Intersexual and intrasexual selection, sneak copulation and male ornamentation in guppies (*Poecilia reticulata*)

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To test the relative importance of female choice, sneak copulation and male dominance as determinants of male mating success in a feral guppy population, we conducted a paired-male mating experiment using the colour patterns of male progeny to score paternity. Sneak copulation and male-male aggression had no noticeable effect on male mating success. Mating success was strongly related to the frequency of female behavioural responses to both displaying and non-displaying males. Relative area of orange colouration and complexity of male colour patterns were positively related to mating success. This substantiates the observed behavioural preference for orange, and strengthens claims that female choice is integral to male mating success. Males in trials in which both females failed to produce offspring showed lower levels of black pigmentation, suggesting a threshold mating preference for this ornament despite it not being correlated with variation in male mating success or attractiveness measures. The implications of these findings for the evolution of multiple secondary sex traits in male guppies are discussed.

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Introducing the concept of sexual selection, Darwin (1871) characterised female choice (intersexual or epigamic selection) and direct intrasexual competition as its two principal mechanisms. Sneak copulation is a third phenomenon that may influence the mating success of males. Sexual selection has recently become a highly productive field of study in behavioural ecology, but it is often difficult to identify which sexually selective processes are occurring in a particular population (Moller 1994). It is certainly possible for both intrasexual and intersexual selection to occur in the same population (Evans & Hatchwell 1992a, b). In this article, we examine the relative importance of sneak copulation, male-male dominance interactions and female choice to male mating success in guppies (*Poecilia reticulata*).

Studies of female mate choice often estimate the strength of preferences by scoring behaviours other than mating (for example Houde 1987, Brooks & Caithness 1995). Proximate measures of male attractiveness used in female choice studies must be shown to be useful predictors of mating success for two reasons. First, arbitrary behaviours might be reified to the status of sexual responses by the observer. More importantly, even if female sexual response is really being measured, it must contribute to variation in male mating success in order to be important to sexual selection (Houde 1988).

We explored the ability of several female response measures to predict mating success in guppies. We also studied behavioural interactions between males, and other male-female interactions so that we could infer the relative importance of female choice, overt male aggression and sneak copulations (= gonopodial thrusts – Farr 1980) to mating success. This is an important goal as there is no consensus in the literature as to whether or not the latter two processes influence differential reproduction of males (Farr 1980, Gorlick 1976, Houde 1988, Kodric-Brown 1992, 1993, Reynolds *et al.* 1993).

Male guppies have several exaggerated secondary sexual

characters including orange (carotenoid), black (melanin), iridescent (structural) and yellow pigments, and exaggerated dorsal and caudal fins. Sexual selection is an obvious possible explanation for the maintenance of exaggerated secondary sexual characters (Moller 1994, Andersson 1994). However, behavioural studies of this population (Brooks & Caithness 1995) have only shown that orange colouration consistently correlates with male attractiveness. We investigated the relationship between secondary sexual characters of males and mating success in order to infer the importance of these traits to the sexually selective processes at work.

Methods

The guppies originated from a small stream about 1 km upstream from the Beachwood mangrove swamp in Beachwood, Durban, South Africa. Forty males and forty naive virgin females were randomly chosen from individuals that had been wild caught as juveniles and separated into single sex stocks (approx 40 fish / 100 l) as soon as their sex could be identified. Fish were kept in a temperature controlled (25° C) room, with fluorescent tube lighting on a 12:12h light:dark cycle.

Behavioural measures and mating

Each pair of males was placed with two virgin females in a 10 l aquarium with brown paper on three sides to standardise background colour. Mating trials lasted seven days, with two ten-minute observation sessions per male on the first two mornings. We combined behavioural data from both days. We scored the female response to every sigmoid display of the focal male as follows (after Houde 1987): no response; orient toward male; orient then glide toward male (swimming smoothly with pectoral fins); or full copulation. Full copulation was infrequent (nine times in 800 minutes of observation), which is one reason breeding experiments are required

to quantify mating success. We used absolute number of each female response, proportion of displays eliciting the response and 'swim-toward' (a non-displaying male) responses (Houde 1987) as measures of male attractiveness. We also noted attempted sneaky copulations (gonopodial thrusts) and male dominance (success) in chase/standoff interactions over females (which is how male-male competition is manifested, if at all, in guppies, Houde 1988, Kodric-Brown 1993).

After the trial, we isolated each female and inspected her tank daily until her first litter was produced, or until three months had elapsed (eight of the 40 females used). Five to six months after birth, we sexed offspring and established the paternity of each male by comparing his colour patterns with those of the two potential sires. Male guppy colour patterns are highly heritable (Endler 1978), and (in this population) very similar between father and son, particularly in the size, shape and placement of orange spots (personal observations from matings of known parentage). For all but three individual offspring, paternity could be clearly established. Each male was considered to have mated with a female if he sired at least one son by her. Thus, males obtained a score of zero, one or two for the number of matings obtained. This method does not precisely measure male mating success because males siring only daughters by a female cannot be credited with mating with that female.

Measuring male attractiveness

We placed each male in a clear-plastic bottle (base 23 mm by 39 mm filled to 40 mm) and photographed him from both sides, including a short section of plastic ruler for calibration. We traced the outlines of the fish and all colour spots from the projected slide onto A4 paper from which we used the digitiser board of a computer to calculate the area of the body and of each spot and the length of the gonopodium and the dorsal fin of each male. This method shows 99.7% repeatability (Brooks & Caithness 1995). We used the mean value for both sides in all bilateral traits.

In addition to considering the male colour pattern components separately, we built a composite measure of male colour pattern complexity. After the method developed by Nicoletto (1993), we ranked each male for the number of spots or patches of each colour (orange, black and iridescent) and then summed those three ranks. This provides a measure of pattern complexity relative to the other males in the sample.

Statistical analyses

All morphological variables conformed to the assumptions of parametric tests, some after transformation to their natural logarithms. We used the arcsine transformation to transform relative areas of orange and black and the proportion of male displays eliciting a glide or an orient response. Other behavioural variables could not be transformed to normality, and non-parametric statistical procedures were used.

We examined (i) the relationship between male ornaments and mating success, (ii) the relationship between male behaviour, female response and mating success, and (iii) the correlations between female response, male behaviour and ornamentation. In analyses (i) and (ii), the more successful male in terms of number of matings (winner) was compared with the less successful (loser) male using paired sample

t-tests or Wilcoxon's signed ranks test for matched pairs. We also compared males who did not sire sons with males who sired sons by one female and by both females using the Jonckheere test for ordered alternatives (Seigel & Castellan 1988) including the data from pairs where males did not differ in mating success. The results of this analysis did not differ from the results of pairwise analysis and are therefore not presented here.

We adjusted the 0.05 α-level of acceptance for the number of tests presented in a table using a sequential Bonferroni readjustment (Rice 1989), to reduce the possibility of a type II error due to the use of multiple tests.

Results

Both orient and glide responses of females to displaying males are sound predictors of male mating success (in terms of winning or losing mating trials as defined above, Table 1). In both cases these behaviours are more strongly related to mating success when divided by display rate. Also, the female swim toward (a non-displaying male) behaviour is significantly higher in males that won their mating trials than in their losing opponents.

Table 1 Female responses to winning and losing males in paired mating trials.

	Winner	Loser	test statistic	P
Orient	13.00	9.38	z = 1.51	0.066
(per 20 min)	(6.93)	(6.80)		
Orient/displays	0.443	0.313	t = 2.58	0.005*
	(0.14)	(0.16)		
Glide	3.56	1.938	z = 2.33	0.010*
(per 20 min)	(2.48)	(2.02)		
Glide/displays	0.128	0.070	t = 3.01	0.001*
	(0.09)	(0.08)		
Swim toward	3.38	1.75	z = 2.06	0.020*
(per 20 min)	(3.00)	(1.57)		

n = 16 pairs in all cases

P = One-tailed probability

z = standard normal approximation of the Wilcoxon signed ranks test statistic

t = Student's t-test for paired samples

* = Significant (P < 0.05) after Bonferroni readjustment

Other behaviours

Male mating behaviours are reported in Table 2. Neither the number of sigmoid displays per unit of time nor the frequency of gonopodial thrusts differed between successful and unsuccessful males. Male dominance interactions were very infrequent (14 events in 800 minutes of observation) and did not covary measurably with mating success.

Cues for female choice

In three trials neither female produced any offspring. We compared the ornamentation of the six unmated males with that of the remaining 34. Of the ten morphological and display characters studied in this experiment, the relative areas

Table 2 Behaviours of winning and losing males in paired mating trials compared

	Winner	Loser	z	P
Displays	29.06	26.43	0.85	0.39
	(10.7)	(12.3)		
Gonopodial thrusts	1.313	1.813	1.12	0.264
	(1.25)	(1.64)		
Dominance	0.313	0.375	0.07	0.94
	(0.60)	(0.80)		

n = 16 pairs in all cases

z   Standard normal approximation of Wilcoxon rank-sum test Statistic

Units are per *20 minute sample* in all cases, standard deviations in brackets

of black and yellow were significantly smaller in the ignored males (2-sample Student's *t* using separate variances: black *t* = 2.963, df = 15.5, individual one-tailed *P* = 0.005, yellow *t* = 2.418, df = 33.4, *P* = 0.011). The first of these results is significant (*P* = 0.050) and the second is not (*P* = 0.099) after sequential Bonferroni readjustment.

Both orange pigmentation and pattern complexity were significantly higher in trial winners than losers, but complexity was not significantly higher after the Bonferroni readjustment (Table 3). In our earlier study, only orange area was found to correlate significantly with female response (Brooks & Caithness 1995). Those findings were corroborated in this experiment, and thus the correlations between the male attributes and female response measures are not presented here. However, in the earlier study we did not

Table 3 Secondary sexual characters of winning and losing males in paired mating trials compared

	d.f.	Winner	Loser	T	P
Orange (% body area)	13	10.30	4.67	5.278	0.000*
		(4 55)	(3.26)		
% Black	13	3.28	3.71	0.834	ns
	?	(1.44)	(1.81)		
% Yellow	13	0.54	0.51	0.112	ns
		(0.82)	(0.83)		
% Irridescence	13	1.34	0.66	0.593	ns
		(1.42)	(0.62)		
Pattern complexity	13	67.04	51.71	2.392	0.032
		(17.3)	(20.3)		
Body area (mm²)	13	102.2	103.5	–0.030	ns
		(17.5)	(21.7)		
Dorsal fin (mm)	9	7.36	6.71	0.742	ns
		(1.83)	(2.52)		
Gonopodium (mm)	9	5.71	5.96	–0.845	ns
		(1.18)	(1.68)		

* = significant (P < 0.05) after Bonferroni readjustment

sd in brackets

measure pattern complexity, so we report the relevant correlation here: complexity was significantly correlated with the proportion of displays eliciting an orient response (Spearman r_s = 0.480, n = 38, 1-t *P* < 0.002) and the proportion glide response (Spearman r_s = 0.330, n = 38, 1-t *P* < 0.025)

Discussion

A common difficulty faced in sexual selection studies is how to separate the importance of female choice from that of intrasexual interactions (Houde 1988, Andersson 1994), and of mating tactics that circumvent choice. The low frequency of sneak copulations and aggressive encounters and their apparent unrelatedness to paternity in this population suggests that they do not significantly effect mating success. We found no support for the assertion that male dominance in guppies is an important determinant of mating success (Gorlick 1976, Kodric-Brown 1992), nor that it influences or is influenced by female choice (which has recently been shown in another guppy population [Kodric-Brown 1993], and northern pintails [Sorenson & Derrickson 1994]). Three caveats apply to our claim that male dominance and sneak copulations are not important to sexual selection in guppies. First, in guppy populations, mating behaviour and mate choice cues are strongly influenced by local predation and environmental conditions (Endler & Houde 1995). Our result may be due to local circumstances within the population we studied rather than a general pattern for guppies. Second, differential conspicuousness of males both to females and to predators under different lighting conditions results in a diurnal cycle in the relative importance of sneak copulation and courtship to mating success (Reynolds *et al.* 1993, Endler 1987). Last, using virgin females may have led us to underestimate the importance of sneak copulation which is more important to males attempting to fertilise unreceptive females (Farr 1980).

Houde (1988) found that the glide response of a female was a reliable predictor of the likelihood that she would mate with a male. Our results indicate that both the initial (orient) female response and the second (glide) response (always preceded by an orient response) are reliable indices of male mating success. While the glide response is more accurate as a measure of mating success than the orient response because it is further along the series of responses before mating, the sampling effort required to observe sufficient glide responses make the more frequently observed orient response a useful measure.

The sigmoid display rate of male guppies is related to male condition (McMinn 1990, Nicoletto 1993) and is thus a potential cue for female choice (McMinn 1990). In this population, the number of male displays in a time period does not influence mating success. However, the length of the sigmoid display could be costly and thus be a means for the female to assess a male's genetic quality (as suggested in birds: Vehrencamp *et al.* 1989, Halliday 1987), suggesting a possible reason for the evolution of this behaviour and the female stepwise response. Male 'attractiveness' to a female is not entirely dependent on the performance of the sigmoid display, because the swim-toward response (in which a female swims toward a non-displaying male) is also a significant predictor of mating success.

The levels of variation in male colour patterns and the mean colour areas and preferences in this population (Brooks & Caithness 1995) are similar to those in medium to low predation wild guppy populations (Endler & Houde 1995). Therefore, it is possible that the results of our study can be generalised to guppies in general.

In this population, males with a greater area of orange pigmentation not only elicit positive female sexual responses (Brooks & Caithness 1995), but they are more successful at obtaining matings than males with smaller areas of orange. We therefore feel it is justified to claim that orange elements of male guppy patterns have been sexually selected by female choice in this population.

The fact that other colour pattern elements are not significantly related to male mating success or female response in this population makes it difficult to explain how they arose and are maintained. The apparent threshold requirement of black pigment before a female will mate with a male is interesting, more so because once males reach this threshold other cues become the focus of choice obscuring any correlation between black and mating success.

Instead of considering the male ornaments as separate characters with distinct signalling functions and evolutionary histories, it may be informative to look at the different colours in a guppy's pattern as a single 'composite ornament'. The success of males with complex colour patterns in attracting females and fertilising their eggs supports the use of this composite measure of the colour pattern.

Endler & Houde (1995) use 'colour contrast' and 'brightness contrast' as measures of the contributions of each spot to the overall mosaic of colour perceived by the female. If different colour areas serve as a single, composite signal to the female, a single female preference for a complex or high-contrast colour pattern could maintain several different patch colours in the population.

Another (but not contradictory) way of looking at two or more colour pattern attributes as the evolutionary results of a single female preference is if some colours act as signal amplifiers (Hasson 1991) of others. For example, black pigment has been shown to behave in a way that is consistent with its being an amplifier of orange area, in males from this population (Brooks 1996). The amplifier evolves because it makes it easier for females to discriminate among males on the basis of another cue (orange) which reflects male quality. The expression of the amplifier is not necessarily correlated with male attractiveness (Hasson 1991). Mechanistically, the black-as-amplifier idea is very similar to the idea that males with high contrast colour patterns (Endler & Houde 1995) are attractive since black probably increases the contrast between orange areas and the visual background.

The finding that males with complex colour patterns are preferred suggest that understanding the origin of multiple ornaments may be improved by considering the possibility of a single preference for a composite ornament. This and the success of other composite measure approaches (Brooks 1996, Endler & Houde 1995) support the assertion that single preference explanations for multiple ornaments deserve theoretical and empirical attention.

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