



Female preference variation has implications for the maintenance of an alternative mating strategy in a swordtail fish

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Female preference variation over space and time could be an alternative to frequency-dependent selection as a mechanism maintaining alternative male reproductive strategies. In the swordtail fish, *Xiphophorus nigrensis*, males have alternative strategies where large courting males (courters) and smaller males (sneakers) have equal fitnesses due to a mating advantage for the courters and a higher probability of reaching sexual maturity for the sneakers. Variation in one of these advantages over space or time may be the mechanism that maintains these two strategies. We examined female preference variation for the courting strategy in *Xiphophorus multilineatus*, a species with the same strategies. Females had an overall significant preference for courters. The strength of this preference was positively related to female size, with smaller females having a weak preference for courters. If the relationship between female size and strength of preference is consistent over space and time, more smaller females would result in a weaker preference for courters, which would increase the relative mating advantage of sneakers. We assessed female size distribution and the frequency of each strategy across space and time and detected significant differences in mean female size across subpopulations and across time, as well as a relationship between mean female size and the relative frequencies of the two male strategies: courters were significantly more common in those samples with the largest females. These results suggest that variation across subpopulations of *X. multilineatus* in female preferences over space and/or time could shift the balance in fitness between the two strategies.

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Species in which individuals of one sex show very different behaviours when competing for mates are fairly common (Taborsky 1994; Gross 1996; Henson & Warner 1997; Brockmann 2001). Such variation has long intrigued evolutionary biologists because it is often not clear what mechanisms allow for the coexistence of multiple reproductive behaviours within the same population. Alternative reproductive phenotypes may be categorized as alternative strategies, mixed strategies or conditional

strategies depending on the degree of genetic variation underlying the phenotypic variation (Gross 1996). Alternative strategies (sensu Gross 1996) are characterized by a genetic polymorphism. Because the genotypes of any alternative strategy with a relative lower fitness would be eventually eliminated from the population, theory suggests that, to be evolutionarily stable, these types of strategies must have equal average fitnesses that will be maintained at equilibrium through negative frequency-dependent selection (Maynard Smith 1982).

There are only a few examples of alternative reproductive strategies where a genetic polymorphism has been demonstrated: the swordtail *Xiphophorus nigrensis* (Zimmerer & Kallman 1989); the marine isopod *Paracerceis sculpta* (Shuster & Wade 1991); the tree lizard *Urosaurus ornatus* (Thompson et al. 1993); the ruff *Philomachus pugnax* (Lank et al. 1995); and the side-blotched lizard *Uta*

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stansburiana (Sinervo & Lively 1996). However, equal average fitnesses have only been suggested for the swordtail, the isopod and the side-blotched lizard (Shuster & Wade 1991; Ryan et al. 1992), and negative frequency-dependent selection has rarely been tested for distinct phenotypes within a sex (Gigord et al. 2001; Sinervo & Zamudio 2001). Furthermore, some researchers have questioned the use of evolutionarily stable strategy (ESS) theory to explain the evolution of alternative reproductive strategies (Austad 1984; Dominey 1984) and some have proposed that other factors affect the maintenance of alternative strategies in a population (Gross 1996; Henson & Warner 1997; Alonzo & Warner 2000; Calsbeek et al. 2002).

Mechanisms other than negative frequency-dependent selection that could maintain alternative reproductive strategies in a population include environmental heterogeneity over space or time, as well as any other mechanism that in general maintains genetic variability (e.g. heterozygote superiority; Austad 1984; Dominey 1984; Calsbeek et al. 2002). Recent studies suggest that female preferences can vary in relation to a multitude of factors (i.e. Marler et al. 1997; Gray 1999; Kodric-Brown & Nicoletto 2001; Calsbeek et al. 2002; Coleman et al. 2004). However, few studies have examined the possibility that variation in female preference could be an alternative to frequency-dependent selection for maintaining alternative reproductive strategies (e.g. Hugie & Lank 1997). Thus, it is important to determine whether there is variation in female preference across space or time that would influence the frequency of male strategies.

A clear example of alternative mating strategies can be found in swordtail fish (*Xiphophorus*). In several *Xiphophorus* species, male size and age at maturation are determined by a series of sex-linked (*P*) alleles: little growth occurs after maturation, resulting in genetically determined male size classes (Kallman 1989). In *X. nigrensis* and *X. multilineatus*, mating behaviours are tightly correlated with male size; males from the smallest size class in both species use a sneak-chase behaviour (sneakers), and males from the larger size classes use courtship (courtiers), and although sneakers court females in the absence of courtiers, courtiers never use sneaking behaviour (Ryan & Causey 1989; Zimmerer & Kallman 1989). Zimmerer & Kallman (1989) demonstrated that *X. multilineatus* males from the largest size class produce more offspring per brood than the smallest males in competitive laboratory trials with one courting male, one sneaker and two females. Because courting males spent more time in association with females, Zimmerer & Kallman (1989) suggested that both male–male competition and female preference for large males provide courting males with a mating advantage over sneakers. Ryan et al. (1990) also found a mating advantage for larger males in *X. nigrensis*. Given field estimates of relative reproductive success (Ryan et al. 1990) and age to sexual maturity (Morris & Ryan 1990), Ryan et al. (1992) used a model to predict the mortality rates necessary to balance the fitness of the two reproductive strategies. Their estimates of mortality rates suggested that the fitnesses of the two strategies are equal because of a balance between sexual selection and

the probability of reaching sexual maturity. In other words, even though large courting males have higher mating success, smaller males can have equal fitness because they reach sexual maturity sooner. The mechanism that might be maintaining this evolutionarily stable state (ESS), however, has not been identified.

In the current study, we examine the potential for variation in female preference to play a role in the maintenance of alternative mating strategies in *X. multilineatus*. If the relatively higher reproductive success of courter over sneaker males is the result of females preferring these larger males, then variation in female preference for courter males could produce spatial or temporal variation in sexual selection, which may ultimately influence the maintenance of these alternative mating strategies. Note that the relative mating success of small sneakers does not have to be greater than that of large courtiers to tip the fitness balance in favour of sneakers, because sneakers have the advantage of reaching sexual maturity sooner. The specific goals for the current study were the following. First, we determined whether *X. multilineatus* females have a preference for courtiers over sneaker males, which would suggest that the mating advantage of courtiers detected by Zimmerer & Kallman (1989) was indeed partly due to female preference. Second, we determined whether there was variation in female preference for the courtiers as compared to sneakers and whether that variation was correlated with female size. Third, we determined whether there was any indication of spatial and/or temporal variation in female preference for male mating strategy by examining variation in female size across space and time. Finally, we determined whether the variation in female size (either between sites, or within sites over time) corresponded to the variation in the frequencies of the different male strategies, which would support the hypothesis that the variation in female preference influences the relative frequency of the alternative mating strategies.

METHODS

Study Organism

The swordtail *X. multilineatus* is found in the Río Coy and some of its tributaries (Río Pánuco basin), in the state of San Luis Potosí, Mexico (Rauchenberger et al. 1990). Males of this species differ in size by more than a factor of two and show size-related differences in mating behaviour (Zimmerer & Kallman 1989) similar to that described for the sibling species *X. nigrensis* (Ryan & Causey 1989). Four size classes of *X. multilineatus* have been identified, and these differ in their Y-linked *P* alleles (from smallest to largest: *s*, *I*, *II*, *L*) and colour genes (see Table 1 in Zimmerer & Kallman 1989; *X. multilineatus* was considered a subpopulation of *X. nigrensis* until recently; Rauchenberger et al. 1990). Therefore, using a combination of body size and colour morphs, the genotype of each male can be ascertained. Males of the *Y-I*, *Y-II* and *Y-L* genotypes show the same mating behaviours (use court displays and never chase) and are considered to have the same mating

strategy (courters). In contrast, males of the Y-s genotype attempt copulations by a sneak-chase strategy (sneakers). Females are homozygous for the *s P* allele, and therefore, females mature at the same size as the small males, but in contrast to the males, continue to grow after reaching sexual maturity (Kallman 1989).

In March 2004, we collected *X. multilineatus* females and males from the Río Coy in the state of San Luis Potosí, Mexico for an experiment that examined female preference for male strategy. In addition, we sampled the Río Coy, and adjacent subpopulations of *X. multilineatus* in the Ríos Oxitipa and Tambaque, tributaries of the Río Coy to obtain the size distribution of females, the proportion of males in each size class and the proportion of juveniles that would mature into courters. In April 2006, we collected *X. multilineatus* females and males from the Ríos Oxitipa and Tambaque to examine female preference for male strategy in these subpopulations. Furthermore, during this second sampling, we also obtained the size distribution of females, proportion of males in each size class and the proportion of juveniles that would mature into courters, to assess whether changes in these variables also occurred within sites across time.

Preference Tests

Before the tests, we measured standard length of all fish without anaesthetizing them. For all tests, we used only mature females, as evaluated by the presence of a brood spot. The brood spot is made up of pigmentation of the tissue surrounding the female reproductive organs and appears when females reach sexual maturity (Meffe & Snelson 1989; Morris & Ryan 1992). We isolated females and males into individual 38-litre tanks at least 1 week before being tested. For tests with females from the Coy subpopulation, we used 13 males from the size classes that use courting behaviour and 10 from the size class that uses sneak-chase behaviour to form male pairs to be used as stimuli. We used 10 males in more than one pair to form 28 different pairs. Pairs consisted of one courter and one sneaker male, and individuals were assigned to pairs in such a way as to maximize as far as possible the number of unique combinations of pairs of males. We used 14 male pairs twice, randomly assigning them to different females. Thus, during the two trials, each female evaluated four males in two different pairs. The mean \pm SE size of courters was 33.9 ± 0.86 mm (range 28.7–38.3 mm), and that of sneakers was 22.3 ± 0.62 mm (range 18.2–25.5 mm). The mean size difference between males within a pair was 12.3 mm (range 6.9–16.4 mm). We tested 28 females (mean \pm SE size = 28.8 ± 0.65 mm, range 21.5–33.8 mm).

For tests with females from the Oxitipa and Tambaque subpopulations, we used seven males from the size classes that use courting behaviour and four males from the size class that uses sneak-chase behaviour to form male pairs to be used as stimuli. We used all males in more than one pair to form 28 pair combinations, containing one courter and one sneaker male. The mean \pm SE size of courters was 33.4 ± 0.89 mm (range 29.1–36.1 mm), and that of

sneakers was 22.1 ± 0.53 mm (range 20.9–23.5 mm). The mean size difference between males within a pair was 11.4 mm (range 5.6–15.2 mm). For the Oxitipa subpopulation, we tested 14 females (mean \pm SE size = 27 ± 0.81 mm, range 22.2–32.6 mm). For the Tambaque subpopulation, we tested nine females (mean \pm SE size = 27.5 ± 1.28 mm, range 24.9–35.4 mm).

Preference tests were carried out in a 225-litre tank divided into five equal sections, with the two outer sections separated from the inner three by glass partitions (inner three sections were indicated only by lines on the tank). We placed males in the outer sections of the choice tank and the female in the middle section inside a clear holding cube. We allowed both males and females to acclimate for 10 min before releasing the female. We made observations from a window covered with one-way vision film in the neighbouring room. We recorded the time that females spent in the inner section adjacent to each male (choice zone) for a 10-min period. Trials of this duration provide the most information, because interactions decrease with the length of the trial. We switched males from one side of the tank to the other between a first and second trial to control for side bias by the females. The time interval between the first and second trials included the transfer time plus 10 min of acclimation. We tested each female with two pairs of males to control for female bias of male characters other than those defining the alternative strategies of particular males. We tested females with the second pair of males (third and fourth trial) after a 7-day period to control for the possibility of carry-over effects between tests. We eliminated trials in which one of the males (either courter or sneaker) did not interact with the female by courting or swimming up and down the glass partition. We compared the total time (for both pairs of males; i.e. for the four trials) that females associated with the sneaker males and the courters across the tests with a paired *t* test. Time associating with a stimulus is a good measure of mating preference in the closely related swordtail *X. nigrensis* (Ryan et al. 1990; Morris et al. 1992) as well as in another live-bearing fish (Bisazza et al. 2001). We used the difference in time spent with courting versus sneaker males as our measure of the strength of female preference. Finally, we examined the relation of the average strength of preference with female size using a linear regression analysis.

Female Size and Male Strategy Distributions

The three subpopulations that we sampled were from partially still pools and backwaters separated by deeper, more rapidly moving parts, and/or by areas with very low water levels. The Río Tambaque merges into the Río Oxitipa, and, after this point, is known as the Río Coy. The Oxitipa and Tambaque subpopulations are separated by 0.75 km river distance, while the Coy subpopulation is separated from the Oxitipa subpopulation by 12.88 km and from the Tambaque subpopulation by 12.67 km. We used seine nets to capture all the fish in the selected area (approximately 12 m²). We measured standard length of all captured *X. multilineatus* individuals and recorded

body and fin coloration of mature males. We assigned mature males to the sneaker or courting strategy based on morphological characters described by Zimmerer & Kallman (1989) and our own observations of the size distributions of the subpopulations under study. Males were identified by the presence of a gonopodium (modified anal fin used as an intromittent organ to transfer sperm), then classified as courters or sneakers based on the presence or absence of a sword and on pigment patterns known to correspond to the genotypes of the different strategies (Zimmerer & Kallman 1989). Because sneakers mature earlier than courters, we identified juveniles that would become courters and combined them with adult courters, thus removing the confounding effect of differences in time to sexual maturity between the two strategies in our analyses. Within each subpopulation, juveniles with no secondary sexual characters (i.e. gonopodium, sword or brood spot in the case of females) that were larger than the largest sneaker collected were assigned as courters (these individuals will mature as courters of the larger size classes) and juveniles that were smaller than the largest sneaker were assigned as juveniles of undetermined sex. Because females and sneakers mature at the same age/size, these immature individuals could still mature as either females or males. To examine the possibility that the variation in the frequency of strategies across subpopulations was due to differences in population growth, we compared the proportion of juveniles across subpopulations. Female size (standard length) was not normally distributed for one of the three subpopulations and lacked homoscedasticity. Thus, we used a Kruskal–Wallis test to compare female size across sites and a Mann–Whitney U test to compare this variable across time. We used chi-square analyses to compare the proportion of males in each subpopulation that were sneakers (smallest size class) and courters (three larger size classes) and the proportion of individuals that were juveniles between sites and across time. Because data from the 2004 sampling of the Oxitipa and Tambaque subpopulations were used in comparisons between sites and across time, we used Bonferroni corrections to adjust the critical alpha levels accordingly (Sokal & Rohlf 1995). The corrected critical levels (α') are indicated along with the statistics for such tests.

RESULTS

For all subpopulations, females spent more time with courters than with sneaker males (paired t tests: Oxitipa: $t_{13} = 5.19$, $P < 0.001$; Tambaque: $t_8 = 9.34$, $P < 0.001$; Coy: $t_{27} = 4.39$, $P < 0.001$; Table 1). The slopes for the relationship between female size and strength of preference of individual subpopulations were not significantly different from each other (test of parallelism: $F_{2,45} = 1.21$, $P = 0.31$). Therefore, for further analysis, we pooled the data between subpopulations. A female's size was significantly related to her average strength of preference, with the time spent with courters increasing with female size (Fig. 1).

Table 1. Mean \pm SE total time (in seconds) that female swordtail fish spent with the sneaker and courter male in each subpopulation

Strategy	Oxitipa		Tambaque		Coy	
	Mean	SE	Mean	SE	Mean	SE
Sneaker	606.2	90.8	485.0	64.9	779.6	64.4
Courter	1575.8	98.6	1727.2	71.3	1395.5	79.2

Female size differed between sites (Kruskal–Wallis test: $H_2 = 16.2$, $N = 142$, $\alpha' = 0.025$ after Bonferroni correction, $P < 0.001$), with a significantly smaller mean size in Río Oxitipa than in Río Coy (nonparametric multiple comparisons: $P < 0.05$; Fig. 2a). The proportion of males using sneaker and courter strategies varied significantly between sites (chi-square test: $\chi_2^2 = 8.63$, $N = 162$, $\alpha' = 0.025$ after Bonferroni correction, $P < 0.02$). A partitioning of the original contingency table (Siegel & Castellan 1988) showed that, across subpopulations, Oxitipa (with the smallest females) had a significantly smaller proportion of courters and a significantly larger proportion of sneakers (Fig. 2b). The proportion of juveniles did not differ significantly between sites (chi-square test: $\chi_2^2 = 0.49$, $N = 103$, $\alpha' = 0.025$ after Bonferroni correction, $P = 0.78$). In addition, the female size distribution differed from normality only for the Tambaque subpopulation in the 2004 sampling (Kolmogorov–Smirnov test: $D = 0.2$, $P < 0.001$; $D < 0.1$, $P = 0.2$ for the other two subpopulations); the data were negatively skewed and significantly

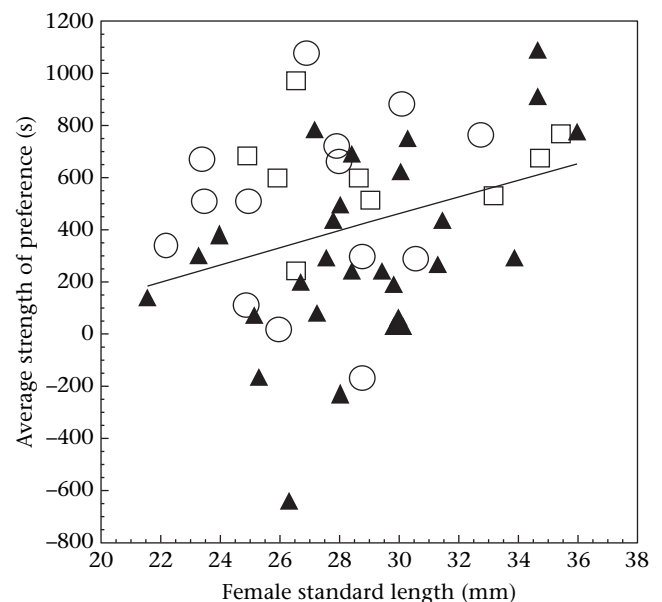


Figure 1. Relationship between female size and average strength of preference (mean difference in time spent with courters and sneakers) for male alternative reproductive strategies in three subpopulations (\blacktriangle : Coy; \circ : Oxitipa; \square : Tambaque) of *X. multilineatus*. The regression line shown is for all (pooled) subpopulations (linear regression: $R = 0.3$, $N = 51$, $F_{1,49} = 5.3$, $P < 0.05$). Negative scores indicate more time with sneaker males and positive scores indicate more time with courters.

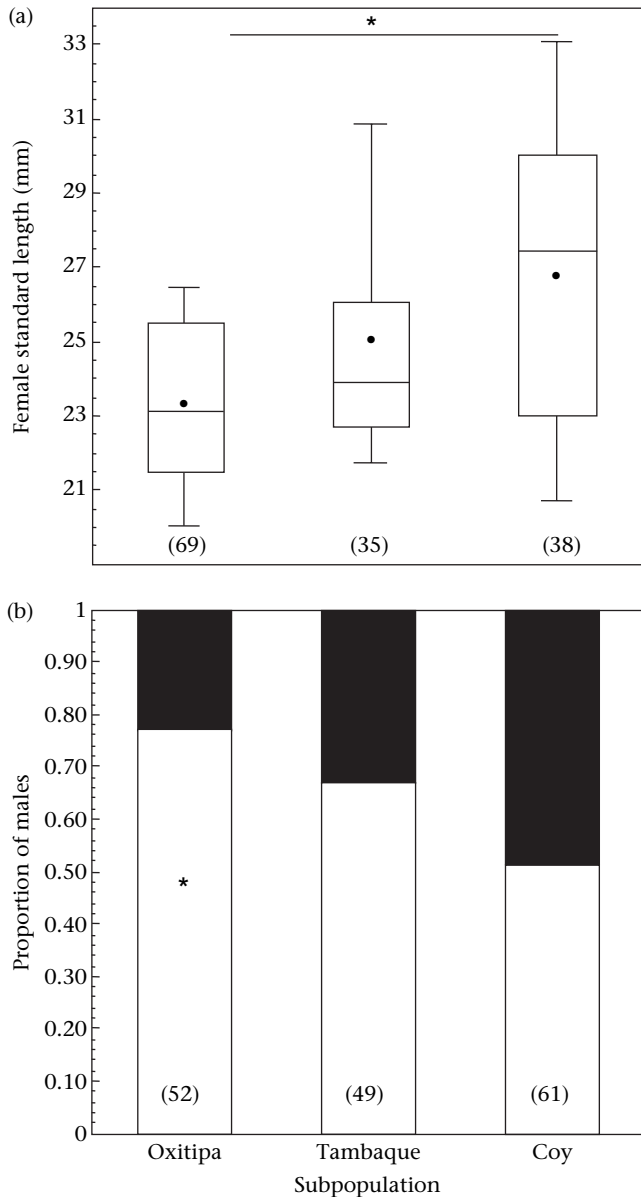


Figure 2. (a) Box plots for female size at each site showing 25th and 75th percentiles (box), median (line within the box), and 10th and 90th percentiles (whiskers). Dots indicate means. Asterisks indicate significant differences between subpopulations in pairwise comparisons. (b) Frequency of males of each strategy (■: courtiers; □: sneakers) at the same three sites. Asterisks indicate proportions that were significantly different from those expected. In both graphs, sample sizes are indicated in parentheses.

fitted a log-normal distribution (Kolmogorov–Smirnov test: $D = 0.18$, $P < 0.2$; Fig. 2a), which might be indicative of a recently established, growing subpopulation (see Discussion).

Female size also differed across time for Oxitipa (Mann–Whitney U test: $U = 644$, $N_1 = 69$, $N_2 = 41$, $\alpha' = 0.025$ after Bonferroni correction, $P < 0.001$), with significantly larger females in 2006 than in 2004 (Fig. 3a). The same pattern was detected for Tambaque, but it was not significant ($U = 381$, $N_1 = 35$, $N_2 = 30$, $\alpha' = 0.025$ after Bonferroni correction, $P = 0.058$; Fig. 3a). The proportion of

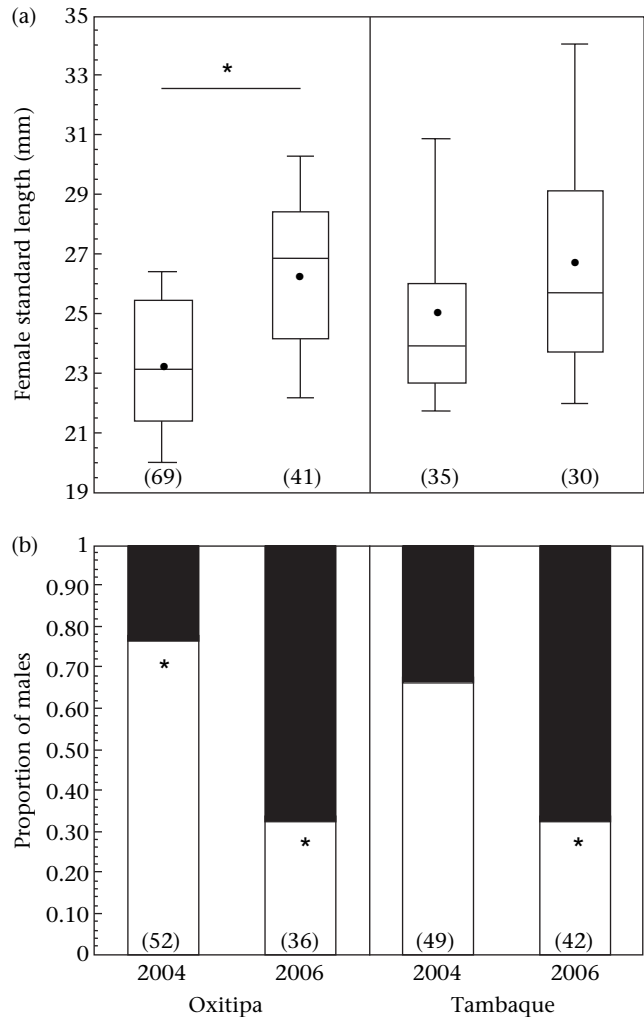


Figure 3. (a) Box plots for female size at two sites (Oxitipa and Tambaque) in 2004 and 2006 showing 25th and 75th percentiles (box), median (line within the box), and 10th and 90th percentiles (whiskers). Dots indicate means. Asterisks indicate significant differences across time. (b) Frequency of males of each strategy (■: courtiers; □: sneakers) at the same sites. Asterisks indicate proportions that were significantly different from those expected. In both graphs, sample sizes are indicated in parentheses.

males using sneaker and courter strategies varied significantly across time for both subpopulations (chi-square test: Oxitipa: $\chi_1^2 = 16.72$, $N = 88$, $\alpha' = 0.025$ after Bonferroni correction, $P < 0.0001$; Tambaque: $\chi_1^2 = 10.48$, $N = 91$, $\alpha' = 0.025$ after Bonferroni correction, $P < 0.01$) with a significantly higher than expected proportion of courtiers in 2006 (Fig. 3b). Finally, the proportion of juveniles differed significantly across time for Oxitipa (chi-square test: $\chi_1^2 = 17.5$, $N = 194$, $\alpha' = 0.025$ after Bonferroni correction, $P < 0.0001$). Specifically, the Oxitipa subpopulation during the 2006 sampling had a lower proportion of adults and a higher proportion of juveniles than expected. In contrast, the proportion of juveniles did not differ significantly across time for Tambaque (chi-square test: $\chi_1^2 = 2.8$, $N = 178$, $\alpha' = 0.025$ after Bonferroni correction, $P = 0.1$).

DISCUSSION

Our results confirm that female preference contributes to the previously detected mating advantage of larger courting males in *X. multilineatus* (Zimmerer & Kallman 1989). Consequentially, any spatial or temporal variation in female preference for courtiers should produce variation across space or time in this advantage for courtiers. Our results show that variation in female preference exists both across subpopulations and within subpopulations over time. We detected a significant relationship between the strength of female preference for the larger courtiers and the size of females, testing females from all three subpopulations. In addition, we detected statistically significant differences in female size both across subpopulations and within one subpopulation over time. Therefore, we suggest that the strength of female preference for courtiers varies both across subpopulations and within subpopulations over time.

Sample sizes used to test for the relationship between female size and the strength of female preference for courtiers in the Oxitipa and Tambaque subpopulations were small; thus, evidence that these relationships are equivalent across sites should be corroborated with larger sample sizes. Nevertheless, a consequence of this apparently constant variation in female preference for courtiers in relation to female body size is that, in subpopulations where smaller females may be more common, sneakers should be more successful than in subpopulations where small females are uncommon. Even when females do not necessarily prefer sneakers, the weak preference of small females for large males increases the likelihood that small females will mate with sneakers more often than will large females. Assuming that all other variables remain constant, we predict that subpopulations with relatively higher frequencies of small females will have relatively higher frequencies of sneaker males, and this should be true when comparisons are being made between subpopulations or within subpopulations at different times. The differences in the frequency of sneaker males that we detected across subpopulations was in the direction predicted by the distribution of female sizes; the subpopulation with the smallest females had fewer courtiers, but when the female size distribution in this subpopulation significantly increased over time, so did the number of courtiers.

Another explanation for the relationship between the variation in female size distribution and frequency of male mating strategies is that subpopulations that have more small females are newly established subpopulations in which larger males may not have had enough time to reach sexual maturity. Since smaller males reach sexual maturity sooner, a newly established subpopulation would be expected to have more small individuals in general (i.e. small females and sneakers) regardless of the strength of female preference for courtiers. However, the relationship detected above was not an effect of large courting males not having reached sexual maturity, because we could estimate the number of juveniles that would become courtiers as the number of juveniles larger than the largest sneaker males, which is also above the size at which females reach sexual maturity. By combining

juvenile courtiers with adult courtiers and comparing the frequency of the two strategies across subpopulations and time, we removed the confounding effect of the different times to sexual maturity between the sneakers and courtiers. In addition, based on a significantly higher than expected proportion of juveniles, the Oxitipa (2006) subpopulation seems to be the only recently established, growing subpopulation in this study; however, this subpopulation in 2006 also had a significantly higher proportion of courtiers. Finally, female size distributions in Tambaque during the 2004 sampling were negatively skewed (Fig. 2a), suggesting a recently established, growing subpopulation. However, females were not significantly smaller (Fig. 2a) and the proportion of small males was not significantly higher (Fig. 2b) in Tambaque in 2004 than in the other subpopulations. Together, our results suggest that differences in the proportion of courtier and sneaker males are not caused by differences in the age distribution between subpopulations.

Alternatively, large females and large males may be more frequent in some subpopulations because large size is favoured by natural selection. However, even in the samples with the largest proportion of large males and females relative to other samples, sneaker (small) males were still relatively well represented among the adult males in the subpopulation (51% for the Coy, 67% for the Tambaque and 33% for the Oxitipa 2006 subpopulations). This finding suggests that a factor other than overall natural selection for large size may be important at least in the Coy and Tambaque subpopulations. In addition, if factors such as low food availability and/or high predation pressure are responsible for the correlation between the frequency of small females and sneaker males, we would also expect to see more juveniles in those same subpopulations. However, our results do not support these alternative explanations, because a significantly high proportion of juveniles occurred only in Oxitipa (2006), where we also found significantly larger females and a significantly high proportion of courtiers. Nevertheless, in future investigations, we plan to extensively examine the potential role of variation in predation pressure in the maintenance of these alternative reproductive strategies.

Variation in female preference could maintain both strategies in the species as a whole (without negative frequency dependence) if there is gene flow between these subpopulations and/or the variation in female size (and thus preference) changes over time. If there is no gene flow between these subpopulations and/or the variation in female size does not change over time, then these subpopulations may be at different ESSt; fewer courtiers will be maintained in the subpopulations that consistently have more small females. In this case, variation in female preference would not be maintaining the variation in male reproductive behaviours, but would be influencing the frequency of each strategy, or ESSt, reached by each subpopulation. We are currently examining the degree of gene flow between these subpopulations as well as determining whether the variation that we detected in female size and preference at each site and over time is common.

Variation in female preference in relation to size and/or factors related to size (i.e. age and experience) has been detected in several other systems. A decline in female preference based on age has been detected in crickets (Gray 1999), and age, experience and female size are all known to affect female preferences in other poeciliid fish (Breden et al. 1995; Marler et al. 1997; Kodric-Brown & Nicoletto 2001; Morris et al. 2006). In bowerbirds, females have been shown to shift their preference from one trait to another with age (Coleman et al. 2004). In all of these cases, there is the potential for spatially or temporally oscillating selection on male phenotypes if there is variation in the female size distributions across space or time.

In summary, we confirmed that female preference for large males gives courters a mating advantage; but we also showed that this preference is weak in small females. A mating advantage is not the only component of fitness; in fact sneakers have the advantage of reaching sexual maturity sooner. This advantage coupled with an enhanced probability to mate with small females suggest that both spatial and temporal variation in female preference influences selection on the alternative male reproductive strategies and thus supports female preference as a potential mechanism for the maintenance of different male reproductive strategies in natural populations. While the correspondence between female preference for courters and their relative proportion could be due to factors other than the selection by female preference, we were able to rule out the differences in time to sexual maturity of the two strategies as the primary factor. Future experiments will attempt to strengthen the support for a causal relationship between these two variables by ruling out other alternatives in natural subpopulations (i.e. variation in predation) as well as examining this relationship in controlled experimental subpopulations.

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