Costs influence sequential mate choice in sticklebacks, *Gasterosteus aculeatus*

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SUMMARY

In nature females can usually choose among males only sequentially. Recent models of sequential choice predict that, with increasing costs of sampling, selectivity for preferred males should decline. In our tests female sticklebacks were highly selective in their sequential choice between dull and bright males when costs were low. With increasing time and energy costs of moving between males, they reduced their selectivity. In particular, when the females had to swim against a current, dull males when met became highly acceptable. The females' response to increased costs of sampling shows that they make the kind of economic decisions predicted by models of sequential choice. The strength of sexual selection by female choice therefore depends on the spatial structure of the population.

I. INTRODUCTION

Female choice for male sexual ornaments is regarded as a major force in sexual selection (see Harvey & Bradbury (1991) for a recent review). Although most of the experimental evidence for female mate preference comes from simultaneous choice situations, under most natural conditions females can choose only sequentially. This is a more demanding task (Real 1990; Bakker & Milinski 1991). However, several studies have shown that females are able to select more preferable males when they can see only one male at a time (see, for example, Brown 1981; Houde 1987; Moore & Moore 1988; Zuk *et al.* 1990; Petrie *et al.* 1991; Bensch & Hasselquist 1992), or at least that females visit several males before mating (see references in Bakker & Milinski 1990; Dale *et al.* 1990, 1992; Pruett-Jones & Pruett-Jones 1990). Only two studies (Bakker & Milinski 1991; Collins 1992) have provided experimental evidence for the influence of recent experience on female choice, demonstrating that females have compared different males across time. Similarly, katydids discriminate among potential mates after they have experienced a higher mate availability (Shelly & Bailey 1992).

Several rules which females could use to make sequential choice both possible and efficient have been modelled (Janetos 1980; Janetos & Cole 1981; Parker 1983; Wittenberger 1983; Real 1990; Slagsvold & Dale 1991). Real's (1990) analysis has shown that costs of sampling and choosing should have a dramatic effect on the female's decision. If costs increase, sampling should be reduced and a female should decrease her selectivity. Costs can consist of long distances between territorial males so that a female has to invest time and energy for sampling. For example, female pied flycatchers having an easy choice (in nest box groups)

choose males with high-quality boxes, whereas females with a more costly choice (single boxes) do not make this distinction (Alatalo *et al.* 1988). Energy demand and competition for mates were suggested as potential costs of searching in this species (Slagsvold *et al.* 1988; Dale *et al.* 1992). Costs of mate choice influence the predictions of several models of sexual selection (see, for example, Pomiankowski 1987).

A male three-spined stickleback fish develops a bright red coloration and claims a territory of up to several square metres at the start of the breeding season. He may collect up to 20 clutches of eggs from different females for each breeding cycle (Wootton 1976). A gravid female can spawn the whole clutch at any time during a few days. The females prefer the more intensely red-coloured male in simultaneous choice situations, as observations (McLennan & McPhail 1990) and experiments (Milinski & Bakker 1990) have shown. Female preference is based almost exclusively on the intensity of the male's coloration, which reveals his physical condition (Milinski & Bakker 1990). However, under natural conditions, the distances (up to several metres) between the males and also vegetation force a female to compare potential mates sequentially. We have shown (Bakker & Milinski 1991) that female three-spined sticklebacks also rate sequentially presented males according to their brightness. Furthermore, a male's attractiveness has a significant effect on the female's rating of the next male; a given male is rated higher when preceded by a duller male than by a brighter one. We moved a female within a cell between males and determined the duration of her 'head-up display' in front of a given male as a measure of her preference for that male. Collins (1992) also used the display rate of female zebra finches when she found an effect of previous experience on female preference.

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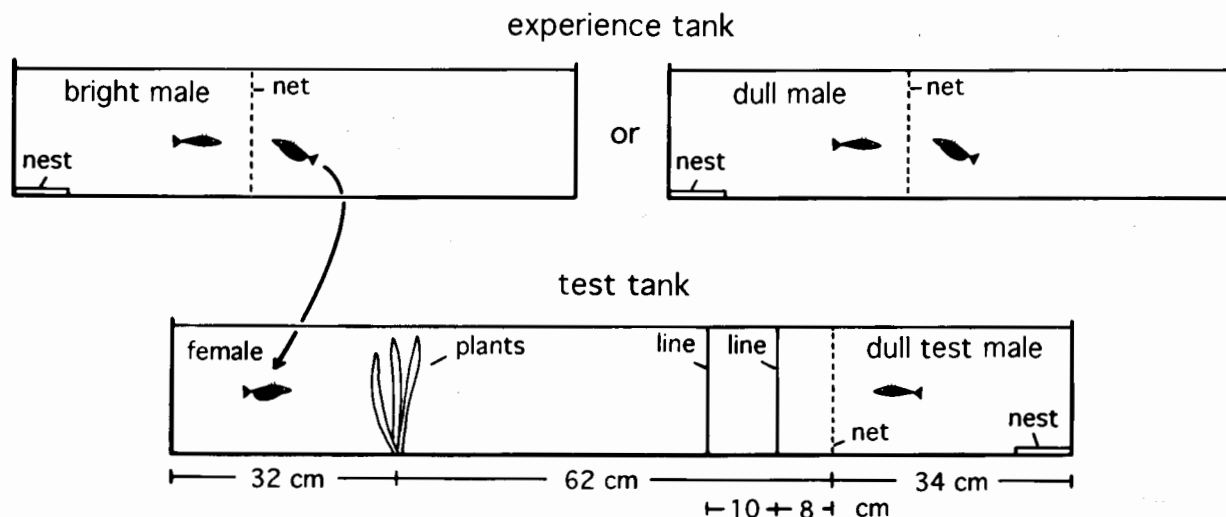*Printed in Great Britain*

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Figure 1. Experimental set-up. A female spent 3 min in the experience tank either with a bright red or a dull (only experiments 1 and 2) male. Then she was transferred to the test tank either directly or after a specified time which she spent either in still or in running water. See text for further explanations.

In the present study we investigate: (i) the previous-male effect, when female sticklebacks have to approach each male from a distance, and whether they can decide how long to stay and display for and how far to move when they leave; and (ii) whether time and energy costs of moving between males reduce the females' selectivity so that a dull male can become attractive even when the female has seen a bright male before.

2. METHODS AND RESULTS

The sticklebacks originated from a Swiss freshwater population near Roche (Montreux). They were caught with a net from tributaries of the Rhône river with flowing water (velocity at surface: 31 cm s^{-1}) during the breeding season (May to July). The females were housed in large communal tanks for several weeks before they were used in choice tests. Males were placed individually into 10 l tanks (temperature 15 °C, L/D = 16 h/8 h). After several weeks, most males had built a complete nest out of filamentous algae in a Petri dish filled with sand. When the males' red breeding coloration seemed to have stabilized, we chose a bright red and two dull males (similar to 9, 2 and 1, respectively, of the scale of Milinski & Bakker (1990)) and transferred them with their nests to experimental tanks. The intensity of courtship and male brightness are positively correlated (Milinski & Bakker 1990). The bright male and the slightly brighter of the two dull males were each placed into an 'experience tank' (80 cm × 21 cm, water level 18 cm), the duller male into the 'test tank' (128 cm × 21 cm, water level 18 cm) (figure 1). In each case the Petri dish with the nest was placed near a short side wall close to an air stone. The test tank contained three plants (*Vallisneria*). Each tank was illuminated by two parallel 40 W fluorescent tubes mounted 35 cm above the water level. The long back wall was covered with white styrofoam from the outside, the two short walls with grey PVC.

Several hours before the start of an experiment, females which were ready to spawn, as judged from their colour, the extension of their bellies, and from the opening of their cloacae, were selected and placed in small tanks (17.8 cm × 34.5 cm, 16.5 cm water level). A partition consisting of a frame with a green net was placed in the test tank and in each of the two experience tanks (figure 1). The long

front walls were covered with a white net to prevent distraction of the fish. A ripe female (not used in experiments) was placed in the large compartment of each experience and test tank until the male courted at a stable level. The males zig-zagged towards the female which displayed her 'head-up posture' while touching the green net.

To minimize disturbance, the females were transferred within a glass pipe containing several decilitres of water. Each female was used only once. We used parametric statistics after appropriate (log$_{10}$) transformation of the data (SYSTAT 5.2 software package for Macintosh). We used one-tailed tests whenever hypotheses in one direction were tested.

(a) Experiment 1: the effect of the sequence of males

Method A female was placed into the large compartment of the experience tank with the dull male. After 3 min she was transferred to the test tank and released behind the plants. We observed both fish with a video camera. After about 1 min she usually swam past the plants and approached the male, which typically started displaying. When she passed a vertical line drawn on the wall 8 cm from the green net, we measured the time she remained in his vicinity, that is until she passed a second vertical line 18 cm from the net (figure 1). If a female did not pass the second line within 300 s, we rated her reaction with this value. She was transferred to her individual small tank where she stayed for about (at least) 1 h until the whole procedure was repeated, starting with transferring her to the experience tank with the bright male. Every second female started with the bright male.

Results The females usually displayed 'head-up' almost all the time they stayed near the dull test male. When they came from the dull male, they stayed for a longer period of time (201.9±38.7 s, mean±s.e.) near the test male than when they had come from the bright male (122.9±45.6 s, $t = 2.26$, d.f. = 7, $p < 0.03$, one-tailed paired t-test). As a rule, they swam back the whole distance until they were again behind the plants once they had passed the second line.

(b) Experiment 2: combined time and energy costs

Method The procedure was the same as in experiment 1, except for an additional round each female had to spend in the experience tank with the bright male and afterwards in the test tank with the dull test male. In the additional round, experience and test were separated by 10 min in which the
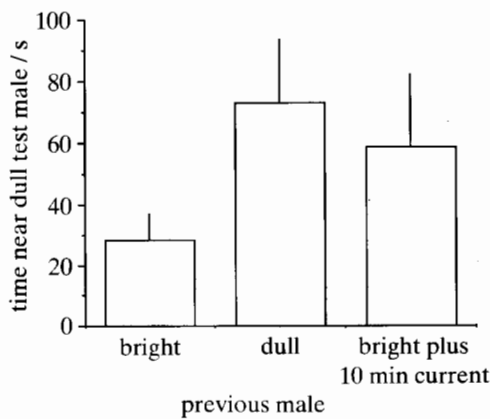
Figure 2. Time (average + s.e.) the females spent near the dull test male after they had come directly either from a bright male or a dull male, or after they had come from the bright male and then had to maintain their position in a water current for 10 min before the test ($n = 12$ females).
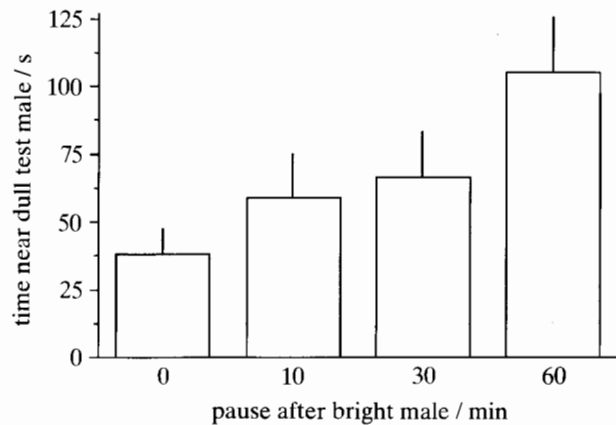

Figure 3. Time (average + s.e.) the females spent near the dull test male after they had come either directly (0 min) from a bright male or after they had spent 10 min, 30 min or 60 min, respectively, in their individual tanks when they came from the bright male ($n = 24$ females).

female had to keep position in a water current of 14.3 cm s^{-1}. The female was placed into a greenish-blue plastic bowl (diameter 36 cm, water level 12 cm) with a grey bucket (diameter 21 cm) in its centre. The water current in the ring channel was generated by the outflow and the protected inflow pipe of a pump outside the bowl. Black vertical stripes on the inside of the bowl allowed the female to orientate (mostly moving slowly upstream) in the current. By this procedure we tried to simulate time and energy costs of moving from the bright to the dull male. Between rounds the female was placed in her individual tank for about (at least) 1 h.

There are six different sequences of the three different procedures to make up a complete block design. Two such blocks (12 females) were completed. Within each block, the sequence of the six sequences was randomized. A new set of three males was used in this experiment.

Results As in experiment 1, the females spent significantly more time with the dull test male when they had come from the dull male than when they had seen the bright male before (figure 2, $t = 3.20$, d.f. $= 11$, $p = 0.004$, one-tailed paired *t*-test). However, when the females had spent 10 min maintaining their position in the water current after they had seen the bright male, they displayed significantly longer near the dull test male than when they had come from the bright male directly (figure 2, $t = 2.02$, d.f. $= 11$, $p < 0.04$, one-tailed paired *t*-test). This suggests that females take time and energy invested on the way from one male to another into account when they decide whether to return to a bright male.

(c) *Experiment 3: costs of time*

Method In this experiment the females saw only the bright male before the dull test male. To separate the time from the energy costs of moving from the bright to the dull male, each female experienced four different waiting times (0 min, 10 min, 30 min and 60 min, respectively) which she, except for the pause of 0 min, spent waiting or slowly moving around in her individual small tank.

There are 24 different sequences of the four waiting times to make up a complete block design. One such block (24 females) was completed. The sequence of the 24 sequences was randomized. Between rounds the female was placed in her individual tank for about (at least) 1 h. The bright male and the dull test male were the same as in the previous experiment.

Results The time the females spent near the dull test male was significantly influenced by the length of the pause between seeing the bright male and the dull test male (figure 3) ($F = 4.39$, d.f. $= 3$, 69, $p < 0.007$, one-way repeated measures ANOVA, two-tailed). After a pause of 30 min the females spent significantly more time with the dull test male than when they had come directly (pause: 0 min) from the bright male ($t = 2.28$, d.f. $= 23$, $p < 0.02$, one-tailed paired *t*-test). This effect was more pronounced after a pause of 60 min ($t = 3.55$, $p = 0.001$).

When the female left the dull test male (i.e. passed the vertical line at 18 cm from the net), she usually moved almost the whole distance (62 cm) back to the plants irrespective of pause length (pause 0 min, 62 cm (median); 10 min, 55 cm; 30 min, 60 cm; 60 min, 60 cm) (d.f. $= 3$, $\chi^2 = 1.10$, $p = 0.78$, Friedman two-way ANOVA, corrected for ties).

Because each pause length appeared equally often in the first, second, third and fourth test, we can investigate whether the dull test male is devalued over the four tests, because the females may learn that they come back to the bright male at least 1 h after they have left the dull test male. The time the females spent near the dull test male was significantly influenced by the number of the test (first test, 110.5 ± 19.4 s (mean $\pm$ s.e.); second test, 62.0 ± 18.0 s; third test, 43.8 ± 12.0 s; fourth test, 51.7 ± 12.3 s) ($F = 4.92$, d.f. $= 3$, 69, $p < 0.004$, one-way repeated measures ANOVA, two-tailed). They spent significantly less time with the dull test male in their second test than in their first test (Fisher's LSD post-hoc test, $p < 0.05$, two-tailed). There was no further significant ($p > 0.05$) reduction of the time near the dull test male, either from the second to the third test or from the second to the fourth test. Thus, females remembered the bright male for longer than 1 h.

There was no significant effect of the number of tests on the females' duration of display near the bright male. Of the 120 s measured, most females displayed for 120 s in each of the four tests (d.f. $= 3$, $\chi^2 = 4.90$, $p = 0.18$, Friedman two-way ANOVA, corrected for ties).

(d) *Experiment 4: costs of energy*

Method As in the previous experiment, the females saw only the bright male before the dull test male. To separate the energy from the time costs of moving from the bright to the dull male, the female spent 20 min either in the water current or in a similar plastic bowl without a current, respectively, before she was transferred to the test tank. Each
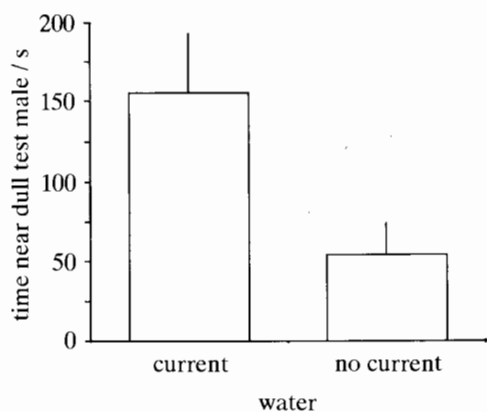
Figure 4. Time (average + s.e.) the females spent near the dull test male after they had come from a bright male and had then spent 20 min either in a water current or in non-moving water before the test (*n* = 8 females).

female met both situations but every second female met the reverse sequence. We chose 20 min because the choice for the dull test male increased only slightly between waiting times of 0 min and 30 min in experiment 3 (figure 3). The energy spent by moving against a current is, however, expected to increase substantially within 20 min (Lester 1971). To make this experiment even more efficient by reducing variation, we used the finding from experiment 3 that choice of the dull test male diminishes mostly between the first and the second trial. Thus, each female had to run through three trials; there were, however, only two different sequences of pauses, i.e. no current, current, no current; and no current, no current, current. The results of the first round were not used for analysis. Eight females were used in this experiment. Another bright male was used, but the dull test male was the same as in the previous experiment. Its display behaviour was very constant at low level.

Results The females stayed for a significantly longer time near the dull test male after they had spent their pause of 20 min in the water current than when they had been in still water (figure 4; *t* = 3.00, d.f. = 7, *p* = 0.01, paired *t*-test, one-tailed). It is unlikely that the females that came from the bowl with the water current stayed longer with the test male because they were exhausted. If they were exhausted they would have stayed near the plants where they were released. Furthermore, the females usually displayed 'head-up' when they were close to the test male.

3. DISCUSSION

In this study the females could actively decide whether to approach a male and for how long to stay with him. The male was presented behind a net so the female could get also tactile information. Under these more natural conditions the previous male effect seemed to be more pronounced than in a previous study in which the female was confined in a plexiglas cell in front of a male (Bakker & Milinski 1991). In that study, the females' display at a given male did not fade out but stopped after a while. Here we found similarly that when the female stopped displaying she swam almost the whole distance back to the place from which she had started to approach the male. Because the male was confined behind a net, we can be sure that the female decided when to leave. When she came from a dull male she stayed for longer with the dull test male than when she came from a bright male. This

suggests that she decided to leave the dull male to return to the bright male that she had seen before. The duration of the female's head-up display in front of a male correlates with her probability to spawn with that male (McLennan & McPhail 1990). Thus, her probability to spawn with the dull test male was lower when she had seen a bright instead of another dull male before.

When the female had to swim in a water current of three body lengths per second (half as fast as that of the site where we had caught the fish) for 10 min after she had seen the bright male, she displayed longer in front of the dull test male than when she had come from the bright male directly. This shows that increased time and energy costs of moving between males reduce the female's selectivity as predicted by theory (Real 1990). When we distinguished experimentally between time and energy costs of moving, we found a pronounced effect of waiting time only after 30 min, whereas moving in a current of natural velocity for 20 min reduced the females' selectivity strongly compared with females who only waited for 20 min after they had seen the bright male and could approach the dull test male. So both time and energy costs of moving influence female decision making as predicted: they become less selective.

Three-spined sticklebacks have no problems maintaining position in a current of four body lengths per second for 480 min; at a speed of more than six body lengths per second, however, they fatigue rapidly (Whoriskey & Wootton 1987). Swimming at a velocity of three body lengths per second for up to 20 min in our experiment was therefore far below the sticklebacks' capability. Nevertheless, swimming is energetically costly. The oxygen consumption of sticklebacks is doubled at a speed of two body lengths per second as compared with one body length per second (Lester 1971). A ripe female has a higher oxygen consumption than a non-gravid one (Giles 1987), and swimming is more demanding for her because her belly is extended by the eggs (Wootton 1976). Thus, a ripe female has energy costs of sampling a number of males, and we found that she takes that into account.

Time should become a cost factor when the female approaches the time when she finally has to spawn (Bakker & Milinski 1991). Only three females in experiment 3 reached this point by the next morning after the experiment. Two of them had increased their time with the dull test male over the 5 h the four tests (plus pauses) lasted in the experiment. The two females provide anecdotal evidence for time to become pressing close to the final spawning time. There was, however, the general trend that the females decreased the time with a dull male when they had previously come back to the bright male after about 1 h. This suggests that time costs, although taken into account, are less pressing than energy costs for a female stickleback that is choosing sequentially among a number of males.

The spatial structure of the population, that is, the distances among male territories, should affect the strength of sexual selection, because females become less selective when sampling potential mates has increasing costs. Hence, there should exist population

differences in the expression of male sexual ornaments dependent on spatial structure and physical environment, e.g. current velocity.

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