Sexual Selection on *P-Alleles* and the Evolution of Mating Asymmetries in Swordtails (*Xiphophorus nigrensis* and *X. pygmaeus*)

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ABSTRACT

In *Xiphophorus nigrensis* from the Rio Choy, Mexico, there are three Y-linked *P*-alleles, *S*, *I*, and *L*, which result in males of small, intermediate and large body size. In nature there was a decrease in the frequency of the Y-linked *S*-allele between the parental and F_1 populations. This selective disadvantage for *S* was the result of female mating preferences based on size-related mating behavior; females preferred courtship, which was not exhibited by small males. Larger males also had greater swimming endurance than smaller males. Swimming endurance might be related to the ability of males to gain access to females. However, despite a change in Y-linked *P*-alleles across this generation, the frequency of size classes did not change. This was due to the production of small XX males fathered by males with Y-linked *I*- and *L*-alleles. The sister species, *X. pygmaeus*, consists of only small males that do not court. Female *X. pygmaeus* exhibited heterospecific mating preferences, preferring large *X. nigrensis* males to their own, smaller, conspecific males.

INTRODUCTION

In species from a wide range of taxa, sexual selection favors larger males either because these males are better able to compete for access to females or because females prefer them as mates (reviewed in Ryan 1985). Most studies of sexual selection on body size are restricted to the phenotype, with little or no ability to document genetic effects. Furthermore, because paternity analysis is often difficult or impossible, differential reproductive success is usually estimated within a generation (e.g. number of matings) instead of across generations (e.g. number of offspring produced). These limitations can be circumvented in studies of swordtails and platys, which seem to offer an ideal model system for studies of sexual selection.

Kallman and his colleagues (reviewed in Kallman 1984) have documented a significant genetic contribution to body size variation in many species of male swordtails and platys (genus Xiphophorus). The heritable basis of body size is due to allelic variation at the pituitary (P) locus. Alleles at this locus determine activation of the pituitary-gondal axis which, in turn, results in secretion of androgens. Since males either cease or drastically reduce growth upon reaching sexual maturity, earlier maturing males are smaller and later maturing males are larger. Females continue to grow after reaching sexual maturity.

In *X. nigrensis* from the Rio Choy, near Tamuin, Mexico, there are three alternative alleles at the *P*-locus on the Y chromosome. These alleles, S, I and L, result in males of three size classes, small (< 26 mm standard length, SL; tip of snout to hypural plate), intermediate (26-31 mm SL), and large (> 31 mm SL). All XY individuals are male. Narrow sense heritability for body size is 92% (Ryan and Wagner 1987). However, there is an autosomal sex determining factor. XX individuals that are homozygous recessive at this autosomal locus are male. The X-linked *P*-locus is fixed for the S-allele, thus XX males are all small. XY and XX small males can be distinguished by the presence of a yellow caudal in XY males (Kallman 1984).

The main purpose of our research with swordtails has been to investigate the influence of sexual selection on *P*-alleles. Here we address four questions: Is there evidence for sexual selection on Y-linked P alleles in natural populations of *X. nigrensis* from the Rio Choy? Is this sexual selection a result of female choice or male competition? What is the phenotypic effect of sexual selection on P alleles? Does female preference for larger males influence interspecific mate preferences?

Sexual Selection on P-Alleles

Ryan et al. (in review) estimated the effect of sexual selection on *P*-alleles. We compared the distribution of *P*-alleles in males sampled in nature to the distribution of sons fathered by these same males. Thus the selection we measured is due primarily to differential mating success and thus is sexual selection.

In February 1987 we captured, measured, temporarily marked, and recaptured 287 males. Recapture rates did not differ significantly among size classes; thus our capture technique did not introduce a bias among size classes. Male body size was used to estimate the Y-linked *P*-allele. XX males (i.e. small males without yellow caudal) were eliminated from the analysis. The frequencies of Y-linked *P*-alleles were: S, 0.22; I, 0.43,; L, 0.35 (Fig. 1).

Twenty seven females were collected and returned to the lab where they gave birth. One hundred twenty three sons were raised to sexual maturity; 25 were XX males. The size of each XY male was used to determine its Y-linked *P*-allele (Fig. 1). The frequencies of the Y-linked *P*-alleles in the F_1 generation were: S, 0.11; I, 0.53; L, 0.36. There is a significant difference in the distribution of P alleles between the parental and the F_1 populations (chi-

square=6.38, df=2, P=.04). The Y-linked *S*-allele is at a distinct disadvantage relative to the *I* and *L* alleles.
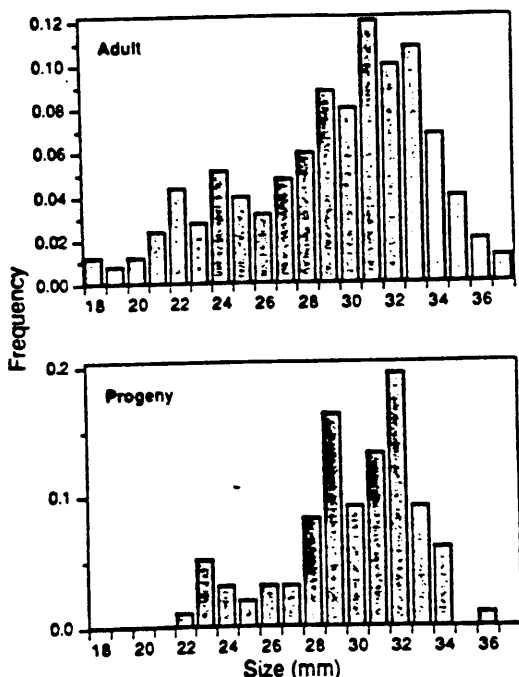

Fig. 1:
The frequency distribution of body sizes of XY males of *X . nigrensis* in nature (adult) and the body size of their sons at sexual maturity (progeny). These data were used to estimate the frequency of Y-linked *P*-alleles in each generation (from Ryan et al. in review).

Sexual Selection due to Female Choice

Ryan et al. (in review) tested the hypothesis that the sexual selection disadvantage of the Y-linked *S*-allele is due to female mating preferences for larger males. Female preference among pairs of males differing in size was determined by comparing the amount of time females associated with each male in a paired choice test. In many cases females preferred the larger male, although this was not always the case (Fig. 2). Neither the absolute nor the relative size difference among males determined female preference. For example, females preferred a 30 mm SL to a 27 mm SL male, but showed no preference between a 23 mm SL and a 28 mm SL male (Fig. 2). These results suggest that a portion of the sexual selection against the Y-linked *S*-allele was due to sexual selection by female choice.

Males that differ in body size can differ in other traits as well. It is not unusual in fish, as well as in other animals, that males of the less preferred or less competitive phenotype evolve alternative mating behaviors (reviewed in Dominey 1984). Ryan and Causey (in review) documented size-related differences in mating behaviors in *X. nigrensis*. Males in the large size class exhibited courtship behavior typified by the sexual display, a behavior in
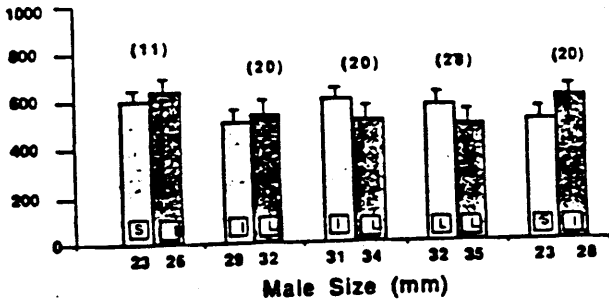
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Fig. 2: The average amount of time (plus one standard error) spent by female X. nigrensis with each conspecific male in a paired choice test. Sample sizes are in parentheses and size class denoted in each histogram bar.
*, P< .05;
**, P<.01;
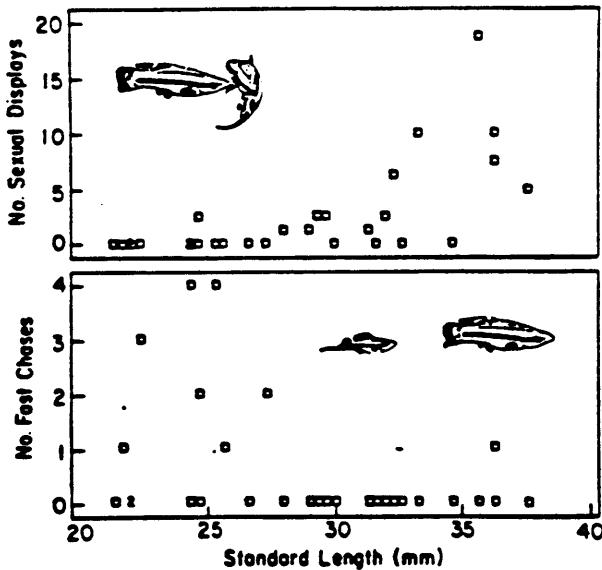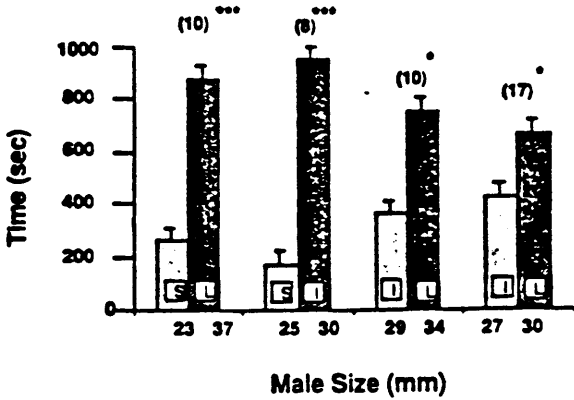***, P<.001, by a paired t test (from Ryan et al. in review).

Fig. 3: The number of sexual displays and fast chases as a function of body size exhibited by single X. nigrensis males paired with conspecific females during 10 minute periods.

which the male bends his body in the shape of a "C" around the front
of the female (Fig. 3). Small males did not exhibit this behavior.
Instead, they rapidly chased females and attempted to force
copulation. This behavior was similar to the "sneaky" or "chase"
alternative mating behavior common in males of many fish species.
The intermediate size class was not typified by a single mating
behavior. Smaller intermediate males chased while larger ones
courted. The threshold for the chase/court dichotomy was
approximately 29 mm SL (Fig. 3).

The threshold for chase/court seems to account for the somewhat
anomalous results of the female choice tests of Ryan, Hews and
Wagner (in review). When females were given a choice of two males
that were on each side of this threshold of 29 mm SL, they preferred
the larger male. If both males were on the same side of the
threshold females tended not to exhibit a preference (Fig. 2). Thus
female choice appears to be influenced not by male size per se, but
instead by mating behaviors correlated to male size.

Sexual Selection due to Male Competition

Our studies of male-male interactions involved with matings are
not yet complete. However, we have documented an aspect of sexual
selection that often is neglected, but can fall under the rubric
male competition (Koenig and Albano 1986). Males can only mate if
they are present in the vicinity of receptive females. In birds
(Gibson and Bradbury 1985) and frogs (Ryan 1985) a large portion of
the variance in male mating success is attributed to the amount of
time a male spends at the mating arena.

In the Rio Choy, *X. nigrensis* feed near algal covered rocks, and
during this time males attempt to mate females. Sometimes this
occurs in rather swift currents, and it appears that smaller males
may have a more difficult time in maintaining their location in
space in these currents. This suggested that differences in swimming
endurance could contribute to some of the variation in mating
success among genotypes.

Ryan (1988) tested this hypothesis by determining the amount of
time five males of each size class could swim in a current of
constant speed (3.9 cm/sec). There was a difference among size
classes in swimming endurance (F=6.88, df=2, P<.005); large males
had the greatest endurance while small does limit a male's access to
females, these data suggest it might contribute to the selective
disadvantage of the Y-linked *S*-allele.

Phenotypic Effects of Sexual Selection.

There is a strong correspondence between Y-linked *P*-allele and
body size, that is, between genotype and phenotype. However, since
some males are XX, the distribution of Y-linked *P*-alleles differs
from the distribution of body size classes. Ryan et al. (in review)
determined the phenotypic effect of selection by comparing the
distribution of body size classes for all males, including XX
individuals, between the parental and F$_1$ generations analyzed
previously (Fig. 4). In the parental population, the frequencies of

small, intermediate, and large males were .32, .37, and .30, respectively. The analogous frequencies in the F₁ population were .29, .42, and .38. These two frequency distributions are not significantly different (chi-square=0.98, df=2, P=.63).
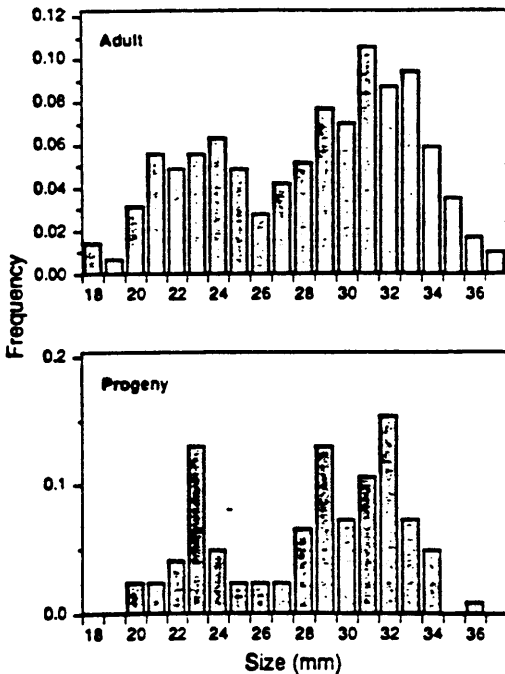

Fig. 4:
The frequency distribution of body sizes of all (i.e. XY and XX) males of *X. nigrensis* in nature (adult) and the body size of their sons at sexual maturity (progeny). These data were used to determine the frequency distribution of body size classes in each generation (from Ryan et al. in review).

Although there were significant changes in allelic frequencies across this generation, there was no phenotypic evolution. This appears to be due to the autosomal sex determining factor, and suggests that the small male size class was maintained by intermediate and large males, either heterozygous or homozygous recessive at the autosomal sex determining locus, mating with females that were heterozygous at this locus. Kallman (1984) estimated the allele *a* to be at a frequency of at least 0.26. Our estimate is even higher than 0.50. Thus the high frequency of *a* enhances the stability of the phenotypic distribution in spite of changes in the distribution of Y-linked *P*-alleles.

What Maintains Y-linked Alleles?

The results reported so far raise two general questions that demand immediate attention. First, what factors maintain the Y-linked *S*-allele? There are a number of possibilities we are investigating. These include differences in probability of reaching

sexual maturity and differences in total reproductive lifespan, as well as frequency dependent selection. Another, more complicated question is why are there XX males? In the Rio Coy population of this species, as well as in the sister species, *X. pygmaeus,* there are no XX males. The answer to the latter question might come from across-population and across-species comparisons.

Mating Asymmetries Between *X. nigrensis* and *X. pygmaeus*.

The sister species *X. pygmaeus* has only XY males and lacks allelic variation at the Y-linked *P*-locus (Kallman 1984). The total size range of *X. pygmaeus* males is smaller than that of *X. nigrensis,* and *X. pygmaeus* males are similar in size to the small and the smaller of the intermediate-sized *X. nigrensis* males. *X. pygmaeus* females have no experience with larger males, thus Ryan and Wagner (1987) determined the mating preference of *X. pygmaeus* females for larger *X. nigrensis* males versus their own conspecific males. Females showed a preference for larger males (Fig. 4). When both the heterospecific and conspecific were 26 mm SL, females still preferred the heterospecific. Male *X. nigrensis* usually have a more well developed sword than do *X. pygmaeus*. This character did not explain the female preference. When the heterospecific was desworded the female still preferred that male.

As with *X. nigrensis,* the heterospecific mating preference of *X. pygmaeus* might be due to size-related behaviors. Ryan and Causey (in review) found that *X. pygmaeus* males were similar to small *X. nigrensis* males in their mating behavior. They never exhibited the sexual display; instead, they too quickly chased a female while attempting to force copulation. This is surprising, since these chase behaviors are thought to evolve as an alternative to courtship behaviors in less preferred males (Dominey 1984). But in *X. pygmaeus,* all males are of the "less preferred" smaller size. Thus it is hardly proper to talk of this as an "alternative" behavior in *X. pygmaeus*.

In the above choice tests in which both males were the same size, the *X. nigrensis* was in the intermediate size class. The experiment was repeated such that the males were the same size but the *X. nigrensis* male was in the small size class. In this experiment, the female no longer preferred the heterospecific. Perhaps in this case too, the female's preference was due to size-related behavioral differences rather than size per se. Unfortunately, these results are not as consistent with the behavioral study by Ryan and Causey (in review). Although when both the *X. nigrensis* and *X. pygmaeus* males were 23 mm SL, we expect neither to exhibit courtship behaviors, we also do not expect the 26 mm SL *X. nigrensis* male to court (Fig. 5).

This demonstration of a mating asymmetry among species raises the question of why *X. pygmaeus* are small and lack courtship, since the evolution of larger size and courting behavior would be favored by sexual selection. There are at least three hypothesis. First, the species *X. pygmaeus* was founded by a small male *X. nigrensis* which lacked courtship. This hypothesis must also account for the historical lack of genetic variation for large size and/or
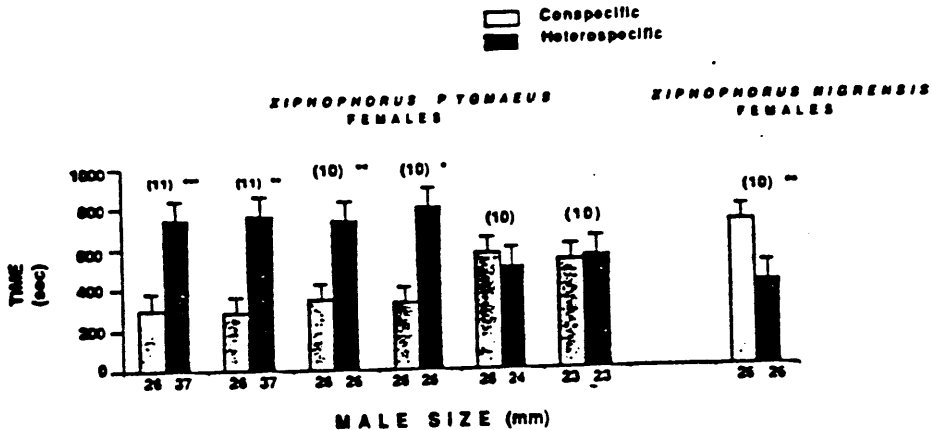
Fig. 5: The average amount of time (plus one standard error)   spent by female *X. pygmaeus* and *X. nigrensis* with each conspecific and heterospecific male in a paired choice test.   Sample sizes are in parentheses.   *, P< .05; **, P<.01; ***, P<.001, by a paired t test (data from Ryan and Wagner 1987).

In the first experiment in which both males were 26 mm SL (on the right), the heterospecific had a well developed sword.   In the second experiment in which both males were 26 mm SL, the sword of the heterospecific was removed, otherwise both males were identical as in the previous experiment.

courtship. Second, there is strong selection against large size and/or courtship in *X. pygmaeus*. For this hypothesis to be true, the selective disadvantage must be strong enough to outweigh the advantage of large size and courtship due to sexual selection. Third, small size and the lack of courtship is ancestral, and larger size and courtship was derived in *X. nigrensis* after it and *X. pygmaeus* diverged from their common ancestor. If true, this hypothesis, like the first, must account for historical lack of genetic variation for size and courtship in *X. pygmaeus*.

PROSPECTUS

The genus Xiphophorus has been the subject of studies from a wide range biological disciplines, including classical genetics (Kallman 1984), population genetics (Morizot and Siciliano 1982), systematics (Rosen 1979), biogeography (Rosen 1978), development (Tavolga 1949), endocrinology (Schreibman 1964), and ethology (Heinrich and Schröder 1986). Conspicuously lacking have been detailed studies of the animal's population biology and behavioral ecology. Given the rich background of information already available, especially the phenomenon of heritable Y-linked variation for body size, and the ease with which these fish can be studied in both the field and the

laboratory, Xiphophorus appears to be an ideal model system for studies of population biology and behavioral ecology, especially sexual selection. This report summarizes the beginning of a long term research program addressing these topics.

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