Positive genetic correlation between female preference and preferred male ornament in sticklebacks
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A NUMBER of population genetics models predict the evolution of male sexual ornaments through female choice[1], but their genetic assumptions and predictions have hardly been investigated[2,3]. A key feature of these models is a positive genetic correlation between male ornaments and female preference for them. I present a test of this prediction at the within-population level with three-spined sticklebacks, Gasterosteus aculeatus, which show conspicuous sexual dimorphism[4]. Intense red males are preferred in various situations[5], but there is great intrapopulation variation in the redness both among wild-caught[6] and among laboratory-bred males[7], which is partly environmentally[8] and may be partly genetic[9,10]. Also, females show considerable intrapopulation variation in their preference for redder males[11], which is partly environmentally[12]. Wild-caught, intense red males and dull males were crossed with a number of females from the same population in a full-sib/half-sib breeding design. Daughters were tested for their preference for more intensely red males, and the sons' coloration was quantified. Both traits showed genetic variation. Also the redness of the sons correlated with the preference for red of the sisters, thus the two traits show positive genetic correlation.

Sexually mature sticklebacks were caught in the spring of 1999 from a Swiss freshwater population near Roche/Montreux, 46°26'N, 8°35'E, which was introduced more than a century ago[13]. In the laboratory, fish were kept under simulated summer conditions; males were housed singly in small tanks, whereas females were stored in female groups[14]. After nest building, males were used in sequential male choice tests of ripe females[15]. Six of the most extremely coloured males, that is, three intense red and three pale red males, served as fathers. They were crossed in a full-sib/half-sib breeding design[16] with 14 females, which covered the whole spectrum of preference phenotypes for redder males as determined in sequential choice tests[17]. Parental effects on offspring traits were excluded by removing clutches from the fathers' nests after fertilization. Progenies were raised and maintained in several small standardized full-sib groups per cross. Before the attainment of sexual maturity the sexes were separated; at each cross a random sample of males was housed individually and a random sample of females was maintained in standardized sister groups.

Two weeks after the completion of the first nest, the males' mammalian intensity of red coloration on the thorax was quantified[18]. Redder fathers produced on average significantly redder sons (Fig. 1), thus indicating additive genetic variation for red intensity. An analysis of variance (ANOVA) of red intensity of sons in full-sib and half-sib families also revealed a significant added variance component among fathers, indicating additive genetic variance of red intensity (Table 1a). Note that the variation among fathers is much greater than among their progenies (Fig. 1). Because the fathers were wild-caught, cages of variation in the intensity of red can be different for fathers and sons, making the regression unsuitable for heritability estimation. Narrow-sense heritability estimation from the sib analysis was not possible owing to the relatively low variance among full-sibs (Table 1a), which was probably affected by the use of extreme father coloration categories as opposed to the use of mothers from the whole spectrum of preference phenotypes. In addition, dominance effects may have hidden the mothers' contribution (see below). A full-sib analysis[20] yielded a rough estimate of heritability of red intensity of 0.25 (i.e. 0.27).

The preference of the female Daphnia for redder males was investigated with a simultaneous-choice design[21]. When ripe, female progeny were given a choice between two courting males that differed in the intensity of red. Females were selected according to their ripeness and used once. When females were still ripe the day after the choice test, we tested them again to assess the repeatability[22] of female choice.

Most females were consistent in their choice: they either preferred the redder male or showed no preference on both days (Fig. 2). For unknown reasons a small number of females preferred the dullest male on the first test day, but showed a clear preference for the redder male on the next (Fig. 2, empty circles). This group of females (relative preference for the redder male 0.35%) included 16 of 97 (16.4%) of the dughter females from red males and 5 from dull males) was left out in the following analyses, although their inclusion does not change the conclusions. Red preferences were significantly correlated between two successive days (r² = 0.41, F = 11.34, df = 1, 16, P = 0.004).

**Fig. 1.** Correlation between the intensity of red breeding coloration of wild-caught fathers and their laboratory-bred sons (average score of sons per father in each) r² = 0.79, F = 150.02, df = 1, 4, P = 0.000 1-tailed. Number of tested sons (number of crosses) from left to right: 21 (5), 31 (3), 203 (2), 81 (5), 285. Paternal effects were ruled out by removing clutches from the nests 3 h after fertilization, and by breeding them artificially[14]. The fish were raised in small standardized full-sib groups under simulated summer conditions (16:8 h light/dark cycle, 15°C) and fed freely. Because parasites might affect male coloration[23], only food items were used that were most likely to be free of parasites. The few fish that caught or were suspended in the first were given an Oridonin injection instead of food. At first signs of developing breeding colouration, about 7 months after hatching, the sexes were separated, and a random selection of males individually housed[15]. Each row of 6 male tanks was illuminated by a 40 W fluorescent tube mounted 15 cm above the tanks. The males were regularly stimulated with ripe females, and most of them had built a nest within 2 months of isolation. Fathers' and sons' intensity of red was quantified 2 weeks after the completion of the first nest using Fischer's procedure[16]. Groups of the males were taken in a standardized set-up and analyzed with a densitometer[24] in the red thorax region, the optical density of red (R, filter 700 nm), green (G, filter 568 nm), and blue (B, filter 450 nm) was measured at 10 defined points (diameter 0.35 mm). An appropriate measure of the intensity of red that is independent of the brightness of a colour is the red index[25] in which the R value corrected for differences in film development is expressed relative to the total colour density (R + G + B) and subtracted from 1 to obtain positive values between 0 and 1. The highest index for red on the thorax was used in the analysis. A proof of the reliability of the method was obtained by a direct comparison[26] of the red index with chroma calculated from reflectance spectra[27] (Spearman's rank correlation coefficient r = 0.80, N = 15, P < 0.005, 1-tailed).

**Fig. 2.** Preference of females for the red male. Female progeny were given a choice between courting males that differed in the intensity of red. Females were selected according to their ripeness and used once.
The repeatability (coefficient of intraclass correlation) of preference was estimated as 0.65 ± 0.14 (x = 0.9). An ANOVA of female preference within and between full-sib and half-sib families indicated the presence of additive genetic variance of preference for redder males, as the added variance component among families was significant (Table 16). The relatively low variance of the daughters' preference among mothers (Table 16) prevented narrow-sense heritability estimation. A full-sib analysis gave a rough estimate of heritability of preference for redder males of 0.63 (x = 0.8). The influence of mothers on both their daughters' red preference and on their sons' red intensity tended to be stronger in crosses with full-sibs than when crossed with red males (between mothers source of variance of daughters' preference in crosses with full-sibs: F = 0.5, df = 5, Ns = 0.77; and it crosses with full-sibs: F = 1.81, df = 6, P = 0.12; for sons' coloration, F = 0.61, df = 5, 47 = 0.69, and F = 1.54, df = 7, 58, P = 0.25, respectively). These results may indicate partial dominance of genes that promote preference for redder males and intense red coloration.

A positive genetic correlation between ornament and preference was indicated by the significant positive correlation between the fathers' intensity of red and their daughters' average preference for red (preferences corrected for the degree to which test males differed in their red intensities; r = 0.77, F = 13.38, df = 1, 4, P = 0.011, l-tailed). Conclusive evidence of this positive genetic correlation is given by the highly significant positive correlation that exists across fathers between the sons' intensity of red and the daughters' preference for redder males (Fig. 3). The phenotypic correlation of 0.994 roughly estimates the genetic correlation between male and female traits because by using group means environmental effects are reduced. A more precise estimate of 0.75 (x = 0.9) was calculated from the slope of the regression line (using red index x 100 and average scores per cross averaged over the different crosses per father: y = 63.13 + 8.507x, r = 0.998, F = 179.61, df = 1, 4, P < 0.0001) and the estimated heritabilities of the male and female traits.

It was unlikely that the strong correlation between male and female traits (Fig. 3) is caused by nonheritable effects common environmental influences were minimized by the applied

FIG. 2 Change in preference for the redder male of 22 rite females between the second successive days (day 1–20) as a function of the preference in day 1. Preference is expressed as % of total duration of head-up display directed at the超sib males. The horizontal line indicates no change, females in a preference less than 30% on day 1 are indicated by empty circles. Females were mated together with their brothers in several small standardized groups per cross. Before the attainment of sexual maturity they were reared by their natural mothers. Each group consisted of 25 randomly chosen females or males in excess of 600 tanks. Each female tank was fitted with a 40-W fluorescent tube hanging off the top of the tanks. Ripe females were selected out of the social groups then placed in a small tank and tested the next day for their preference for redder males. In the choice test, females were enclosed in a plasmaplast tube and offered a simultaneous choice between two counter-mating males that differed in their intensity of red coloration, and could not interact with each other. The criterion of the female's head-up courtship posture while viewing a one of the two males was measured during a 2 min period after 5 min of acquaintrance. This measure correlates positively with the probability to spawn with that male. Three different substr and four different red males were used in five different combinations. Positions of the males were regularly changed. After the choice test, females were put back in their individual tanks and after spontaneous spawning they were marked by clipping, the tip of one or more toes and put back in their sister groups.

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methods of raising and maintaining fish were and apparently low as indicated by the relatively small variance of both traits between mothers crossed with the same father (Table 1). Precise estimates of genetic variances and covariances require large sample sizes and may thus pose practical problems in quantitative genetic studies of sexual selection. This study clearly demonstrates significant genetic variation in male ornament and female preference and positive genetic correlation between these, but precise estimates of the genetic variables cannot be retained with such data sets: the estimates may be inflated by the use of full-sib comparisons, the estimated genetic correlation has a large standard error, and the heritability estimates are not significant.

Data on genetic variation in female mating preference and genetic covariation between female preference and preferred male traits are scarce and partly ambiguous.13-15 These genetic variables are critical in population genetics models of sexual selection. During the evolution of mate ornaments, both 'fisherian' and 'good genes' models predict a positive genetic correlation between preference and male ornament. At equilibrium, in the Fisher process a positive genetic covariance will typically be maintained in both major gene13 and polygenic models.16 The genetic covariance can even be maintained when female choice is costly,17 which is likely in this stickleback population. In major gene models of 'good genes' sexual selection, there are no internal equilibria, and the male and/or female trait will become fixed, but in polygenic models it is usually assumed that genetic variation in both traits is maintained and that this is a positive genetic covariance at equilibrium.

Thus one cannot distinguish whether a fisherian or good genes process, alone18 or in combination19-20, maintains genetic variation and covariance of the evolved male and female traits in this stickleback population. My findings confirm the genetic predictions of models of sexual selection, but cannot exclude alternative hypotheses without further investigations. For example, the possibility of multiple introductions of sticklebacks in these waters, either through repeated introductions from one source population or a single introduction from more than one source, is difficult, if not impossible, to rule out, and may have caused a transitory genetic correlation between male and female traits.


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