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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 an 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*

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 (courters), and although sneakers court females in the absence of courters, courters 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 (ESSt), 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 courters 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 courters over sneaker males, which would suggest that the mating advantage of courters detected by Zimmerer & Kallman (1989) was indeed partly due to female preference. Second, we determined whether there was variation in female preference for the courters 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