Positive genetic correlation between female preference and preferred male ornament in sticklebacks

Theo C. M. Bakker

University of Bern, Zoologisches Institut, Abteilung Verhaltensökologie, Wohlenstrasse 50a, CH-3032 Hinterkappelen, Switzerland

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[4]. Here I test this prediction at the within-population level with three-spined sticklebacks, *Gasterosteus aculeatus*, which show conspicuous sexual dichromatism[5]. Intense red males are preferred in various situations[6–10], but there is great intrapopulational variation in redness both among wild-caught[6,10] and among laboratory-bred males[11], which is partly environmental[6] and may be partly genetic[12,13]. Also, females show considerable intrapopulational variation in their preference for redder males[6,8,9], which is partly environmental[8,9]. 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 their sisters, thus the two traits show positive genetic correlation.

Sexually mature sticklebacks were caught in the spring of 1990 from a Swiss freshwater population (near Roche/Montreux, 46°26′ N, 6°55′ E) which was introduced more than a century ago[14]. In the laboratory, fish were kept under simulated summer conditions; males were housed singly in small tanks, whereas females were stored in female groups[6]. After nest building, males were used in sequential mate choice tests of ripe females[8].

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[15] with 14 females, which covered the whole spectrum of preference phenotypes for redder males as determined in sequential choice tests[8]. Paternal 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; of 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' maximal intensity of red coloration on the throat was quantified[16]. 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 1*a*). Note that the variation among fathers is much greater than among their progenies (Fig. 1). Because the fathers were wild-caught, causes of variation in red intensity may 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 mothers (Table 1*a*), which was probably effected 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[15] yielded a rough estimate of heritability of red intensity of 0.23 (s.e.[17] = 0.27).

The preference of the female progeny for redder males was investigated with a simultaneous choice design[6]. 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, they were tested again to assess the repeatability[15] 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 duller 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 <35%: 11 out of 97 females tested, 6 daughters 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 ($r^2 = 0.41$, $F = 11.34$, d.f. = 1, 16, $P = 0.004$).

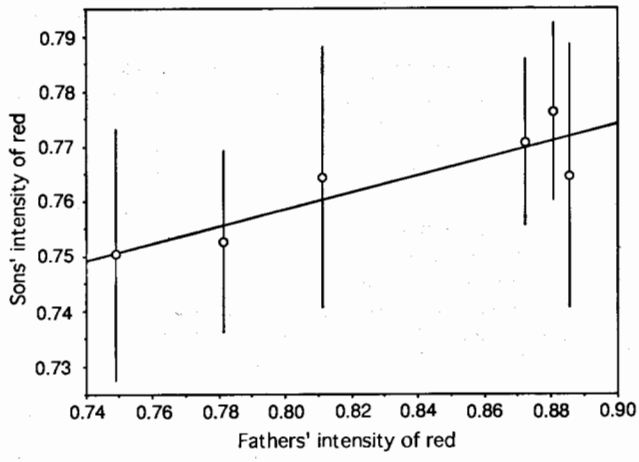

FIG. 1 Correlation between the intensity of red breeding coloration of wild-caught fathers and their laboratory-bred sons (average score of sons per father ±s.d.) ($r^2 = 0.79$, $F = 15.02$, d.f. = 1, 4, $P = 0.009$, 1-tailed). Number of tested sons (number of crosses) from left to right: 21(3), 7(1), 20(3), 9(2), 8(1), 38(5). Paternal effects were ruled out by removing clutches from the nests 1 h after fertilization, and hatching them artificially[11]. The fish were raised in small standardized full-sib groups under simulated summer conditions (16:8 h in light/dark cycle, 15 °C) and fed freely. Because parasites might affect male coloration[6], only food items were used that were most likely to be free of parasites. The few fish that caught or were suspected to have caught an *Oodinium* infection were not used in the tests. At first signs of developing breeding coloration, about 7 months after hatching, the sexes were separated, and a random selection of males individually housed[6]. Each row of 6 male tanks was illuminated by a 40 W fluorescent tube mounted 10 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 Frischknecht's procedure[16]. Slides of the males were taken in a standardized set-up and analysed with a densitometer[16]. In the red throat region, the optical density of red (R, filter 700 nm), green (G, filter 546.1 nm), and blue (B, filter 435.8 nm) was measured at 10 defined points (diameter 0.5 mm). An appropriate measure of the intensity of red that is independent of the brightness of a colour, is the red index[16], in which the R value (corrected for differences in film development) is expressed relative to the total colour density (R+G+B) and substracted from 1 to obtain positive values between 0 and 1. The highest index for red on the throat was used in the analyses. A proof of the reliability of the method was obtained by a direct comparison[10] of the red index with chroma calculated from reflectance spectra[34,35] (Spearman rank correlation coefficient $r_s = 0.80$, $N = 15$, $P < 0.0015$, 1-tailed).

TABLE 1 Sib analyses

	Full-sib and half-sib families				Full-sib families			
Source	d.f.	SS	F	P	d.f.	SS	F	P
(a) Sib analysis of variation in red coloration								
Between fathers	5	1,398,703	4.95	<0.02	5	808,872	2.03	<0.10
Between mothers (same fathers)	9	508,391	0.48	>0.75				
Within progenies	88	10,354,026			42	3,347,355		
(b) Sib analysis of variation in red preference								
Between fathers	5	2,049.13	4.48	<0.04	5	2,828.11	2.64	<0.05
Between mothers (same fathers)	8	731.88	0.52	>0.75				
Within progenies	47	8,348.70			31	6,641.62		

Sib analysis[15] of variation in *a*, the intensity of red male coloration, and *b*, female preference for redder males. Red intensity data (red index ×100) were square-transformed to meet the normality assumption for ANOVA (Kolmogorov–Smirnov test on transformed scores, $D=0.072$, $N=123$, P (Lilliefors) $=0.112$, 2-tailed)[33]. The daughters' preferences were corrected for the degree to which their test males differed in red intensity by taking the residuals from the regression of preferences (y) on ranked differences in red intensity (x) between the test males of each pair ($y=56.54+2.88x$, $r^2=0.09$, $F=8.07$, d.f. $=1$, 84, $P<0.003$, 1-tailed). The distribution of standardized female preferences did not significantly deviate from normality (Kolmogorov–Smirnov test, $D=0.070$, $N=86$, P (Lilliefors) $=0.346$, 2-tailed)[33]. The variation of red intensity of sons and of red preferences of daughters in full-sib and half-sib families were analysed with a two-level nested ANOVA with unequal sample sizes (left columns)[33]. Variation among the most reliable full-sib family of each father (that is the one with the greatest number of scored sons or daughters) was analysed with a single classification ANOVA with unequal sample sizes (right columns)[33]. Progeny was produced using a full-sib/half-sib breeding design[15]; over 2 weeks, 3 red and 3 dull males were crossed with 14 different females, 1–5 per male, which had been used the same day or the day before in sequential mate choice tests[8]. A few weeks after the first cross, three of the mothers became ripe again, and were crossed with a male from the other colour category. Because of the independence of the data set, their progeny was not used in the analyses except in the full-sib analysis and in the separate analyses for red and dull fathers (see below).

The repeatability (coefficient of intraclass correlation) of preference was estimated as 0.65 ± 0.14 (s.e.[17]) (single classification ANOVA, $F=4.73$, d.f. $=17, 18$, $P<0.001$).

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, because the added variance component among fathers was significant (Table 1*b*). The relatively low variance of the daughters' preference among mothers (Table 1*b*) prevented narrow-sense heritability estimation. A full-sib analysis gave a rough estimate of heritability of preference for redder males of 0.43 (s.e.[17] $=0.37$).

The influence of mothers both on their daughters' red preference and on their sons' red intensity tended to be stronger in crosses with dull fathers than when crossed with red males (between mothers source of variation of daughters' preference in crosses with red males: $F=0.50$, d.f. $=5, 26$, $P>0.77$; and in crosses with dull males: $F=1.81$, d.f. $=6, 43$, $P<0.12$; for sons' coloration, $F=0.61$, d.f. $=5, 47$, $P>0.69$, and $F=1.34$, d.f. $=7, 58$, $P<0.25$, 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; $r^2=0.77$, $F=13.30$, d.f. $=1, 4$, $P=0.011$, 1-tailed). Conclusive evidence of this positive genetic correlation is given by the highly significant positive correlation that existed across fathers between the sons' intensity of red and the daughters' preference for redder males (Fig. 3). The phenotypic correlation of 0.998 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 (s.e.[15,18] $=0.31$) was calculated from the slope of the regression line (using red index × 100 and average scores per cross averaged over the different crosses per father: $y=-651.13+8.507x$, $r^2=0.998$, $F=1709.01$, d.f. $=1, 4$, $P<0.0001$) and the estimated heritabilities of the male and female trait[15,19].

It is unlikely that the strong correlation between male and female traits (Fig. 3) is caused by nonheritable effects[20]: common environmental influences were minimized by the applied

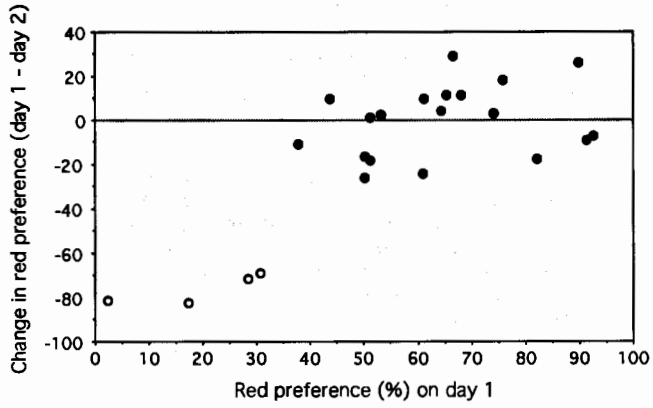

FIG. 2 Change in preference for the redder male of 22 ripe females between two successive days (day 1–day 2) as a function of the preference on day 1. Preference is expressed as % of total duration of head-up display directed at the redder male. The horizontal line indicates no change. Females with a preference less than 35% on day 1 are indicated by empty circles. Females were maintained together with their brothers in several small standardized groups per cross. Before the attainment of sexual maturity they were separated from their brothers by placing 15 randomly chosen sisters of each cross in 60-l tanks. Each three female tanks was lit by a 40 W fluorescent tube lying on top of the tanks. Ripe females were selected out of the sister groups, each was isolated in a small tank, and tested the next day for their preference for redder males. In the choice test, females were enclosed in a plexiglass cell and offered a simultaneous choice between two courting males that differed in their intensity of red coloration, and could not interact with each other[6]. The duration of the female's head-up courtship posture while pointing at one of the two males was measured during a 2 min period after 1 min of acclimatization. This measure correlates positively with the probability to spawn with that male[7]. Three different dull 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 spines and put back in their sister groups.

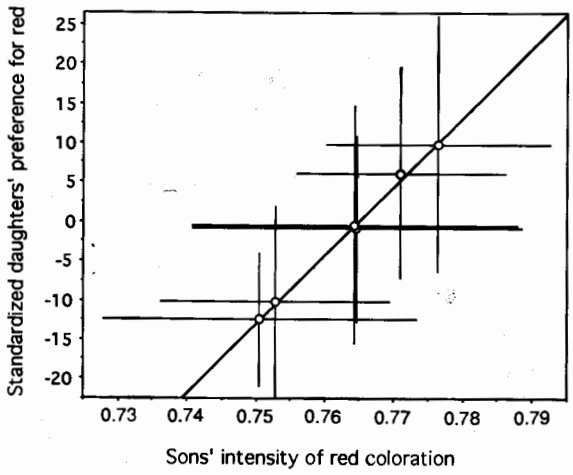

FIG. 3 Correlation across fathers ($N=6$) between the average (±s.d.) red intensity of sons ($N=103$ sons, for numbers of sons and crosses per father, see Fig. 1) and the average (±s.d.) preference of daughters for redder males ($N=61$ daughters, numbers of tested daughters per father from left to right: 7, 5, 15, 25, 5, 4). The daughters' preferences were corrected for the degree to which their test males differed in their red intensities. The regression equation is: $y=-667.60+872.94x$ ($r^2=0.997$, $F=1038.20$, d.f.$=1, 4$, $P<0.0001$). In producing offspring, matings were forced, that is I determined beforehand to which male a particular ripe female would be released. Criteria that were used in making up mating pairs were that both categories (red and dull) of males could reproduce about equally often, and that within each category the males were crossed with the whole spectrum of relative female preferences for intense red males, as had been determined in the sequential choice tests[8]. With a few exceptions, the females spawned with their imposed males.

methods of raising and maintaining fish and were 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[15] 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 them, but precise estimates of the genetic variables cannot be obtained 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[2,3] and partly ambiguous[21-23]. These genetic variables are critical in population genetics models of sexual selection. During the evolution of male 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 gene[24,25] and polygenic models[19,26-28]. The genetic covariance can even be maintained when female choice is costly[29], which is likely in this stickleback population[9]. In major gene models of 'good genes' sexual selection, there are no internal equilibria, and the male and/or female trait will become fixed[1]. But in polygenic models it is usually assumed that genetic variation in both traits is maintained and that there is a positive genetic covariance at equilibrium.

Thus one cannot distinguish whether a fisherian or good genes process, alone[19,24-30] or in combination[31,32], maintains genetic variation and covariation 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. □

Received 11 December 1992; accepted 26 February 1993.

1. Maynard Smith, J. *Trends Ecol. Evol.* **6,** 146-151 (1991).
2. Heisler, L. *et al.* in *Sexual Selection: Testing the Alternatives* (eds Bradbury, J. W. & Andersson, M. B.) (Wiley, Chichester, 1987).
3. Bakker, T. C. M. *Neth. J. Zool.* **40,** 617-642 (1990).
4. Kirkpatrick, M. & Ryan, M. J. *Nature* **350,** 33-38 (1991).
5. Darwin, C. *The Descent of Man, and Selection in Relation to Sex* (Murray, London, 1871).
6. Milinski, M. & Bakker, T. C. M. *Nature* **344,** 330-333 (1990).
7. McLennan, D. A. & McPhail, J. D. *Can. J. Zool.* **68,** 482-492 (1990).
8. Bakker, T. C. M. & Milinski, M. *Behav. Ecol. Sociobiol.* **29,** 205-210 (1991).
9. Milinski, M. & Bakker, T. C. M. *Proc. R. Soc.* B**250,** 229-233 (1992).
10. Bakker, T. C. M. & Mundwiler, B. *Behav. Ecol.* (in the press).
11. Bakker, T. C. M. *Behaviour* **98,** 1-144 (1986).
12. Hagen, D. W. & Moodie, G. E. E. *Evolution* **33,** 641-648 (1979).
13. Bakker, T. C. M. in *The Evolutionary Biology of the Threespine Stickleback* (eds Bell, M. A. & Foster, S. A.) (Oxford Univ. Press. Oxford, in the press).
14. Laurent, P. J. *J. Fish. Res. Bd. Canada* **29,** 867-875 (1972).
15. Falconer, D. S. *Introduction to Quantitative Genetics* 3rd edn (Longman, Harlow, 1989).
16. Frischknecht, M. *Evol. Ecol.* (in the press).
17. Becker, W. A. *Manual of Quantitative Genetics* 4th edn (Academic Enterprises, Pullman, 1985).
18. Robertson, A. *Biometrics* **15,** 469-485 (1959).
19. Lande, M. *Proc. natn. Acad. Sci.* **78,** 3721-3725 (1981).
20. van Noordwijk, A. J. in *Population Biology and Evolution* (**eds** Wöhrman, K. & Loeschcke, V.) (Springer, Heidelberg, 1984).
21. Kearns, P. W. E., Tomlinson, I. P. M., Veltman, C. J. & O'Donald, P. *Heredity* **68,** 385-389 (1992).
22. O'Donald, P. & Majerus, M. E. N. *Heredity* **69,** 521-526 (1992).
23. Ritchie, M. G. *Trends Ecol. Evol.* **7,** 328-329 (1992).
24. Kirkpatrick, M. *Evolution* **36,** 1-12 (1982).
25. Seger, J. *Evolution* **39,** 1185-1193 (1985).
26. Heisler, I. L. *Heredity* **55,** 187-198 (1985).
27. Kirkpatrick, M., Price, T. & Arnold, S. J. *Evolution* **44,** 180-193 (1990).
28. Barton, N. H. & Turelli, M. *Genetics* **127,** 229-255 (1991).
29. Pomiankowski, A., Iwasa, Y. & Nee, S. *Evolution* **45,** 1422-1430 (1991).
30. Iwasa, Y., Pomiankowski, A. & Nee, S. *Evolution* **45,** 1431-1442 (1991).
31. Kirkpatrick, M. *J. theor. Biol.* **119,** 263-271 (1986).
32. Tomlinson, I. P. M. *Heredity* **60,** 283-293 (1988).
33. Sokal, R. R. & Rohlf, F. J. *Biometry* 2nd edn (Freeman, New York, 1981).
34. Endler, J. A. *Biol. J. Linn. Soc.* **41,** 315-352 (1990).
35. Endler, J. A. *Vision Res.* **31,** 587-608 (1991).

ACKNOWLEDGEMENTS. I thank M. Frischknecht and B. Mundwiler for help in quantifying male coloration, M. Milinski and A. Pomiankowski for helpful comments, W. Rowland (Indiana University) for facilities, C. Baube for allowing me to measure and analyse reflectance spectra, A. Pomiankowski, A. van Noordwijk, C. Wedekind and F. Weissing for discussions, A. van Noordwijk for statistical advice, and the Swiss National Science Foundation and the Swiss Academy of Science for financial support.