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Larger swordtail females prefer asymmetrical males

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Many organisms, including humans, find symmetry more attractive than asymmetry. Is this bias towards symmetry simply a by-product of their detection system? We examined female preference for symmetry of the pigment pattern vertical bars in the swordtail fishes *Xiphophorus cortezi* and *Xiphophorus malinche*. We found a relationship between preference for symmetry and female size, with larger and thus older females spending significantly more time with the asymmetrical video animation as compared to the symmetrical video animation. The preference for asymmetry we report demonstrates that even if females can detect symmetrical males better, this does not preclude subsequent selection on females to prefer symmetrical or asymmetrical males. In addition, because the preference was correlated with female size, past studies may have missed preference for either asymmetry and/or symmetry by not examining the relationship between female preference and size/age or by measuring a limited size/age distribution of females. In both of the species of swordtail fishes examined, a high proportion of males are asymmetrical by more than one bar. We suggest that female preference may be maintaining fluctuating asymmetries in these fishes.

Keywords: fluctuating asymmetry; *Xiphophorus*; variation in female preference; vertical bars

1. INTRODUCTION

The bias that many different organisms have for symmetry (Osorio 1996; Møller & Thornhill 1998) has been explained by two different hypotheses. First, the 'good genes' models for the evolution of female preference for symmetry suggest that preference for symmetry evolved because females gain indirect advantages by mating with symmetrical males (Watson & Thornhill 1994). Fluctuating asymmetries (FA) are random deviations from perfect symmetry in bilateral traits. The detection of negative associations between FA and various measures of fitness, such as viability, fecundity or growth rate (reviewed by Møller 1997) has lent support to the 'good genes' hypothesis. In contrast, studies of pattern identification using neural networks suggest that a bias towards symmetry may be a by-product of detection systems alone. Specifically, these models suggest that preferences for symmetry may have evolved to help detect patterns irrespective of orientation (Enquist & Arak 1994), through a preference for average traits which are often the most symmetrical traits (Johnstone 1994), or due to other visual functions like edge and line detection (Osorio 1996). Discriminating between these two hypotheses has been difficult, as both are supported by female preference for symmetrical traits. However, models that suggest this preference is a by-product of detection systems would not be supported by a preference for asymmetry. A preference for asymmetry would suggest that a biased ability to detect symmetry does not preclude subsequent selection on females to either prefer the higher quality symmetrical males, or to prefer lower quality asymmetrical males due to some cost to mating with symmetrical males that outweighs the benefits of mating with high quality males.

The vertical bar pattern found on many species of swordtail fishes (*Xiphophorus*) is a secondary sexual trait known to attract females and deter rival males (Morris *et al.* 1995). Preference for bar number symmetry has been detected in the swordtail *Xiphophorus cortezi* (Morris & Casey 1998; Morris 1998; Merry & Morris 2001). The males of *X. cortezi* and several other northern swordtail species darken their bars during courtship and use a 'figure-eight' display in which they swim back and forth in front of a female, which would provide females with an opportunity to assess bilateral bar symmetry. Rauchenberger *et al.* (1990) suggested that the barring pattern on *Xiphophorus malinche* was more asymmetrical than in closely related species. Therefore, we were interested in determining if *X. malinche* females might prefer asymmetrical males. We compared female preferences for bar number symmetry in *X. malinche* and *X. cortezi* using the same video animations. We also determined if *X. malinche* males were more asymmetrical in bar number than *X. cortezi* males.

2. MATERIAL AND METHODS

Xiphophorus malinche males and females were collected from the Río Claro in the state of Hidalgo, Mexico, and *X. cortezi* males and females from the arroyo La Conchita near the town of Xilitla in the state of San Luis Potosí, Mexico. Females were measured and isolated into individual tanks at least one week prior to being tested. A subset of these females were re-measured 4–14 months after being tested to determine growth rates.

The animations of swordtail males used in this study were created using LIGHTWAVE 3D v. 5.6 (Newtek). Details of how these were created can be found elsewhere (Morris *et al.* 2003). Animations were based on a male *X. cortezi*, which is closely related to *X. malinche* and has the same body shape (J. A. Moretz & M. R. Morris, unpublished data). The two animations used to make the stimulus videos differed only in the skin applied to the wire frame: 'symmetrical' treatment had seven bars on both sides and the 'asymmetrical' treatment animation had six bars on one side and eight bars on the other. The skin had no other distinguishing pigment patterns, a state found in both species. The male in the animation swims back and forth, providing the female with views of both sides.

Preference tests were conducted in a 37.9 l tank visually divided into three equal compartments. High definition video monitors were placed at either end of the tank, each connected to a VCR. Both sides of the test aquarium were covered with cardboard and a mirror was placed at an angle on top of the tank so that the movements of the female could be observed. The female was given a 10 min acclimation period in a clear Plexiglas square tube in the centre of the choice tank before each trial. Each tape consisted of a 3 min lead-in which the females were exposed to the background colour and lighting, but no fish animation. The female was released after the animated fish had appeared on the monitors for 1 min. The time the female spent in the third of the aquarium closest to an animation was recorded for 8 min and 40 s. The entire procedure was then repeated, switching the sides on which stimulus videos were shown. Difference in the total time spent with each animation was used as a measure of strength of preference. Each female was tested twice to provide an estimate of consistency. We

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examined the possibility that time between tests (range 1–13 days) might influence the strength of preference that females exhibited, and found no relationship (linear regression: $R=0.026$, $N=20$, $F_{1,18}=0.012$, $p=0.91$, *X. malinche*; $R=0.16$, $N=19$, $F_{1,17}=0.45$, $p=0.51$, *X. cortezi*). Therefore, this variation was not considered in any of the further analyses. While female preferences for video animations of males compared to live males have not been assessed in *X. malinche*, females of *X. cortezi* have been shown to respond similarly to video animations and live males (Morris *et al.* 2003). The time that females spend associating with males has been shown to be a good measure of female mating preference in the closely related swordtails *Xiphophorus nigrensis* (Ryan *et al.* 1990; Morris *et al.* 1992) and *Xiphophorus multilineatus* (documented as *X. nigrensis* in Zimmerer & Kallman 1989), as well as in another live-bearing fish (Bisazza *et al.* 2001).

Males were measured for standard length (SL) and were scored for bar number symmetry, or the absolute difference in total number of bars on the left as compared to the right side (table 1). We compared the number of males that were symmetrical, asymmetrical by one bar and asymmetrical by two bars within and across the two species with a chi-square test. We also tested for a relation between male size (standard length) and bar number asymmetry using logistic regression.

3. RESULTS

We found no significant differences in the average time females spent associating with the symmetrical video animation as compared to asymmetrical video animation (*X. malinche* symmetry mean = 906.75 ± 442.88 s, asymmetry mean = 951.3 ± 435.18 s, paired *t*-test: $t_{19}=-0.232$, $p=0.819$; *X. cortezi* symmetry mean = 754.21 ± 492.95 s, asymmetry mean = 1185.95 ± 484.65, paired *t*-test: $t_{18}=-1.943$, $p=0.068$), although *X. cortezi* females seemed to have a tendency to prefer the asymmetric video animation. Female size, however, was significantly related to average strength of preference (difference in time spent with each animation) in both species (figure 1), with the time spent with the asymmetric image increasing with female size. To examine the possibility of having two groups of females with opposite preferences based on size, we used the regression equation from this relationship to divide the females into two size groups. The estimated 'switch size' was 37.6 mm in *X. malinche* and 38.1 mm in *X. cortezi* (figure 1). Females larger than this size had a significant preference for the asymmetrical video animation, while females smaller than this size tended to prefer the symmetric video animation in both species (figure 2). The small number of females below the switch size in the current study may have contributed to our inability to detect a significant preference for symmetry. In addition, we detected a relationship between female size and consistency in preference (absolute difference in strength of preference between test 1 and test 2; log-likelihood ratio test: $\chi^2_1=8.803$, $N=20$, $p<0.01$, for *X. malinche*; $\chi^2_1=3.412$, $N=19$, $p=0.065$, for *X. cortezi*), with larger females being more likely to be consistent in their preference. Lower consistency of smaller females would make it more difficult to detect a significant preference in females below the switch size. Females of both species continued to grow in the laboratory (*X. malinche*; mean growth 0.04 mm d^{-1}, $n=6$; *X. cortezi* mean growth 0.02 mm d^{-1}, $n=11$), suggesting that larger females are on average older females in these two species.

Table 1. The percentage, number and standard length of males with symmetrical and asymmetrical bar numbers in *Xiphophorus malinche* and *X. cortezi*. (All bars, whether broken or shorter were counted to determine the symmetry score. Males were from the American Museum of Natural History collections (AMNH) and from our own collections from the same two sites where females were collected for the preference tests. Collections for *X. malinche* males: $N=23$ AMNH #88338; $N=20$ collected in 2002 and 2003. Collections for *X. cortezi* males: $N=15$ AMNH #45333; $N=12$ collected in 2000; $N=6$ collected in 2004.)

	X. malinche		*X. cortezi*	
	per cent (*n*)	mean size ± s.d. (mm)	per cent (*n*)	mean size ± s.d. (mm)
symmetrical (0)	23.3 (10)	44.6 ± 3.29	42.4 (14)	40.4 ± 7.35
asymmetrical (±1)	46.5 (20)	41.8 ± 5.00	39.4 (13)	40.4 ± 7.25
asymmetrical (± > 1)	30.2 (13)	43.2 ± 4.77	18.2 (6)	35.2 ± 4.75

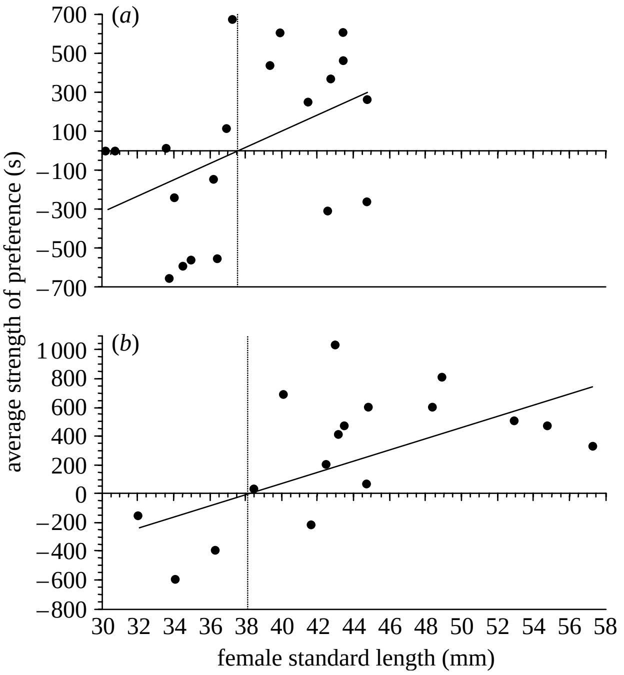

Figure 1. The relationship between female size and average strength of preference for (*a*) *X. malinche* and (*b*) *X. cortezi*. Negative scores indicate more time with symmetrical bar pattern and positive scores indicate more time with asymmetrical bar pattern. Linear regression: $R=0.444$, $N=20$, $F_{1,18}=4.424$, $p<0.05$, *X. malinche*; $R=0.531$, $N=19$, $F_{1,17}=6.674$, $p<0.05$, *X. cortezi*. The 'switch size' (size where the predicted average strength of preference is zero, denoted by a dotted line) is based on the linear regression equations.

There was no significant difference in the number of males that were symmetrical as compared to asymmetrical between the two species (table 1, chi-square test, $\chi^2_2=3.48$, $N=76$, $p=0.18$). In addition, there was no relationship between male size and the asymmetry scores for either species (log-likelihood ratio test: $\chi^2_2=2.61$, $N=43$, $p=0.27$, for *X. malinche*; $\chi^2_2=2.76$, $N=33$, $p=0.25$, for *X. cortezi*). However,

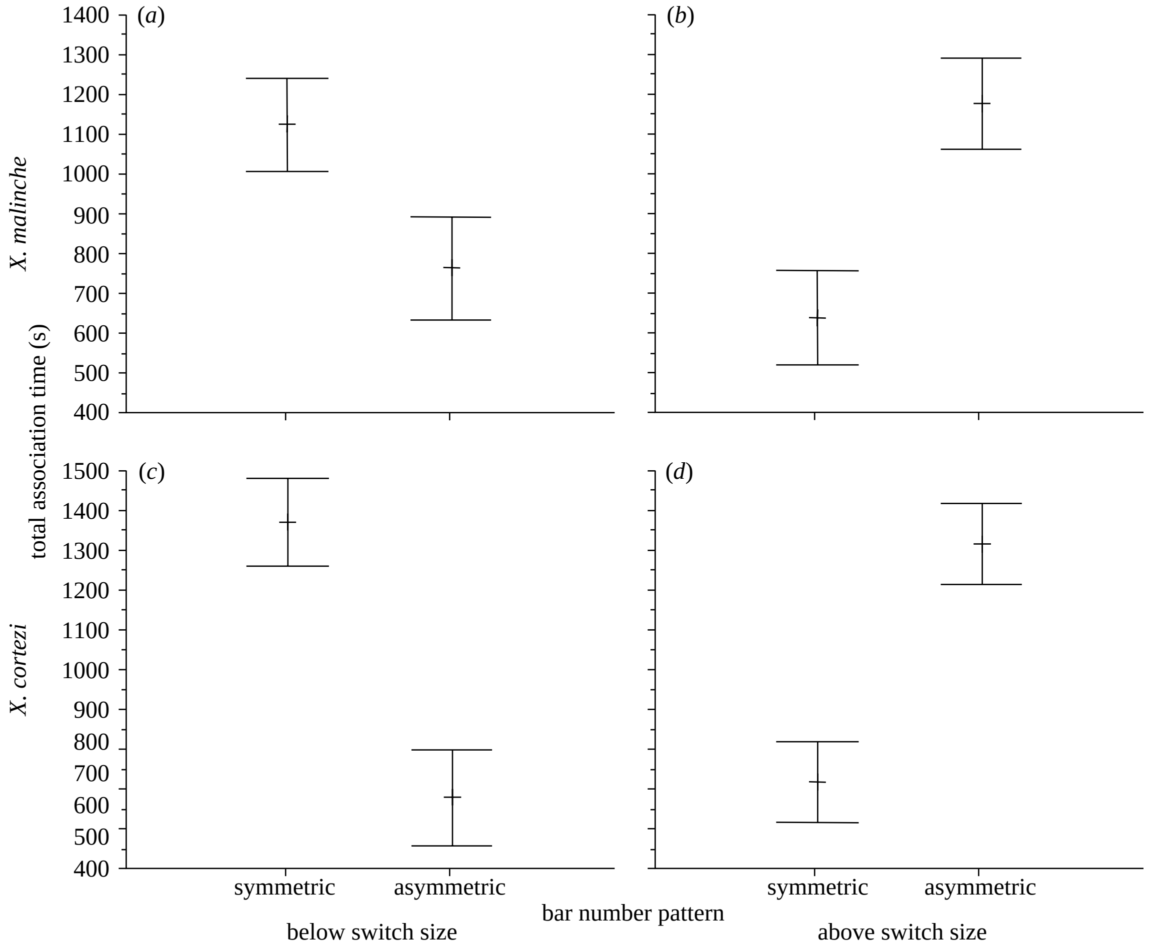

Figure 2. Means and standard errors of time spent with symmetric and asymmetric animations for females above and below the 'switch size'. *Xiphophorus malinche* females below the 'switch size' (*a*) tended to prefer the symmetric animation (paired *t*-test: $t_{10}=1.486$, $p=0.168$), while females above the 'switch size' (*b*) had a significant preference for the asymmetric animation (paired *t*-test: $t_8=-2.368$, $p<0.05$). Similarly, *X. cortezi* females below the switch size (*c*) tended to prefer the symmetric animation (paired *t*-test: $t_2=3.009$, $p=0.09$), while females above the 'switch size' (*d*) had a significant preference for the asymmetric animation (paired *t*-test: $t_{15}=-3.004$, $p<0.01$).

X. malinche did have a higher proportion of asymmetrical males than symmetrical males (chi-square test, $\chi_1^2=12.3$, $N=43$, $p=0.0001$), which was not true for *X. cortezi* (chi-square test, $\chi_1^2=0.76$, $N=33$, $p=0.384$).

4. DISCUSSION

The preference for asymmetry demonstrated here suggests that a biased ability to detect symmetry does not preclude subsequent selection on females to prefer either symmetrical or asymmetrical males. We know of only one other study that has presented evidence of preference for asymmetry (Oakes & Barnard 1994), but those results were interpreted as preference for trait size. We found no relationship between male size (SL), a known target of female mating preferences in poeciliids (i.e. Ryan *et al.* 1990; Ptacek & Travis 1997), and the degree of bar asymmetry in either species. It is hard to know how common a size-related preference for asymmetry may be, as variation in female preference for symmetry has not, to our knowledge, been previously examined (although see Mazzi *et al.* 2004). Past studies may have missed preferences for symmetry or asymmetry by assessing only average female preferences or a limited size range of females. For example, the previous studies of preference for symmetry in *X. cortezi* females (Morris & Casey 1998; Morris 1998; Morris *et al.* 2003) missed a preference for asymmetry as the average sizes of the females tested were smaller than the average size of the females tested in the current study.

Female swordtails have been noted to continue to grow after sexual maturity, although at a slower rate (see Kallman 1989; Marcus & McCune 1999). We detected growth after sexual maturity by females in both species used in this study. All females used in this study were wild caught, and therefore differences in preferences could reflect differences in experience. A decline in female preference based on age has been detected in crickets (Gray 1999). Experience of interacting with males (Breden *et al.* 1995; Marler *et al.* 2001) as well as age alone (Kodric-Brown & Nicoletto 2001) are known to affect the strength of female preferences in poeciliid fishes. In bowerbirds, females have been shown to shift their preference from one trait to another with age (Coleman *et al.* 2004). The results from our study suggest yet another way in which female preferences can vary, as females had different preferences for the same trait depending on size. These results also suggest that the high degree of fluctuating asymmetry in bar number in these two species could be a result of the preference for asymmetry we detected in larger females.

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