Female preference for trait symmetry in addition to trait size in swordtail fish

Molly R. Morris

Department of Biological Sciences, Ohio University, Athens, OH 45701, USA

Interest in fluctuating asymmetries, random deviations from perfect bilateral symmetry, has spread to studies of sexual selection because of the intriguing idea that females could use the degree of asymmetry of a male trait to assess the genetic quality of potential mates. The evidence that females prefer males with symmetrical sexual signals, however, remains controversial. A problem that applies to most previous studies is that preference for trait size can be misinterpreted as preference for symmetry, even when overall trait size is held constant, if females assess trait size by the largest minimum on one side. If overall trait size is equal between males, the asymmetrical males will have the maximum and minimum trait size, and so preference to mate with symmetrical males could actually reflect a preference to avoid males with the minimum trait on one side. *Xiphophorus cortezi* females preferred males with symmetrical bar numbers when the minimum number of bars was held constant. The strength of female preference for the symmetrical males was negatively correlated with the strength of preference the same females had for bar number. These results clearly demonstrate that females preferred trait symmetry in addition to trait size.

Keywords: female mate choice; fluctuating asymmetries; *Xiphophorus*; vertical bars; multiple mate preferences

1. INTRODUCTION

Fluctuating asymmetries (Van Valen 1962) of sexually selected male traits have attracted much interest owing to the growing number of studies suggesting that they are correlated with measures of genetic quality and that symmetrical males have greater mating success than asymmetrical males (for a review, see Watson & Thornhill 1994). Demonstrating that females assess and prefer symmetry is necessary but not sufficient for accepting the hypothesis that females use symmetry to choose and mate with genetically superior males. However, the evidence that females prefer males with symmetrical sexual signals has remained controversial, partly because studies have failed to separate preference for symmetry from preference for trait size assessed by the largest minimum trait on one side. Preference for trait size can be examined while symmetry is held constant, but manipulations of symmetry will be confounded by trait size as long as there is the possibility that females use the maximum or minimum size of a trait on one side to assess trait size. The ideal study would demonstrate that the strength of female preference for trait size could be diminished by increasing levels of trait asymmetry, suggesting that females prefer both trait size and trait symmetry.

Vertical bars are a polygenetic pigment pattern (Zimmerer & Kallman 1988) found in many *Xiphophorus* fish (see figure 1). The bars are known to function as a signal that attracts females and deters rival males in at least two species (Morris *et al.* 1995; Morris & Ryan 1996). Males darken their bars during courtship (Morris

et al. 1995) and use a 'figure-eight' display (Ryan & Causey 1989) in which a male swims back and forth in front of a female, providing the female with an opportunity to assess bilateral bar symmetry. In addition, the bars appear to be well-suited to provide information about symmetry. Number of bars is a relatively discontinuous character, and therefore bar number from side to side would provide a comparatively unambiguous signal about symmetry. The discontinuous nature of the bars also means that problems associated with the analysis of continuous characters (Palmer & Strobeck 1986) can be avoided.

In a previous study, *Xiphophorus cortezi* females preferred males with symmetrical bar numbers when overall bar number was held constant (Morris & Casey 1998). The current study examined the possibility that the preference for symmetrical males previously detected was actually due to a preference for the largest minimum number of bars on one side. Preference for symmetry was examined while holding the minimum number of bars constant, giving symmetrical males fewer bars overall than the asymmetrical males. Female responses were compared in three tests with the same pairs of males. In the first two tests, pairs of males differed in bar symmetry and the minimum number of bars was held constant. In the third test, males differed in bar number and bar symmetry was held constant. By examining the responses of the same females across all three tests, it was possible to determine if there was a relation between variation in the strength of female preference for bar number and variation in the strength of female preference for symmetry when confounded by bar number.

Figure 1. The left (a) and (b) right side of an *X. cortezi* male asymmetrical for bar number. This male was naturally asymmetrical by one bar (eight bars on the left and seven bars on the right). The proximal bar on the right side was removed by freeze-branding, which increased the male's bar asymmetry to two (eight bars on the left and six bars on the right).

2. MATERIAL AND METHODS

Male and female *X. cortezi* were collected from Arroyo La Conchita at Xilitla, San Luís Potosí, Mexico, and held in separate compartments of divided tanks throughout the experiment. In total, 26% of *X. cortezi* males from Arroyo La Conchita that had bars were naturally asymmetrical by two or more bars, and average $\pm$ s.d. bar symmetry was not significantly different than zero (Morris & Casey 1998). Bar number is correlated with male size among those males that have bars (Morris & Casey 1998). Bar width varies across males, but a correlation between bar number and area covered by melanophores is likely. In this sense, bar number is a measure of trait size.

Males of similar sizes (within 2 mm) were paired to control for the influence of body size on preference. Symmetry in bar number from side to side, as well as total bar number, were manipulated using freeze-branding (Raleigh *et al.* 1973), a technique previously used with *X. cortezi* and closely related species to successfully remove bars without altering male behaviour (Morris *et al.* 1995; Morris & Ryan 1996; Morris & Casey 1998). 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 than the other. Before the first test, the pairs of males were freeze-branded so that both males had the same minimum number of bars, but one male had either one, two, or three more bars on one side than the other side (asymmetrical number), and the other male had the same number of bars on both sides (symmetrical; see table 1). It was randomly determined which side of a male would have more bars for the asymmetrical treatment, and the most posterior bars were removed (see figure 1). Therefore, males with fewer bars had not

only a smaller proportion of their body covered by melanophores, but had the bars spaced out over a smaller proportion of their sides.

Before the second test, bars were again removed from each male, reversing which male was symmetrical within a pair while keeping the minimum number of bars on the two males equal (table 1). Differences other than bar number and symmetry between males, including their natural bar number and bar symmetry, were controlled for by re-testing females with the same pair of males. The same pairs of males and their associated females were used in a third test to determine if females preferred males with more bars. Males were branded a third time before test 3 so that one male had two more bars than the other male, but both males were either symmetrical ($n=7$) or asymmetrical by the same number of bars ($n=1$; table 1). The tests were completed in order (test 1, test 2 and then test 3) for eight of the nine pairs of males; the males in pair 6 were used in test 3 before test 2.

Simultaneous choice tests were done in an aquarium (45 cm $\times$ 41 cm $\times$ 80 cm) divided into five equal sections. The two end compartments were divided from the centre three by glass plates, which ensured that females only received visual cues. The middle three compartments were divided by lines marked on the aquarium. A test female was placed in an opaque tube in the centre section, and the test males were placed in the two end compartments. All three fish were allowed to acclimatize for 10 min. When the tube was raised, the time the female spent in the sections adjacent to each male was measured during a 20 min test period. The centre section was considered neutral, and gave females the opportunity to choose not to associate with either male. After the first trial, the female was returned to the opaque tube, the males were switched end to end, and all fish were allowed to acclimatize for 10 min. The procedure was then repeated in a second trial to control for any side bias. It was randomly determined which male would be on the right side for the first trial. Tests in which both males did not darken their bars in both trials were eliminated from the analysis.

The total time females spent in the section in front of each male was summed across both trials and compared with the males of a pair with an unpaired *t*-test. The null hypotheses tested were that females would spend the same amount of time with the males of a pair, regardless of which male was symmetrical or had more bars. In addition, I compared the time females spent on the right side of the tank in the first trial to the time spent on the right side of the tank during the second trial with a paired *t*-test. The second analysis of a subset of the data compares only the times females spent on the right side of the tank between trials, and can therefore analyse simultaneous choice data without the potential for violating assumptions of independence. The number of females that spent more time with the symmetrical males in test 1 and then switched between males when I switched which male was symmetrical, and spent more time with the symmetrical male in test 2 was compared to a null of 25% (50% in test 1 and 50% in test 2) with a binomial test. The mean difference in the total time females spent with one male over the other was used as an indicator of the strength of preference. The mean strength of preferences for test 1 and test 2 (preference for bar symmetry confounded by bar number) were calculated for each female and correlated against the same female's strength of preference in test 3 (bar number). The relative time females associate with males in simultaneous choice tests appears to be a good indicator of mate preference in these fish, as indicated by studies of the closely related species, *X. nigrensis* (Ryan *et al.* 1990; Morris *et al.* 1992).

Table 1. *Female preferences for bar symmetry and bar number*

(One female was tested with the each pair of males in all three tests. A male's bar number on both sides (left side-right side) and the time (s) the female associated with him in each test are shown.)

male pair	test 1				test 2				test 3			
	male 1		male 2		male 1		male 2		male 1		male 2	
	bar no.	time (s)	bar no.	time (s)	bar no.	time (s)	bar no.	time (s)	bar no.	time (s)	bar no.	time (s)
1	6/8	211	6/6	2019	4/4	1158	5/4	987	3/3	1138	4/4	1038
2	6/8	1570	6/6	499	4/4	640	4/6	1308	3/3	478	4/4	1579
3	10/7	1028	7/7	957	5/5	1397	5/7	619	4/4	827	5/5	1430
4	6/8	471	6/6	1453	4/4	1226	6/4	829	3/3	803	4/4	1309
5	8/6	822	6/6	1202	4/4	1177	6/4	780	3/3	651	4/4	1450
6	5/6	547	5/5	1577	3/3	1004	5/3	1170	3/4	676	5/4	1275
7	6/8	637	6/6	1053	4/4	1116	4/6	916	3/3	738	4/4	1487
8	7/9	589	7/7	1113	5/5	1160	7/5	926	5/5	782	6/6	1332
9	10/8	882	8/8	1175	6/6	1352	6/8	737	—	—	—	—
mean time (s.d)	750 (390)		1226 (425)		1136 (221)		919 (215)		761 (188)		1362 (165)	
	$t=2.87$ $p=0.02$				$t=2.12$ $p=0.05$				$t=6.78$ $p=0.0004$

Figure 2. Strength of preference for symmetry was calculated as the mean difference in time (s) spent with the symmetrical male over the asymmetrical male in test 1 and test 2 for each female. The asymmetrical males had 1–3 bars more than the symmetrical males. Other differences between the two males were controlled for by reversing which male was symmetrical between the two tests. Strength of preference for bar number was calculated as the difference in the time (s) spent with the male with more bars over the male with fewer bars in test 3. There was a significant negative correlation between preference for symmetry and preference for bar number ($R^2=0.71$, d.f. = 7, $F=14.7$, $p=0.009$). Also shown are 95% confidence intervals of the mean.

3. RESULTS

Females spent significantly more time with the symmetrical males as compared with the asymmetrical males (test 1 and test 2, table 1), and significantly more time with the males with more bars as compared with males with fewer bars (test 3, table 1). In addition, the times females spent on the right side of the test aquarium with the symmetrical males were significantly greater than the times spent on the right side with the asymmetrical males (test 1, paired $t=2.5$, $p=0.04$; test 2, paired $t=2.28$, $p=0.05$). Females also spent significantly more time on the right side of the test tank with males with more bars

than on the right side with males with fewer bars (test 3, paired $t=5.1$, $p=0.001$). Out of the nine females, six switched their preferences for one male over the other when I switched which male was symmetrical between the first and second tests, spending more time with the symmetrical male in both tests, which was statistically significant (binomial test, null = 0.25, $p=0.009$, table 1). Preference to associate with symmetrical over asymmetrical males when the minimum number of bars was held constant (symmetrical males had fewer bars overall) infers that females do have a preference for symmetry, not simply a preference for trait size assessed by the largest minimum on one side. The results also suggest that whereas females have a preference for bar number (test 3), preference for bar symmetry can override this preference within the ranges of these variables tested (test 1 and test 2).

A large degree of the variation in strength of female preference for the symmetrical males with fewer bars over the asymmetrical males with more bars can be explained by variation in the strength of female preference for bar number. Females with stronger preferences for bar number had weaker preferences for symmetrical males with fewer bars (figure 2), further suggesting that females preferred both symmetry and bar number.

4. DISCUSSION AND CONCLUSIONS

Studies designed to demonstrate that females assess and prefer symmetrical male traits during mate choice have been wrought with difficulties and criticisms. In some cases it has been difficult to separate preference for symmetry of a trait from other correlated characters; for example, tail symmetry in barn swallows, *Hirundo rustica* (Møller 1992), also affects flight performance (Møller 1991), and so females may actually assess males according to flight performance, not symmetry. 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, but his assumption that females perceived the white part of the tail feather as 'not there' has been questioned (Swaddle & Cuthill 1994b). In the most convincing study, Swaddle & Cuthill (1994b) demonstrated preference for symmetry of chest barring on finches.

The most recent criticism of studies of female preference for symmetry suggests that preference for size of the trait has been misinterpreted as preference for symmetry. Previous studies of preference for symmetry have attempted to detect preference for symmetry separately from preference for trait size by holding the overall trait size (the sum of both sides) constant (for example, see Møller 1992; Swaddle & Cuthill 1994b). These studies have assumed that the mechanism females use to assess trait size involves summing overall trait size. Females could assess and prefer greater size of a bilateral trait not only by summing both sides and preferring the male with the greatest size, but also by detecting and preferring the maximum trait on one side, or detecting and avoiding the minimum trait on one side. Therefore, in studies where overall trait size was held constant, female preference for larger trait size could have been mistaken for preference for symmetry if the mechanism females used to choose males with larger trait size was to avoid males with the minimum trait on one side. It is also possible that preference for symmetry has been missed in studies where trait size was held constant. For example, although female paradise whydahs (*Vidua paradisaea*) preferred males with asymmetrical tail feathers when overall tail length was held constant, such a result might be due to female preference for long tails (if they perceived the asymmetrical males as having longer tails) rather than a preference for asymmetry (Oakes & Barnard 1994).

Many studies now suggest that mate choice decisions are based on several preferences (for example, see Burley 1981; Baker *et al.* 1986; Brooks & Caithness 1995). Preference for symmetry and preference for trait size may be two of a number of preferences that interact to result in mate choice. If preference for trait symmetry is stronger than preference for trait size, preference for symmetry can be detected even when the minimum trait size on one side is held constant so that the symmetrical males have smaller traits overall (i.e. this study of *X. cortezi*). However, if preference for trait size is stronger, as may be the case in the paradise whydahs (Oakes & Barnard 1994), preference for symmetry could best be detected by demonstrating that the strength of preference for tail length is reduced when long-tailed males are relatively asymmetrical.

Preference by *X. cortezi* females for males with symmetrical bar numbers is consistent with 'good genes' models of sexual selection which predict that females choose mates based on traits that reflect male genetic quality (Møller & Pomiankowski 1993). However, a relation between bar symmetry and male genetic quality needs to be quantified before further consideration is given to the possibility that 'good genes' models can explain the evolution of this strong female preference. In *X. cortezi*, bar symmetry may provide information about the probability that an individual is a hybrid as vertical bars are more variable in the hybrids of several different

species of *Xiphophorus*, including offspring from *X. cortezi* and *X. montezumae* crosses (Atz 1962).

X. cortezi females clearly preferred males with more bars, although preference for symmetry was stronger than preference for bar number within the range of these variables tested. Females could use bar number to assess male size. It is also possible that an increase in bar number could simply increase male detectability, owing to an increase in pigmented area. It has been suggested that females should enforce reliability in a multiple signalling system by being more responsive to more reliable signals (Møller *et al.* 1998). If both bar number and bar symmetry provide information about male quality, the female-enforced reliability hypothesis would predict that bar symmetry is a more reliable indicator of male quality than bar number in *X. cortezi*.

These results demonstrate, I believe for the first time, that females assess and prefer symmetry of a trait in addition to trait size. Depending on how females assess trait size and the relative strength of the two preferences, incorrect conclusions about the presence, as well as the absence, of a preference for symmetry could have been made in previous studies. Future studies need to consider the possibility that preference for trait size may confound the detection of preference for trait symmetry and make the appropriate comparisons.

I thank the Mexican Government, Hector Espinosa of Universidad Nacional A Mexico and Angelica Narvaez of the US Embassy for assistance with the permit; Kevin de Queiroz for valuable suggestions on the manuscript, assistance in field collections, and the photographs in figure 1; Michael Webster for valuable comments on the manuscript; Willem Roosenberg for discussions of statistics; Andra Doherty, Neal Irving and Amanda Hoffman for assistance in data collection and fish care; and Shawn Lester and Sharon Ward for assistance in fish care. This work was supported by a grant from the Montgomery College Foundation.

REFERENCES

- Atz, J. W. 1962 Effects of hybridization on pigmentation in fishes of the genus *Xiphophorus*. *Zoologica* **47**, 153–181.
- Baker, M. C., Bjerke, T. K., Lampe, H. & Espmark, Y. 1986 Sexual response of female great tits to variation in the size of males' repertoires. *Am. Nat.* **128**, 491–498.
- Brooks, R. & Caithness, N. 1995 Female choice in a feral guppy population: are there multiple cues? *Anim. Behav.* **50**, 301–307.
- Burley, N. 1981 Mate choice by multiple criteria in a monogamous species. *Am. Nat.* **117**, 515–528.
- Møller, A. P. 1991 Sexual ornament size and the cost of fluctuating asymmetry. *Proc. R. Soc. Lond. B* **243**, 59–62.
- Møller, A. P. 1992 Female swallow preference for symmetrical male sexual ornaments. *Nature* **357**, 238–240.
- Møller, A. P. 1993 Female choice for apparently symmetrical sexual ornaments in the barn swallow *Hirundo rustica*. *Behav. Ecol. Sociobiol.* **32**, 371–376.
- Møller, A. P. & Pomiankowski, A. 1993 Fluctuating asymmetry and sexual selection. *Genetica* **89**, 276–279.
- Møller, A. P., Saino, N., Taramino, G., Galeotti, P. & Ferrario, S. 1998 Paternity and multiple signaling: effects of a secondary sexual character and song on paternity in the barn swallow. *Am. Nat.* **151**, 236–242.

- Morris, M. R. & Casey, K. 1998 Female swordtail fish prefer symmetrical signals. *Anim. Behav.* **55**, 33–39.
- Morris, M. R. & Ryan, M. J. 1996 Sexual difference in signal–receiver coevolution. *Anim. Behav.* **52**, 1017–1024.
- Morris, M. R., Batra, P. & Ryan, M. J. 1992 Male–male competition and access to females in *Xiphophorus nigrensis*. *Copeia* **1992**, 980–986.
- Morris, M. R., Mussel, M. & Ryan, M. J. 1995 Vertical bars on male *Xiphophorus multilineatus*: a signal that deters rival males and attracts females. *Behav. Ecol.* **4**, 274–279.
- Oakes, E. J. & Barnard, P. 1994 Fluctuating asymmetry and mate choice in paradise whydahs, *Vidua paradisaea*: an experimental manipulation. *Anim. Behav.* **48**, 937–943.
- Palmer, R. A. & Strobeck, C. 1986 Fluctuating asymmetry: measurement, analysis, patterns. *A. Rev. Ecol. Syst.* **17**, 391–421.
- Raleigh, R. F., McLearen, J. B. & Groff, D. G. 1973 Effects of topical location, branding techniques, and changes in hue on recognition of cold brands in Centrarchid and Salmonid fish. *Trans. Am. Fish. Soc.* **102**, 637–641.
- Ryan, M. J. & Causey, B. A. 1989 'Alternative' mating behavior in the swordtails *Xiphophorus nigrensis* and *Xiphophorus pygmaeus*. *Behav. Ecol. Sociobiol.* **24**, 341–348.
- Ryan, M. J., Hews, D. K. & Wagner, W. E. Jr 1990 Sexual selection on alleles that determine body size in the swordtail *Xiphophorus nigrensis*. *Behav. Ecol. Sociobiol.* **26**, 231–237.
- Swaddle, J. P. & Cuthill, I. C. 1994a Female zebra finches prefer symmetric males. *Nature* **367**, 165–166.
- Swaddle, J. P. & Cuthill, I. C. 1994b Female zebra finches prefer males with symmetric chest plumage. *Proc. R. Soc. Lond. B* **258**, 267–271.
- Van Valen, L. 1962 A study of fluctuating asymmetry. *Evolution* **16**, 125–142.
- Watson, P. J. & Thornhill, R. 1994 Fluctuating asymmetry and sexual selection. *Trends. Ecol. Evol.* **9**, 21–25.
- Zimmerer, E. J. & Kallman, K. D. 1988 The inheritance of vertical barring (aggression and appeasement signals) in pygmy swordtail *Xiphophorus nigrensis* (Poeciliidae, Teleostei). *Copeia* **1988**, 299–307.