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# Female sticklebacks use male coloration in mate choice and hence avoid parasitized males

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**AN important problem in evolutionary biology since the time of Darwin has been to understand why females preferentially mate with males handicapped by secondary sexual ornaments[1–3]. One hypothesis of sexual selection theory is that these ornaments reliably reveal the male's condition[4–6], which can be affected for example by parasites[4,7–13]. Here we show that in the three-spined stickleback (*Gasterosteus aculeatus*) the intensity of male red breeding coloration positively correlates with physical condition. Gravid females base their active mate choice on the intensity of the male's red coloration. Choice experiments under green light prevent the use of red colour cues by females, and males that were previously preferred are now chosen no more than randomly, although the courtship behaviour of the males remains unchanged. Parasitization causes a deterioration in the males' condition and a decrease in the intensity of their red coloration. Tests under both lighting conditions reveal that the females recognize the formerly parasitized males by the lower intensity of their breeding coloration. Female sticklebacks possibly select a male with a good capacity for paternal care[14] but if there is additive genetic variation for parasite resistance, then they might also select for resistance genes, as proposed by Hamilton and Zuk[4].**

At the start of the breeding season, male three-spined sticklebacks develop a bright red coloration due to carotenoids[15], and it has been shown that females prefer artificially coloured males over colourless males[16]. In another fish species, the guppy (*Poecilia reticulata*), female choice is based not only on the expression of these pigments[17–21], which may be indicative of fitness[17], but also on courtship behaviour[22,23].

Twenty-four male three-spined sticklebacks with developed breeding coloration were placed individually into tanks (17.8 cm × 34.5 cm, with a water level of 16.5 cm and at a temperature of about 18 °C) separated by grey opaque partitions. Each pair of tanks was illuminated for 16 hours per day by a 60 W reflector bulb (Osram Concentra PAR-EC). Each male was stimulated with a ripe female enclosed in a plexiglass cell (11 cm × 7.5 cm, water level 16 cm) placed close to the front wall of its tank for five minutes daily to accelerate its nestbuilding behaviour[24]. After six days, all the males had a complete nest built in a Petri dish provided close to the backwall and were courting vigorously.

Two students scored the intensity of the red breeding coloration on a 10-point scale (1, dullest male; 10, brightest male) for each male when it courted a female. There was general agreement between the students ($r = 0.71$). Males designated 1 and 2, 3 and 4, and so on, according to increasing colour rank, were defined as pairs for presentation to ripe females. To avoid the right male always being brighter, positions were randomized within pairs. In a separate tank positioned centrally in front of each pair of neighbouring tanks, the cell containing a single gravid female was placed; her choosing process between the two males was video-recorded for a 5-min period after 1 min of acclimatization. Females were previously selected for their readiness to spawn, that is, to adopt and maintain the head-up courtship posture while pointing towards one of the two males. On each day before we gave a female the opportunity to choose between males, we estimated the difference of red colour between the members of each pair on a 5-point scale (0, no difference; 1, slight but distinct difference; 2, pronounced difference, and intermediates). Each of four females chose between each of the 12 pairs of males (female 1 made 12 choices on day 1, female 2 made 12 choices on day 2, and so on). The intensity of the red breeding coloration correlated significantly positively with the males' condition factor (Fig. 1*a*). Therefore, females could judge the well-being of a male from the intensity of its red coloration. Although the experiment was designed so that the males of each pair were very similar in coloration, there was a significant preference by the females for the brighter male in each pair, this preference being intensified as the difference in coloration increased (Fig. 1*b*).

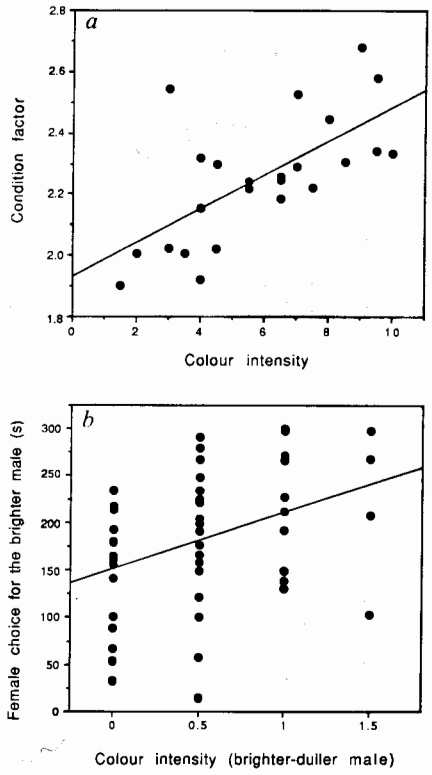


FIG. 1 *a*, Correlation between the intensity of red breeding coloration (average score of 2 students) and the condition factor (100 × weight (g)/length (cm)$^{2.76}$) of 24 reproductive male sticklebacks ($y = 1.93 + 0.056x$, $r^2 = 0.44$, $F = 17.27$, d.f. = 1,22, $P < 0.0004$, 1-tailed). The condition factor, used as a standard practice in fisheries ecology, is regarded as a good indicator of the general well-being of teleost fishes[37]. It assumes that heavier fish of a given length are in better condition. *b*, Correlation between the difference in colour intensity scored daily of 12 pairs (brighter-duller male) and active female choice for the brighter male (measured in seconds, see text) by 4 different females, one on each day. The pooled regression is significant ($y = 151.0 + 59.1x$, $r^2 = 0.14$, $F = 7.62$, d.f. = 1,46, $P < 0.004$, 1-tailed). Pooling of females is allowed for[28] and recommended[38], because the slopes of regression lines of the 4 females were not significantly different ($F = 0.60$, d.f. = 3,40, $P = 0.62$, 2-tailed) and the subsequent analysis of covariance revealed no significant differences either ($F = 2.13$, d.f. = 3,43, $P = 0.11$, 2-tailed).

To investigate whether this preference is ultimately based on coloration or on some related character such as courtship behaviour, we repeated the same experiment using 15 new pairs of males; females could choose between males under white light and under such light conditions that they were almost unable to assess differences in the intensity of red coloration (males and females had not been used in the previous experiment). To achieve this, males and females were kept under white and green light (Osram Concentra PAR-EC Belcolor, 80 W) on alternate days. After the 30 males had built nests in their individual tanks, we ordered the tanks according to the intensity of the inhabitant's red coloration; six students then estimated the difference in red colour between the members of each pair on the 5-point scale (median value of all possible correlations among the students, $r = 0.77$). The difference in intensity of coloration (brighter-

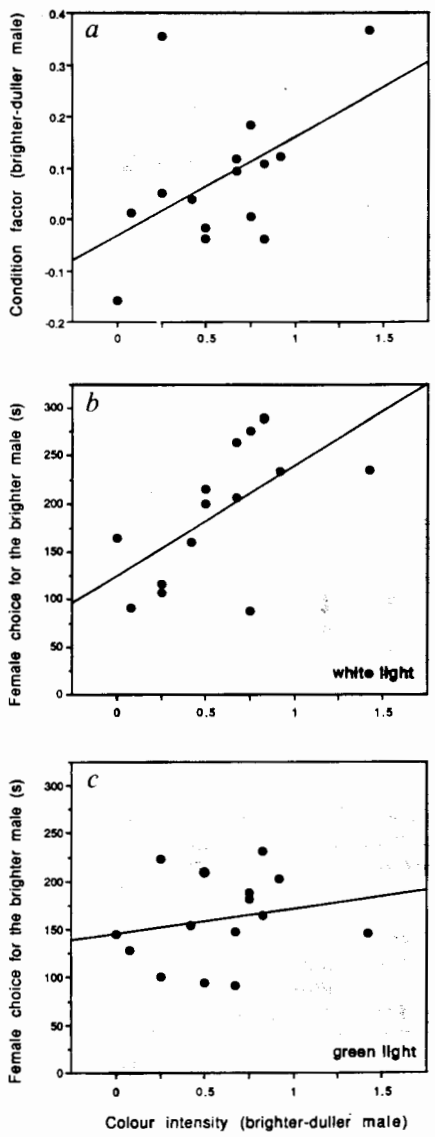


FIG. 2 Difference in the intensity of red breeding coloration of 15 pairs of reproductive male sticklebacks (brighter–duller male) (average score of 6 students) in relation to: *a*, the difference in condition factor (100 × weight (g)/length (cm)$^{2.89}$) ($y = -0.034 + 0.192x$, $r^2 = 0.25$, $F = 4.25$, d.f. = 1,13, $P < 0.03$, 1-tailed); *b*, average active female choice of the brighter male by 3 different females per pair (13 females in total) under white light ($y = 125.09 + 120.2x$, $r^2 = 0.38$, $F = 7.97$, d.f. = 1,13, $P < 0.01$, 1-tailed); *c*, average active female choice of the brighter male by 3 different females per pair (11 females in total) under green light ($y = 144.80 + 26.17x$, $r^2 = 0.04$, $F = 0.60$, d.f. = 1,13, $P = 0.75$, 2-tailed). The 15 average choices under either light condition are treated as independent sample units because the experiment shown in Fig. 1*b* did not reveal significant differences among females choosing between 12 pairs of males. To test whether the intensity of the green light (330 lx at the botton of the tank) was sufficient to prevent females from dropping below the point at which the Purkinje shift occurs, when their ability to distinguish different intensities of red, for example, would be impeded, we submitted three females to a discrimination test under white light at a lowered intensity of 160 lx. Two red colour cards (1.7 × 2.1 cm) of the same hue and grey value, but of different colour intensities (Munsell System no. 7.5 R, 5/14 and no. 7.5 R, 5/2) were presented simultaneously to the fish and positions were alternated randomly. Approaching the less-red card was rewarded with food. In 50 training sessions, two fish achieved 80% and one 100% correct responses (approaching the less-red card within a distance of 5 cm before a reward) in the last 10 trials (as chi$^2 = 2.31$, d.f. = 2, $P = 0.32$, 2-tailed, reveals homogeneity among the 3 females, the 30 choices were pooled, 26:4 was significantly different from 1:1, chi$^2 = 16.13$, d.f. = 1, $P < 0.001$, 1-tailed). Thus, even at half the intensity of the green light, females could discriminate between different intensities of red colour when in white light.

duller male) correlated positively with the difference in condition (Fig. 2*a*).

Under white light, each of 13 different ripe females was allowed to choose between males from as many different pairs as she was reliably willing to (3.5 pairs per female on average), as determined by her head-up posture during the whole trial. Hence, the first female was allowed to choose between the males of the dullest pair, thereafter between the males of the brighter neighbouring pair and so on, until she had to be replaced by the second female, and so on. Under green light, 11 other females were used (4.0 pairs per female). Thus, each pair of males was confronted with three different females in either experiment. To establish whether the courtship behaviour of the males varies under the different light conditions, we confronted each male alone with the cell containing a ripe female under both green and white light for a 4-min period. The number of zigzags performed in the male's courtship display[25], regarded as a reliable measure of male courtship intensity[26,27], was counted during the last three minutes. The males' courtship intensity did not differ significantly in the two lighting situations (mean number of zigzags under white light, 46.5, s.d. = 28.9; under green light, 42.1, s.d. = 25.7; $P > 0.10$, Wilcoxon matched-pairs signed-ranks test, 2-tailed) and differences between pair members did not change significantly (zigzags for the brighter male of a pair, 61.5%, s.d. = 28.6, under white light; and 53.0%, s.d. = 29.2, under green light; $P > 0.10$, Wilcoxon matched-pairs signed-ranks test, 2-tailed). Also the females' willingness to react to male courtship as depicted by duration of head-up posture was not influenced by the type of light (3.5 pairs per female and 4.0 pairs per female, respectively; see above). A female that was allowed to enter the male's territory under green light went through the normal spawning sequence immediately.

Under white light, females preferred the redder male again (Fig. 2*b*), whereas under green light the trend in favour of the brighter males was not significant (Fig. 2*c*); the slope of the regression under white light is significantly greater than that under green light ($F = 2.99$, d.f. = 1,26, $P = 0.05$, 1-tailed). This indicates that the females based their choice primarily on differences in male red coloration. This makes sense functionally, because colour intensity correlates significantly with condition (see Fig. 2*a*; partial correlation[28] when courtship intensity was kept constant, $r = 0.54$, $P < 0.03$, 1-tailed), whereas courtship intensity does not correlate significantly with condition ($r = 0.00$, $P > 0.10$, 1-tailed; partial correlation when colour intensity kept constant, $r = -0.23$, $P > 0.10$, 1-tailed). The conclusion that female choice is based mainly on colour cues is confirmed by partial correlations under white light. The correlation between choice and intensity of red coloration when intensity of courtship is kept constant is $r = 0.54$ ($P < 0.03$, 1-tailed), whereas the almost significant correlation between choice and intensity of courtship ($r = 0.42$, $P = 0.06$, 1-tailed) is far from significant when intensity of red coloration is kept constant ($r = 0.25$, $P > 0.10$, 1-tailed). Also, in guppies no correlation between orange breeding coloration and display rate has been found[20], but contrary to our results, female guppies respond not only to the colour of the males' orange spots, but also to their contrast against background skin under filtered light[29].

To investigate whether parasites influence both the males' red breeding coloration and the result of active female choice, we infested the brighter male of each pair with the ciliate *Ichthyophthirius multifiliis*, a serious and widespread fish disease known as 'white-spot'[30]. On five successive days, 50 ml of water contaminated with tomites (the infective stage) were taken from the tank of a formerly heavily infected stickleback and poured twice daily into the tank of each fish. Uncontaminated aliquots of water were added to the tanks of control fish. After several days, infected fish developed from a few to about 50 visible white cysts, which dropped off the fish after a few days. Two fish died after infection. To prevent reinfection, all tanks (including those of control fish) were treated with *Faunamor*. Surviving fish continued to court stimulus females. Four of the six students

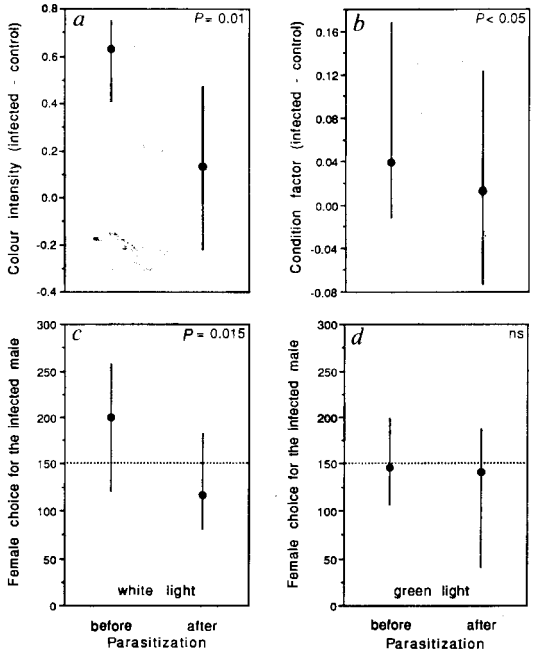


FIG. 3 Median difference of 13 pairs of reproductive male sticklebacks (infected − control) before parasitization of the brighter male with *I. multifiliis* and after recovery from the parasite *a*, in the intensity of red breeding coloration (average score of 4 students) before (median correlation between students, $r = 0.60$) and after infection (median $r = 0.59$) (the same students had difficulties in finding differences in intensity of the red coloration between the males after the more brightly coloured male of each pair had been parasitized. As the students' ability to detect differences should have improved with experience, the students' second scoring was conservative); and *b*, in the condition factor (100 × weight (g)/length (cm)$^{2.89}$). Median active female choice of the infected male of each pair before parasitization with *I. multifiliis* and after recovery from the parasite *c*, by 3 different females (1 female per pair) under white light, and *d*, by 4 different females (1 female per pair) under green light. Before parasitization females chose the brighter males significantly longer under white light than under green light ($P < 0.01$, Wilcoxon matched-pairs signed-ranks test, 1-tailed). After parasitization the respective difference was not significant ($P > 0.10$, Wilcoxon matched-pairs signed-ranks test, 2-tailed). This is expected because both the colour difference (Fig. 3*a*) and the condition difference (Fig. 3*b*) between the males had almost disappeared after parasitization of the brighter male. Bars give quartiles, dotted line indicates no preference, probabilities after Wilcoxon matched-pairs signed-ranks tests, 1-tailed, N.S. $P > 0.10$.

who had scored the males' red coloration were asked to repeat their estimation of pair differences; they were not informed about the earlier parasitization of half of the males. Once more, ripe females which had not been used in previous experiments were allowed to choose between males of the remaining 13 pairs both under white (3 different females) and green light (4 other females) so that each pair of males was presented with one female in each experiment.

Parasitization caused a significant decrease in the intensity of the males' red coloration (Fig. 3*a*) and in their condition factor (Fig. 3*b*). Females significantly reduced their earlier preference for the males that were formerly brighter under white light (Fig. 3*c*), but under green light the males' parasitization had no significant effect on female choice (Fig. 3*d*). This implies that the females detected the prior parasitization of the males by their decreased intensity of breeding coloration, which is a necessary condition for coloration to be judged as a revealing handicap[4,6].

Under natural conditions, brighter males might obtain better territories by dominance interactions, a factor that was excluded in this study. Intersexual selection on male stickleback red breeding coloration seems, however, to be more important than intrasexual selection, because male sticklebacks develop more erythrophores[31] and 'flush' their red colours more strongly[32] after presentation with a ripe female than after exposure to a rival male. Furthermore, the overall intensity and intermale variation of red coloration is greatest during the courtship stage of the breeding cycle[33]. By contrast to the males, the females' visual sensitivity for red coloration periodically increases at the beginning of the reproductive season and reaches a higher level than that of males[34].

The females probably did not make use of the male's courtship intensity for their decision-making because courtship intensity is a poor predictor of condition. Perhaps even a sick or convalescent male can muster energy for the display when need arises, but it is harder for him to bluff the long-term drain on his resources revealed by his lack of colour. Nevertheless, the zigzag display may help the females to recognize a reproductive male stickleback.

In all, the intensity of the red breeding coloration seems to be a revealing handicap[4,6] for a male's condition because it correlates significantly positively with the condition of our wild-caught males and decreases when the males' condition is experimentally reduced by parasitization. Therefore, any agent (including parasites) influencing a male stickleback's condition probably affects the intensity of its breeding coloration and consequently the female's choice. Why do female sticklebacks prefer males of superior condition? As the male cares for the eggs and the fry for about 10 days after spawning[26], she might prefer a strong male with a high probability of survival for this period[14]. Even if she prefers a strong male for paternal care, she cannot avoid simultaneously selecting for genes favourable for parasite resistance if there is additive genetic variation for parasite resistance in the population. Although present evidence is ambiguous with respect to *I. multifiliis*[35], there are indications of such a variation in fish[36]. Therefore the Hamilton–Zuk process[4] may be an auxiliary factor in species with paternal care. □

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