

# Population-level mating patterns and fluctuating asymmetry in swordtail hybrids

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**Abstract** Morphological symmetry is a correlate of fitness-related traits or even a direct target of mate choice in a variety of taxa. In these taxa, when females discriminate among potential mates, increased selection on males should reduce fluctuating asymmetry (FA). Hybrid populations of the swordtails *Xiphophorus birchmanni* and *Xiphophorus malinche* vary from panmictic (unstructured) to highly structured, in which reproductive isolation is maintained among hybrids and parental species. We predicted that FA in flanking vertical bars used in sexual signalling should be lower in structured populations, where non-random mating patterns are observed. FA in vertical bars was markedly lower in structured populations than in parental and unstructured hybrid populations. There was no difference in FA between parentals and hybrids, suggesting that hybridisation does not directly affect FA. Rather, variation in FA likely results from contrasting mating patterns in unstructured and structured populations.

**Keywords** *Xiphophorus* · Hybridisation · Population structure · Mating patterns

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## Introduction

Fluctuating asymmetry (FA) is often considered an indicator of an individual's ability to buffer against environmental stress (Parsons 1992) or developmental 'noise' (Van Valen 1962). Negative associations between asymmetry of morphological traits and fitness metrics such as fighting ability, growth and survival have been reported in a broad range of taxa (Polak 2008). Studies of FA have shown that females often prefer to mate with symmetric males or that FA is negatively correlated with male reproductive success (reviewed in Møller 1997; Møller and Thornhill 1998), demonstrating that symmetry is sometimes the direct target of selection or can be favoured due to correlation with traits under selection. While relationships between symmetry and fitness are by no means ubiquitous (Clarke 1998; Palmer 2000), in those systems where symmetry itself or traits exhibiting variation in symmetry are under selection, strong female choice could generate selection for reduced FA.

Within the genus *Xiphophorus*, males of some species are polymorphic for the expression of dark, pigmented vertical bars on each side of the body, while in other species, males do not express bars (Rauchenberger et al. 1990). These bars are intensified during courtship and male–male aggression, and the presence and number of vertical bars is subject to both intra (Zimmerer and Kallman 1988; Moretz 2005) and intersexual selection (Morris et al. 1995). In *Xiphophorus birchmanni*, females prefer males with vertical bars over males without (Fisher et al. 2009). Female swordtails generally prefer males with symmetrical vertical bars (Morris 1998; Morris and Casey 1998). However, the largest *Xiphophorus cortezi* and *Xiphophorus malinche* females may prefer males with some degree of asymmetry, potentially maintaining FA in natural populations of *Xiphophorus* (Morris et al. 2006).

In tributaries of the Rio Pánuco basin of Mexico, *X. birchmanni* and *X. malinche* co-occur and produce fertile, viable hybrids. *X. malinche* are restricted to highland sites, *X.*

*birchmanni* to lowlands, and hybrid or mixed hybrid/parental populations are found at intermediate elevations (Rosenthal et al. 2003; Culumber et al. 2011). Hybrid sites vary considerably in population-genetic structure, ranging from random-mating, unstructured hybrid swarms, to populations with distinct subpopulations of hybrids and both parental species (Culumber et al. 2011). This structure is unlikely to be due to immigration of parentals, and selection against hybrids is weak or absent. This suggests that non-random mating could be maintaining population structure by promoting reproductive isolation among subpopulations (Culumber et al. 2011; Culumber et al. 2012; Culumber et al. submitted).

Here, we evaluated the relationship between population-genetic structure and vertical bar FA. Given the importance of vertical bars in sexual signalling, we should expect non-random mating to result in lower FA, either via inter or intrasexual selection acting directly on symmetry (Morris 1998) or indirectly via sexual selection on traits correlated with FA (Møller 1997). We first investigated whether hybridisation affects FA by comparing the two parental species and hybrids. Having ruled out an effect of hybridisation on FA, we tested two hypotheses regarding the influence of non-random mating on vertical bars. If non-random mating generates stronger selection in structured populations, then (1) we should observe a greater frequency of males with vertical bars, and (2) FA should be lower in males bearing the trait.

## Materials and methods

### Collecting, trait measurements and genotyping

Adults were collected from 2 *X. malinche*, 2 *X. birchmanni* and 12 hybrid localities (Supplementary Fig. 1). Asymmetry was calculated as the difference in bars between left and right sides of the body divided by the total number of bars. Individuals from hybrid populations were genotyped for four single nucleotide polymorphism markers (see Supplementary Materials and Methods). Population structure was classified based on data and analyses in Culumber et al. (2011) using standard population genetic procedures. Those genetic analyses were used to classify populations as parental, random mating hybrid populations (unstructured) or non-random mating hybrid populations (structured). Trait measurement, genotyping and population classification are detailed in the Supplementary Materials and Methods.

### Statistical analysis

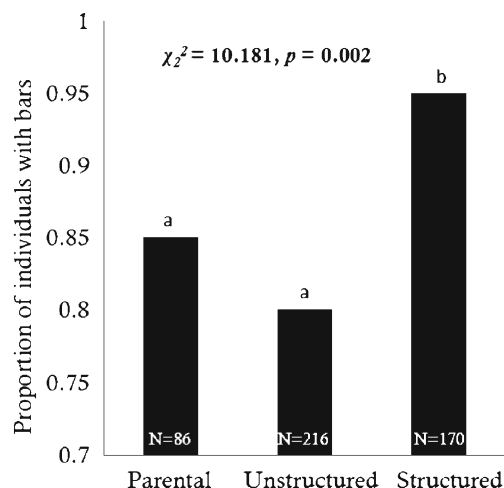
Data were checked for normality, directional asymmetry and anti-symmetry prior to analyses of FA (Supplementary Materials and Methods). We performed separate non-parametric

Kruskal–Wallis analysis of variance (ANOVA) to test for an effect of population type (parental, unstructured and structured) and species (*X. birchmanni*, *X. malinche* and hybrids) on FA. Post hoc Mann–Whitney *U* tests were used to make pairwise comparisons, when a significant relationship was observed in Kruskal–Wallis ANOVAs. Barless individuals were excluded from asymmetry analyses. We constructed contingency tables and used Pearson's  $\chi^2$  to evaluate differences in bar prevalence (proportion of males with bars) among population types and species. Where significant differences were observed, we used Pearson's  $\chi^2$  to evaluate pairwise differences. ANOVA was used to test for an effect of population type on female size to determine whether female size could explain variation in FA with post hoc comparison by Fisher's LSD. When multiple comparisons were made, *p* values were Bonferroni corrected. All analyses were conducted in SPSS 17.0 (SPSS Inc.)

## Results

### Bar prevalence

There was a significant difference in the prevalence of males with bars among population types ( $\chi^2=18.974$ ,  $p<0.001$ ; Fig. 1). Bar prevalence was significantly higher in structured compared to unstructured ( $\chi^2=19.171$ , d.f.=1,  $p<0.01$ ) and parental populations ( $\chi^2=8.219$ , d.f.=1,  $p=0.021$ ). Unstructured and parental populations did not differ in bar prevalence ( $t=0.935$ , d.f.=1,  $p>0.99$ ). There was no difference in the proportion of barred males among species ( $\chi^2=3.645$ , d.f.=2,  $p=0.132$ ; Proportions: *X. birchmanni*, 0.92 barred,  $n=96$ ; *X. malinche*: 0.84 barred,  $n=109$ ; hybrids: 0.84 barred,  $n=234$ ).



**Fig. 1** Proportion of males with bars in each population type. Bars with different letters indicate significant differences ( $p<0.05$ )

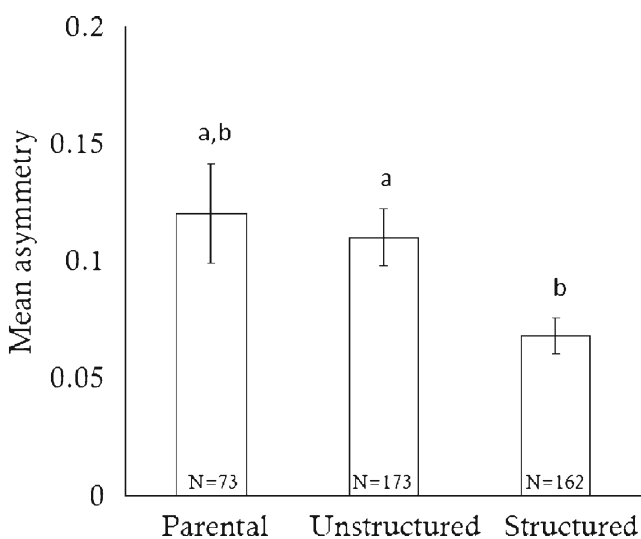
## Fluctuating asymmetry

There was a significant effect of population type on FA ( $H=11.254$ , d.f.=2,  $p=0.004$ ; Fig. 2). Structured populations had significantly lower FA than unstructured populations ( $U_1=11,247$ ,  $p=0.003$ ). The difference between structured and parental populations was significant before but marginally non-significant following correction for multiple tests ( $U_1=4,853$ ,  $p=0.071$ ). Unstructured and parental populations did not differ in FA ( $U_1=6262$ ,  $p>0.99$ ). There was no difference in FA among *X. birchmanni*, *X. malinche* and hybrids ( $H=0.069$ , d.f.=2,  $p=0.966$ ).

Female standard length varied by population type ( $F_{2, 294}=25.962$ ,  $p<0.001$ ). Females from structured populations were significantly larger ( $n=79$ ,  $47.5\pm 0.78$  mm, mean $\pm$ SE) than those from both parental ( $n=74$ ,  $41.8\pm 0.68$  mm) and unstructured ( $n=144$ ,  $42.0\pm 0.47$  mm) hybrid populations ( $p<0.01$  for both). Females from parental and unstructured populations did not differ in standard length ( $p>0.99$ ).

## Discussion

Fluctuating asymmetry in vertical bars covaried with population structure: males from structured populations, where mating is non-random, were more symmetrical than males from unstructured and parental populations. Further, males from these structured populations were more likely to have vertical bars than males from other populations.



**Fig. 2** Mean bar asymmetry ( $\pm$  SE) among males of different population types. Bars with different letters indicate significant differences in post hoc comparisons ( $p<0.05$ ). The difference between structured and parental was marginally nonsignificant after correction for multiple comparisons

*X. birchmanni* (Fisher et al. 2009) and other swordtails (Morris et al. 1995; Morris 1998; Morris and Casey 1998) prefer vertical bars, and preferences for symmetry are widespread in swordtails (Morris 1998; Morris and Casey 1998). Reduced FA in structured populations could result if females have stronger preferences for bars and symmetry or as an indirect consequence of generally increased selection on other traits due to non-random mating. In other populations, where mating is random, selection is relaxed, and FA is elevated. Similar patterns of increased FA resulting from relaxed indirect selection on correlated traits have also been observed in Pecos pupfish (*Cyprinodon pecosensis*, Kodric-Brown 1997) and sticklebacks (*Pungitius pungitius*, Trokovic et al. 2012).

Morris et al. (2006) showed that female preference for bar symmetry in *X. cortezi* and *X. malinche* varied with body size, with the largest females preferring asymmetrical bars. Greater FA in parental and unstructured populations could therefore be a consequence of differences in female size distributions among population types. However, in our study, female size was greatest in structured populations, where FA was the lowest. Size-associated preference variation, thus, did not drive population-level patterns of FA. While we cannot rule out tradeoffs between growth rate and developmental stability, fish in structured populations were the most symmetrical and also the largest, which is opposite in direction to that expected if such tradeoffs existed (Morris et al. 2012).

Males' vertical bar symmetry was unaffected by hybridisation per se. Studies in other systems have found that hybrids have either higher FA, (Gamier et al. 2006; Albarrán-Lara et al. 2010), lower FA (Alibert et al. 1994) or neither (Lu and Bernatchez 1999; Pelabon et al. 2004). Our findings suggest that hybrids have similar FA to that of parentals, and that hybridisation in this system either does not measurably affect developmental stability, or that any effects are countered by selection favouring hybrid male traits (Fisher et al. 2009).

Since parental and unstructured populations have generally higher FA than is observed in structured populations, we can rule out differences in historical frequencies of parentals or immigration from parental populations as alternative explanations for lower FA. If structured populations are epicentres of hybridisation, low FA could be an historical artefact of stronger sexual selection via heightened mate discrimination and assortative mating among sympatric parentals before the breakdown of reproductive isolation. This alternative, nonetheless, would point to mating patterns as maintaining variation in FA among parental populations and lower FA in present day structured populations. Regardless of whether selection acts directly or indirectly on vertical bar symmetry, this study demonstrates that population-level mechanisms such as variation in mating patterns can have important implications for the maintenance of fluctuating asymmetry and other fitness-related traits.

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