Female swordtail fish prefer symmetrical sexual signal

MOLLY R. MORRIS* & KENNETH CASEY†

*Department of Biology, M ontgomery College, Rockville, M D 20850, U.S.A.
†Department of Zoology, University of Texas at Austin

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Abstract. It has been proposed that females use the symmetry of secondary sexual traits to differentiate between potential mates. The vertical bars on male swordtail fish function as a signal that attracts females and deters rival males in one swordtail species. In addition, male courtship behaviour of most Xiphophorus species incorporates serial lateral presentations, which provide females with a clear opportunity to assess males for bilateral symmetry. We tested the hypothesis that X. cortezi females prefer males with a symmetrical bar number by determining whether females switched their preference between two males when we switched which male had a symmetrical number of bars. The ability to manipulate the bars without influencing other male traits allowed us to control for male characters correlated with bar symmetry that females might prefer. The degree of asymmetry in bar number we used was within the degree of asymmetry found for this trait in nature. Females switched their preference between a pair of males when we switched which male was symmetrical for bar number. We discuss the possibility that females prefer a symmetrical bar number as well as an alternative hypothesis.

Fluctuating asymmetries are random deviations from perfect bilateral symmetry in a morphological trait for which differences between the right and left sides have a mean of zero and are normally distributed (Van Valen 1962). Fluctuating asymmetries result from the inability of individuals to undergo identical development on both sides of their bodies (Leary & Allendorf 1989). Interest in fluctuating asymmetries has recently spread to studies of sexual selection because of their potential to link a component of male viability with criteria used by females in discriminating between mates (Watson & Thornhill 1994). Some secondary sexual traits show greater fluctuating asymmetries than other morphological traits (e.g. Møller & Pomiankowski 1993; Møller & Höglund 1991), and differences between conspecifics in their ability to develop symmetrically are heritable (Thornhill & Sauer 1992; Møller & Pomiankowski 1993). It has been proposed that females could use the symmetry of sexually selected traits to discriminate between potential mates based on ‘genetic quality’ (Møller 1990; Møller & Pomiankowski 1993).

Symmetry of sexually selected traits contains information about male genetic quality, and symmetrical males have greater mating success (Watson & Thornhill 1994). It has not been demonstrated, however, that females prefer symmetry in naturally occurring sexual signals. In some cases, it has been difficult to separate preference for the symmetrical trait from other correlated characters; for example, tail symmetry in barn swallows, Hirundo rustica (Møller 1992) affects flight performance (Møller 1991). Other studies have demonstrated preference for symmetry of unnatural traits, for example, coloured leg-bands on zebra finches, Taeniopygia guttata (Swaddle & Cuthill 1994a), and painted tail feathers in barn swallows (Møller 1993). Møller (1993) painted the swallow tail feathers in an attempt to control for the affect of asymmetry on flight performance and assumed that females perceived the white part of the tail feather as ‘not there’. Preference for symmetry has also been demonstrated for chest barring on finches (Swaddle & Cuthill 1994b), but it is not known whether this trait functions as a sexual signal.
The signal that we examined is a pigment pattern of dark vertical bars (Fig. 1). Vertical bars are widespread throughout the genus Xiphophorus as well as in other genera of poeciliid fishes (e.g. Heterandria, Phallichthys). Examination of F1 and back-crossed hybrids among several of the Xiphophorus species, including X. cortezi, suggests that the bars have a polygenic basis (Atz 1964). The bars are composed of hundreds of closely associated melanophores, and males can vary the intensity of their bars by dispersing or concentrating the pigment within the melanophores (Gordon 1931). In several species, including X. cortezi, the bars are a secondary sexual trait found on sexually mature males and they intensify in relation to social interactions; the bars become darker on aggressive males and males courting females (Franck 1964; Zimmerer & K allman 1988; M orris et al. 1995). The bars function in both deterring rival males and attracting females in the closely related species X. multilineatus (M orris et al. 1995).

We tested X. cortezi females to determine whether they preferred males with symmetrical bar numbers over males with asymmetrical bar numbers. We controlled differences between pairs of males other than bar symmetry by testing a female twice with the same pair of males; we determined whether females switched the time they spent associating with one male over another when we switched which male had symmetrical bar numbers. We also examined the degree of bilateral asymmetry in bar numbers for one population of X. cortezi to determine whether the asymmetry we used in our experiment was within the natural range of asymmetry for this trait.

### MATERIALS AND METHODS

We collected Xiphophorus cortezi from two sites in México (the Río Axtla, San Luis Potosí and the Arroyo La Conchita at Xiltla, San Luis Potosi). We recorded male size (standard length) and the number of bars on both sides for the males collected from the Arroyo La Conchita (Table I). Males were anaesthetized with MS222 before measurements were taken, which ensured that the bars were fully expressed when counted. Because

<table>
<thead>
<tr>
<th>Symmetrical (0)</th>
<th>35 (8)</th>
<th>12.5 (4.00)</th>
<th>36.8 (6.47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymmetrical (± 1)</td>
<td>39 (9)</td>
<td>13.4 (3.13)</td>
<td>39.8 (7.20)</td>
</tr>
<tr>
<td>Asymmetrical (± 2)</td>
<td>17 (4)</td>
<td>12.0 (3.65)</td>
<td>37.5 (1.52)</td>
</tr>
<tr>
<td>Asymmetrical (± 3)</td>
<td>9 (2)</td>
<td>15.0 (5.66)</td>
<td>29.8 (8.13)</td>
</tr>
<tr>
<td>Males without bars</td>
<td>N =11</td>
<td>—</td>
<td>37.3 (5.90)</td>
</tr>
</tbody>
</table>

### Table I. Bar symmetry (number of bars on left – number of bars on right side) for X. cortezi males
the bars are a discrete trait, the problems associated with measuring the fluctuating asymmetry of quantitative traits (Palmer & Strobeck 1986) can be avoided. We kept males and females in separate 2.5-litre tanks throughout the experiment to increase sexual interactions during testing. Stimulus males were pairs of males of similar sizes (within 2.0 mm; Table II). We tested females with pairs of stimulus males from the same site from which they were collected, and each female was tested with the same pair of males twice.

Bar Manipulations

Before the first round of tests, the stimulus males from a pair were freeze-branded so that both males had the same total number of bars. One male, however, received an asymmetrical number of bars (two more bars on one side compared to the other), and one male a symmetrical number of bars. The freeze-branding technique (Raleigh et al. 1973) has been used to examine the function of the bars as a sexually selected signal in related swordtail species (Morris et al. 1995; Morris & Ryan 1996) and is an effective method of removing the bars without altering the behaviour of the males. We randomly determined which side of a male would have more bars for the asymmetrical treatments. Males from each pair were freeze-branded an equal number of times during each manipulation, branding between the bars in cases where fewer bars were removed from one male compared to the other. When bars were removed, they were the posterior most bars (Fig. 1). Although the behaviour of the males did not appear to be influenced by freeze-branding, we controlled for any possible influences of this procedure, because males within a pair always received the same number of freeze brands in the same general locations. Before the second round of tests, we removed two bars from one side of each male, which reversed which male was symmetrical within a pair while keeping the total number of bars on the two males equal.

### Preference Tests

Simultaneous choice tests were used to test for female preference. Results from this type of choice test in the closely related species X. nigrensis are highly related to other indicators of mate choice (Morris et al. 1992; Morris & Ryan 1993), in addition to male reproductive success in the field (Ryan et al. 1990b). Tests were conducted in an aquarium (45 × 41 × 80 cm) divided into five equal sections (Fig. 2). The two end compartments were divided from the centre three by glass plates, which ensured that females received only

<table>
<thead>
<tr>
<th>Male size (mm)</th>
<th>Test 1</th>
<th>Test 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bars (R/L)</td>
<td>Time (s)</td>
</tr>
<tr>
<td>45.1</td>
<td>6/6</td>
<td>1374</td>
</tr>
<tr>
<td>43.3</td>
<td>7/5</td>
<td>424</td>
</tr>
<tr>
<td>38.1</td>
<td>3/3</td>
<td>1162</td>
</tr>
<tr>
<td>39.2</td>
<td>2/4</td>
<td>654</td>
</tr>
<tr>
<td>37.2</td>
<td>8/8</td>
<td>1398</td>
</tr>
<tr>
<td>35.3</td>
<td>9/7</td>
<td>685</td>
</tr>
<tr>
<td>31.0</td>
<td>5/5</td>
<td>1465</td>
</tr>
<tr>
<td>31.0</td>
<td>6/4</td>
<td>562</td>
</tr>
<tr>
<td>32.0</td>
<td>6/6</td>
<td>910</td>
</tr>
<tr>
<td>31.5</td>
<td>7/5</td>
<td>737</td>
</tr>
<tr>
<td>34.1</td>
<td>4/4</td>
<td>1386</td>
</tr>
<tr>
<td>35.0</td>
<td>3/5</td>
<td>504</td>
</tr>
</tbody>
</table>

The male that had the symmetrical number of bars in test 1 was freeze-branded to have an asymmetrical number of bars in test 2.
visual cues. The middle three compartments were divided by lines marked on the aquarium. We placed a test female in an opaque tube in the centre section, and the test males in the two end compartments. All three fish were allowed to acclimate for 10 min. When the tube was raised, we measured the time that the female spent in the two sections adjacent to the males for 20 min. The centre section was considered neutral and gave the females the opportunity to choose not to associate with either male. Trials in which both males did not actively interact with the female by swimming up and down the divider were eliminated. In all cases where the males interacted with the female, the bars were expressed. After the first observation period, the female was returned to the opaque tube, the males were switched end to end, and all fish were allowed to acclimate for 10 min. The test was then repeated to control for any position preference. The total time that a female spent with a male across both observation periods was used as an indication of relative preference.

We compared the total time females spent with the symmetrical males compared to the asymmetrical males in both rounds of tests with Wilcoxon matched-pairs signed-rank tests. We also compared the preference of each female in the first round of tests to her preference in the second round of tests to determine whether female preferences switched when we switched which male had a symmetrical bar number. These data were analysed with a binomial test.

RESULTS

Bar Symmetries

Not all *X. cortezi* males from the Arroyo La Conchita population have bars. Excluding males without bars (N=11), the total number of bars was positively correlated with male size (r^2=0.2, P=0.03). The average ± SD bar symmetry on males with bars was −0.13 ± 1.39 (range=−3–+3, N =23), and was not significantly different from zero (t= −0.449, P =0.66). Twenty-six per cent of the males with bars had a degree of asymmetry in bar number equal to or greater than the degree of asymmetry we used in this study (Table I).

Female Preference

Female *X. cortezi* preferred males with a symmetrical bar number over asymmetrical bar number in simultaneous choice tests. Females spent significantly more time with the symmetrical males compared to the asymmetrical males in both tests (test 1: mean ± SE time with symmetrical males=1282.5 ± 85.44 s, time with asymmetrical males=594.3 ± 48.43 s, Wilcoxon signed-rank test, Z =−2.2, N =6, P =0.01; test 2: time with symmetrical males=1230.7 ± 59.07 s, time with asymmetrical males=720.5 ± 23.21 s, Z =−2.2, N =6, P =0.01 (Table II). We also compared the number of females that switched and that maintained their preference when we switched which male was symmetrical between rounds of tests. All six females reversed their preference between the two tests, associating with whichever male received the symmetrical number of vertical bars (Binomial test, N =6, r =6, P =0.036; Table II). These results indicate that female *X. cortezi* fish preferred to associate with males that were symmetrical for bar number over males that were asymmetrical for this trait when the total bar number was held constant.

DISCUSSION

Mounting evidence suggests that symmetry of male secondary sexual traits may provide females with information about male viability, and that symmetrical males have greater mating success than asymmetrical males (*Watson & Thornhill 1994*). Demonstrating that females prefer males with symmetrical sexual signals and that this preference has affected the design of sexually selected male traits has been difficult. Two problems faced in previous studies were identified by *Swaddle & Cuthill (1994b)*. First, it must be...
possible to manipulate the symmetry of the male trait without changing other correlated traits that females might prefer. Vertical body bars are a visual signal that we could manipulate with freeze-branding, a technique that did not influence male behaviour or other male traits. We were able to show a complete reversal of each female’s preference between a pair of males by changing only the symmetry of the signal, which rules out preference for traits correlated with symmetry of the signal. The potential strength of selection for symmetry appears to be strong in X. cortezi. Even though pairs of males may have differed to some degree in several other characters that could potentially influence female choice (body size: Ryan et al. 1990b; sword length: Basolo 1990; other pigment patterns), a change in bar symmetry alone could override these differences and cause a female to change her preference from one male to another. Second, the traits tested must be natural traits, and preferably within the range of asymmetry found in nature, to suggest that female preference for symmetry has affected the design. Vertical bars are found on males throughout all of the populations of X. cortezi, and the degree of asymmetry that we used in our choice tests was within the range of asymmetry in bar number for one of the populations of X. cortezi tested.

A third type of problem for studies designed to examine female preference for symmetrical male traits involves separating preference for symmetry from preferences for trait size assessed by minimum or maximum expression of the trait on one side. Because we controlled the total number of bars, the asymmetrical male had both the greater and the lesser number of bars on one side. Therefore, females that associated with symmetrical males may have been expressing a preference for males with the largest minimum number of bars, rather than for males with a symmetrical number of bars. This possibility applies not only to our study, but also to all studies in which females showed a preference for symmetrical males when the average expression of the trait was held constant. This problem was identified by Oakes & Barnard (1994) when they demonstrated that female paradise whydahs, Vidua paradisaea, preferred males with asymmetrical tails when average tail length was held constant. Oakes & Barnard pointed out that females may actually have preferred the male with the longest single tail feather rather than asymmetrical males. Studies that show preference for symmetry cannot avoid this problem either, however. Although preference for trait size can be tested in an independent experiment (Brookes & Pomiankowski 1994) asymmetry of a trait and size of the trait can not be manipulated independently. By holding the average size of the trait constant, the asymmetrical male will have both the maximum and minimum expression of the trait.

Growing evidence shows that mate choice decisions are based on multiple cues (Burley 1981; Baker et al. 1986; Brooks & Caithness 1995). Preference for symmetry and preference for trait size may be two of several preferences that interact to result in female choice of mates. If preference for exaggeration of the male trait is stronger than preference for symmetry of the male trait, it will be impossible to separate the effects of preference for symmetry and preference for largest minimum trait in the type of study we have presented; the necessary test would require that females prefer a male with symmetrical bar number over an asymmetrical male that has more bars on average, so that the minimum number of bars on one side would be the same for both males. Alternatively, single stimuli preference tests, in which the responses of females with individual males are scored and correlated with the quantitative differences between males for the trait, may provide the type of data that can be used to separate statistically preference for symmetry and preference for largest minimum trait expression.

Two of the courtship displays that have been described for swordtail fish provide females with an opportunity to assess bilateral symmetry. At least one of the courtship displays found in X. cortezi and one of the displays found in other poeciliid fishes incorporates lateral displays to the female in which the male alternates frequently from side to side. The ‘figure-eight’ display (Ryan & Causey 1989), in which males swim back and forth in front of the female in a figure-eight pattern, is used by almost all of the northern swordtails including X. cortezi. Males of the southern swordtail X. helleri use a courtship display in which they alternate lateral presentations by swimming back and forth in front of the female (Clark et al. 1954; Franck 1964). Even the platyfish within the genus Xiphophorus use a behaviour in which females could assess bilateral symmetry: the display ‘side change over head’ (Haas 1993), in
which the male flips his tail back and forth over the female’s head, presenting the female with serial lateral views. It had been suggested that the serial lateral presentations of the courtship displays of swordtails might accentuate the sword (Rosen 1960) or limit the ability of the females to flee (Franck 1964). The results of this study suggest that the function of these components of the courtship displays should also be examined in relation to bilateral assessment by females and their preference for symmetry.

Assuming that the preference we detected was for symmetry, preference for symmetrically barred males could evolve because symmetry reflects individual male quality in relation to the ability of an individual to cope with genetic and/or environmental stress. As such, the results of this study would be consistent with ‘good genes’ models of sexual selection, which predict that females choose mates based on traits that reflect male quality (Møller 1990; Møller 1992; Møller & Pomiankowski 1993). These results would not exclude other models of mate choice, such as direct selection (Kirkpatrick & Ryan 1991), sensory exploitation (Ryan 1990; Ryan et al. 1990a) and selection for mate recognition (Johnstone 1994). For example, the vertical bars are more variable in the hybrids of several species of Xiphophorus, including offspring from X. cortezi and X. montezumae crosses (Atz 1962); bar asymmetry therefore may provide information about the probability that an individual is a hybrid. Further studies of the relationship between bar symmetry and male quality, genetic correlations between female preference and male trait, and the evolutionary history of preference and trait will make it possible to consider which of the various models for the evolution of female preference can explain preference for males with a symmetrical bar number.

The vertical bars appear to be ideally designed as a signal to provide information about symmetry. The number of bars is a discrete character, so bar number from side to side would provide a particularly unambiguous measure of symmetry. Further study is needed, however, to determine the exact nature of female preference in relation to the bars in this species. The preference we detected, whether for symmetrical males or males with the largest minimum number of bars, would select for symmetrical males when the total number of bars is equal. Female preference for males with the largest minimum number of bars would select against the addition of bars if not added symmetrically. In either case, X. cortezi females are assessing bars on both sides of a male, and bilateral assessment could have influenced the design of the vertical bars as a sexually selected signal as well as the serial lateral presentations of the courtship display that accentuates this signal.

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REFERENCES


