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 females preferentially mate with males handicapped by secondary sexual ornaments [1]. One hypothesis of sexual selection theory is that these ornaments reliably relate the male's condition [2,3], which can be affected for example by parasites [4-6]. 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 color 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. Parasitism 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 [7,8] but if there is additive genetic variation for parasite resistance, then they might also select for resistance genes, as proposed by Hamilton and Zuk [9]. At the start of the breeding season, male three-spined sticklebacks develop a bright red coloration due to carotenoids [10], and it has been shown that females prefer artificially coloured males over colourless males [11]. In another fish species, the guppy (Poecilia reticulata), female choice is based not only on the expression of these pigments [12,13], which may be indicative of fitness [14], but also on courtship behaviour [15,16]. Twenty-four male three-spined sticklebacks with developed breeding coloration were placed individually into tanks (17.8 cm x 4.5 cm, with a water level of 16.5 cm and at a temperature of about 18°C) separated by grey opaque partitions. Each male was housed in a tank illuminated for 16 hours per day by a 60 W reflector bulb (Osram Concave PAR-EC). Each male was stimulated with a ripe female encased in a plexiglass cell (13 cm x 7.5 cm, depth 16 cm) and placed on the front wall of its tank for five minutes daily to accelerate its nestbuilding behavior [17]. After six days, all the males had a complete nest built in a Petri dish provided close to the backwall and were counting vigorously. Two studies measured the intensity of the red breeding coloration on a 10-point scale (1, dullest male; 10, brightest male) for each male when it counted a female. There was general agreement between the students (r = 0.71). Males designated 1, 2, 3 and 4, and so on, according to increasing colour rank, were paired as males 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 neighboring tanks, the cell containing a single gravid female was placed; her choosing process between the two males was video-recorded for a 5-minute period after 1 minute 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 the 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. 1a). 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. 1b). 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 experiments). To achieve this, males and females were kept under white and green light (Osram Concave PAR-EC Bicolor, 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-
duller male) correlated positively with the difference in condition (Fig. 2a). Under white light, each of 13 different rife 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 bright 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 separate trials. To establish whether the courtship behaviour of the males varied under the different light conditions, we confronted each male along with the cell containing a rife female under both green and white light for a 4-min period. The number of zigzags performed in the male's courtship display was regarded as a reliable measure of male courtship intensity, was counted during the last three minutes. The males' courtship intensity did not differ significantly in the two light situations (mean number of zigzags under white light: 46.5, s.d. = 28.9; under green light: 42.1, s.d. = 27.5; F = 0.10, Wilcoxon matched-pairs signed-ranks test, 2-tailed) and differences between pair members did not change significantly (zigzags for the brightest male of a pair: 61.5%, s.d. = 26.6, under white light; and 33.6%, 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. 2b), whereas under green light the trend in favour of the brighter male was not significant (Fig. 2c); 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. 2a); partial correlation was significant when courtship intensity was kept constant (r = 0.54, P < 0.03, 1-tailed), whereas courtship intensity does not correlate significantly with condition (r = 0.09, P > 0.10, 1-tailed); partial correlation when colour intensity kept constant (r = 0.32, P = 0.1) was also significant. The condition of the teneral female that choice is based on mainly on colour cues is confirmed by partial correlations under white light. The correlations are much stronger for the brighter males of a pair than for the duller males; across the range of the fish sampled, the correlation increases from 0.2 on the teneral stage to 0.5 in the adult stage. To investigate whether parasites influence both the males' red coloration and the result of active female choice, we infected the brighter male of each pair with the ciliate Ichthyophthirius multifiliis, a serious and widespread fish disease known as 'white spot'26. On five successive days, 50 ml of water contaminated with tomites (the infective stage) were taken from the tank of a freshly washed infected fish and poured directly into the tank of each fish. Uncontaminated aliquots of water were added to the water used for control fish, and control fish were also infected with fish from a new batch of the collected fish, which changed 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 Furanid. Surviving fish continued to court stimulus females. Four of the six surviving males correlated positively with the difference in condition (Fig. 2a).
FIG. 3 Median difference of 13 pairs of reproductive male sticklebacks (inflected - control) before parasitization of the brighter male with 1 multicolor and after recovery from the parasite at the intensity of red breeding coloration (average score of 4 students) before median correlation between students, r = 0.00 and after infection (median r = 0.50) the same students had difficulties in finding differences in intensity of red coloration between the males after the more brightly colored male of each pair had been parasitized. As the students' ability to detect differences should have improved with experience, the students' second scoring was conserved; and in the condition factor (100 × weight g/length cm²). Median active female choice of the inflected male of each pair before parasitization with 1 multicolor and after recovery from the parasite a by 3 different females (1 female per pair) under white light, and by 4 different females (1 female per pair) under green light. Before parasitization females choose the brighter males significantly longer under white light than under green light (P < 0.001, 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 color difference (Fig. 3a) and the condition difference (Fig. 3b) 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, H5. P < 0.10.

who had scored the males' red coloration were asked to repeat their estimate 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. 3c) and in their condition factor (Fig. 3b). Females significantly reduced their earlier preference for the males that were formerly brighter under white light (Fig. 3c), but under green light the males' parasitization had no significant effect on female choice (Fig. 3d). 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.

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 and their red colours more strongly after presentation with a ripe female than after exposure to a rival male. Furthermore, the overall intensity and intra-male variation of red coloration is greatest during the courtship stage of the breeding cycle. 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.

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 for a male's condition because it correlates significantly positively with the condition of our wild-caught males and decreases when the male's 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, they might prefer a strong male with a high probability of survival for this period. 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 J. multifiliis, there are indications of such a variation in B. Therefore the Hamilton-Zuck process may be an auxiliary factor in species with paternal care.