and drab). * P values are for pair-wise comparisons using a t -test (all probabilities are two-tailed, d.f. = 26)

Trait	Colourful	Drab	P^*
Carotenoids (%)	19.64 \pm 1.85	3.42 \pm 0.86	<0.0001
Total colouration (%)	31.66 \pm 2.63	6.27 \pm 1.16	<0.0001
Body length (L_S)	17.41 \pm 0.36	16.74 \pm 0.51	0.29

(Houde, 1997). Moreover, the present observation that females apparently based their preferences on variation in body colouration, rather than behavioural or other phenotypic male traits, strongly suggests that they were sexually motivated.

An additional reason for performing this simple preference test was to determine whether the morphological traits identified as predictors of male reproductive success during post-copulatory sexual selection (Evans *et al.*, 2003; Pilastro *et al.*, 2004) are also favoured during precopulatory episodes of mate choice. The data presented here indicate that this is the case and support earlier predictions that sperm competition and cryptic female choice can result in directional selection on male traits that are preferred during pre-copulatory episodes of sexual selection.

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