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A negative correlation between trait and mate preference in *Xiphophorus pygmaeus*

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Abstract. Variation in female mating preferences was compared with variation in male traits across populations of *Xiphophorus pygmaeus*. Females of this species prefer the large courting males of *X. nigrensis* to their own small, non-courting males. It was predicted that if large, courting *X. pygmaeus* males were to occur, they would be preferred. The discovery, distribution and subsequent spread, of large *X. pygmaeus* males provided an unusual opportunity to analyse how sexual selection might act on a trait not currently fixed in a species. The present study showed variation between populations in female preference for large male body size that was negatively correlated with the presence of large males. This variation in preference persisted over a 5-year period. Female preference for larger body size and courtship are evolutionarily decoupled from the male traits of large size and courtship in *X. pygmaeus*. This pattern is not consistent with indirect selection models (e.g. runaway selection), which predict that female mating preferences and male traits evolve in concert due to a genetic correlation between preference and trait. © 1996 The Association for the Study of Animal Behaviour

Several models have been proposed to explain how female mating preferences evolve (Kirkpatrick & Ryan 1991; Maynard Smith 1991). Good genes and runaway models predict that female preferences coevolve with preferred male traits because of a genetic correlation between the preference and the trait that arises due to non-random mating (Pomiankowski 1988). These are models of indirect selection (Kirkpatrick & Ryan 1991), because preferences evolve due to selection on male traits rather than selection directly on female preferences. In contrast, some direct selection models predict that female mating preferences and male traits are evolutionarily decoupled: preferences can arise before the traits they favour, as in sensory exploitation or sensory bias models (Basolo 1990; Ryan et al. 1990a), and preferences for existing traits can be lost because of costs associated with the preference (Kirkpatrick 1987; Pomiankowski 1987).

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Two types of studies have examined the predictions of these various models. Studies of parent–offspring correlations (Bakker 1993) and selection experiments (Houde 1994; Wilkinson & Reillo 1994) have shown that genetic correlations between mate preference and male sexual ornaments that are necessary for indirect selection models do exist in some cases. Other studies have used the comparative method to examine the relationship between female preference and male traits across populations and species (Basolo 1990, 1995; McLennan 1991; Proctor 1992; Hill 1994; Ryan & Rand 1995). Although comparative studies cannot be used to demonstrate that genetic correlations exist between preference and trait (a positive relationship between preference and trait across populations or species supports both indirect and direct selection models: Houde 1994), they can be used to demonstrate that genetic correlations do not exist, if either preference or trait are absent in a given population. Comparative studies can also add to understanding of the evolution of female preferences, because they examine the patterns of preference and trait evolution over larger time scales than do selection experiments, and in natural rather than artificial selection regimes.

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In this comparative study, we examined variation in female preferences and male traits between populations of the swordtail fish *Xiphophorus pygmaeus*. *Xiphophorus pygmaeus* was named for the small size of the males in this species (range 18–29 mm standard length, snout to hypural plate: Hubbs & Gordon 1943) compared with the males in closely related species *X. nigrensis* (size range 20–41 mm) and *X. multilineatus* (size range 22–42 mm). Female *X. pygmaeus* prefer larger courting *X. nigrensis* males to their own smaller non-courting males (Ryan & Wagner 1987). Thus females showed a preference for traits that did not exist in their own population (i.e. large body size and/or courtship), suggesting that female choice would favour males with these traits if they occurred.

The discovery of 'large' *X. pygmaeus* males (>29 mm) in one population of *X. pygmaeus* (Morris & Ryan 1995) provided us with an opportunity to examine the relationship between female preferences for large males and the presence of large males across populations. Large *X. pygmaeus* males do not court, sometimes have a gold colour, do not possess well developed swords and have a slender body shape (Morris & Ryan 1995). These traits also occur in smaller *X. pygmaeus* males and in the small males of the closely related species *X. nigrensis* and *X. multilineatus*, but are lacking in the large males of these latter two species (Kallman 1989; Rauchenberger et al. 1990). The size at which males reach sexual maturity and stop growing in *X. nigrensis* and *X. multilineatus* is controlled by a series of alleles of the pituitary locus (*P*) on the Y chromosome (Kallman 1989). Although two alleles at the Y-linked *P* locus produce two overlapping small size classes in *X. pygmaeus* (Kallman 1989), the genetic basis of the large males in *X. pygmaeus* is not known (Morris & Ryan 1995). Rauchenberger et al. (1990) placed *X. pygmaeus* in a clade with *X. nigrensis* and *X. multilineatus*. In a competing phylogeny of *Xiphophorus* (Meyer et al. 1994), *X. pygmaeus* was placed in a clade with *X. birchmanni*. Male *X. birchmanni* vary in size and use courtship in a manner similar to *X. nigrensis* and *X. multilineatus* (Haas 1993). Therefore, our conclusions about the evolution of body size and courtship that are based on comparisons of closely related species are not affected by the differences between the two phylogenies.

The geographical distribution and spread of the large male phenotype provided a unique natural experiment to analyse how a trait that is either becoming newly established or re-established in a species affects female preferences. Large *X. pygmaeus* males were first discovered in 1988 at El Nacimiento, San Luis Potosí, Mexico, an upstream site that had not been previously sampled (Morris & Ryan 1995; Fig. 1). In 1989, large males were found at Huichihuayan, a site downstream from El Nacimiento that had been extensively sampled in 1972 without detection of large males (Morris & Ryan 1995; Fig. 1). Large males were also found at La Y Griega Vieja in 1990 but only in very low frequencies (Fig. 1). La Y Griega Vieja had been sampled without detection of large males since 1951 (previously Chimalaco; Morris & Ryan 1995). Five years after the discovery of large males, we analysed the temporal and geographical distribution of large males throughout the Huichihuayan, Tancuilín and Axtla river systems. Over this period, the percentage of large males was greatest at El Nacimiento and decreased downstream from this site. In addition, the overall size of males at La Y Griega Vieja increased from 1988–1989 to 1990–1993. Therefore, large *X. pygmaeus* males were geographically restricted, but the temporal and spatial pattern of this phenotype suggested a recent spread (Morris & Ryan 1995).

We addressed the following three questions in this study. (1) Is there variation in female preference for larger male size between populations of *X. pygmaeus*? (2) Do preference and trait covary across populations, as predicted by several of the models of the evolution of female preference? (3) Were there changes in female preference for large size that correlated with the spread of the large male phenotype?

GENERAL METHODS

We conducted three experiments to examine the nature of female preference across populations of *X. pygmaeus*. Female mating preference was estimated by measuring the time females spent associating with one male versus another. Methods for female choice experiments are given in Ryan & Wagner (1987) and Ryan et al. (1990b). Briefly, a large aquarium was divided into three sections by two clear Plexiglas walls. The central section was also divided into three sections (one

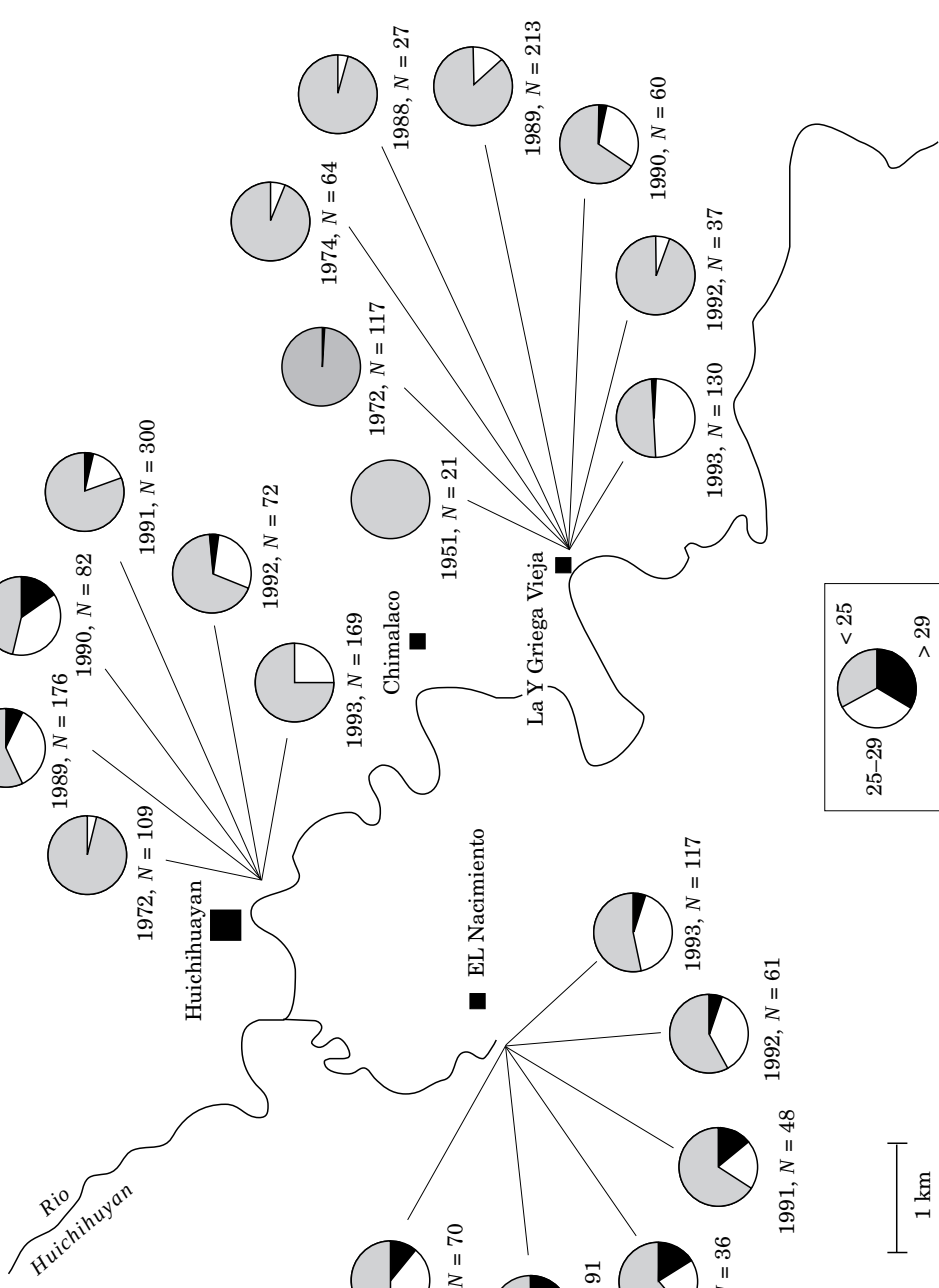


Figure 1. Locations and size distributions of the three populations of *X. pygmaeus* examined. Dates of collection and sample sizes are given below each pie chart.

section next to each end compartment and a neutral zone in the middle) by markings on the bottom and sides of the tank. Females and males were isolated from the opposite sex for at least 30 days prior to testing. Because this is the period of gestation, in most tests females would not have been mated since they last gave birth. One male was placed at each end of the aquarium. Males were physically separated from the females to control for effects of aggressive interactions between the males, which influence male access to females in the closely related species *X. nigrensis* (Morris et al. 1992). A female was placed in the centre in an opaque tube. After the female acclimated for 10 min, the tube was removed and the time the female spent in the section adjacent to each male's compartment was recorded for either 10 min (experiments conducted in 1988) or 20 min (experiments conducted in 1993 and 1994). The males were then switched between the two sides and the procedure was repeated. Testing the same female again after switching the males from end to end corrects for any side bias or position preference not related to the males. Tests in which the behaviour patterns of the two males were not similar were excluded from analysis. In most tests, males followed the movements of the female along the glass divider. In all of the experiments described, we controlled for any preference differences between the two colour morphs (blue and gold; Morris and Ryan 1995) by matching males for colour.

Each female and each pair of males were used only once in any given experiment. The difference in the amount of time a female spent with one male compared with the other male was assumed to indicate a relative strength of preference. Data were analysed using a paired *t*-test. Any negative correlation between the time spent with one male over another, which could arise because each female had a simultaneous choice between a large and a small male, would make the test more conservative (Toothaker & Miller 1996). The degree to which these times could be negatively correlated was also reduced by providing a neutral zone in the centre of the test aquaria, where females could choose not to associate with either male. When experiments were repeated in subsequent years, one-tailed tests were used when there was an a priori prediction of preference.

Female mating preferences in many fish are difficult to assess directly. Our studies of the closely related species *X. nigrensis* have shown, however, that results from preference tests as described above (female preference for large males, Ryan et al. 1990b) are consistent with the results from three other types of studies. In a study designed to measure the receptive behaviour of females placed in a tank with one male, large males elicited more receptive behaviours than small males (Morris & Ryan 1993). A field study of the access that males had to females showed that large males had greater access to females than small males (Morris et al. 1992). Finally, a paternity analysis using genetic markers showed that in nature large males produced more offspring than small males (Ryan et al. 1990b).

EXPERIMENT 1: PREFERENCES FOR LARGER MALES (1988)

Methods

Table I summarizes all of the preference tests performed in 1988. We first tested females from La Y Griega Vieja, where there were no large males, to determine whether they preferred large males from El Nacimiento and Huichihuayan over smaller males from their own population. Each female was tested with a different pair of males. One male from each pair tested was a large male from either El Nacimiento or Huichihuayan, and one was a smaller male from La Y Griega Vieja. Second, to determine whether females from La Y Griega Vieja preferred larger males within the size range of males found in their own population, females were given a choice between pairs of males from La Y Griega Vieja. Third, we tested females from both El Nacimiento and Huichihuayan with pairs of larger and smaller males from these sites. Finally, we determined whether there was a difference in preference between the population without large males (La Y Griega Vieja) and those with large males (Huichihuayan and El Nacimiento) by comparing the average differences in time females spent with the larger males compared with the smaller males from within their own populations with an unpaired *t*-test.

Results

La Y Griega Vieja females spent significantly more time with larger El Nacimiento and Huichihuayan males (mean $\pm$ SD = 652 $\pm$ 157 s)

Table I. Preferences for larger males

Females from:	*N*	Treatment	$\bar{X}$ (SE, range) male size difference (mm)
1988			
LYGV	11	Larger male from N or H versus smaller male from LYGV	8.3 (3.00, 3.3–12.5)
LYGV	10	Larger male from LYGV versus smaller male from LYGV	4.7 (1.85, 2.1–7.6)
N and H	26	Larger male from N or H versus smaller male from N or H	7.3 (2.75, 3.0–12.5)
1993			
LYGV	10	Larger male from N versus smaller male from LYGV	4.4 (2.60, 1.6–8.1)
LYGV	10	Larger male from LYGV versus smaller male from LYGV	4.8 (1.92, 1.4–8.5)
LYGV, N and H	10 of each 30 total	Larger male from N versus smaller male from N	4.9 (2.42, 1.8–10.8)

LYGV=La Y Griega Vieja; N=El Nacimiento; H=Huichihuayan.

than with their own smaller males (379.0 ± 130.5 s; paired $t=3.29$, $df=10$, $P=0.009$; Fig. 2a). La Y Griega Vieja females also spent significantly more time with larger males from within their own population (689.0 ± 198.9 s) compared with their own smaller males (319.0 ± 109.2 s; paired $t=3.97$, $df=9$, $P=0.003$; Fig. 2b). Therefore, even within the range of male sizes in their own population, females from this site showed a preference for larger males. Females from a combination of the Huichihuayan and El Nacimiento sites, the only sites with large males at this time, did not show a preference for larger males (501.0 ± 247.7 s) over smaller males from these sites (542.0 ± 248.6 s; paired $t=-0.44$, $df=25$, $P=0.67$; Fig. 2c). Thus, in the population with no large males, larger males were preferred, and where large males are typically found, no preference for larger males was detected.

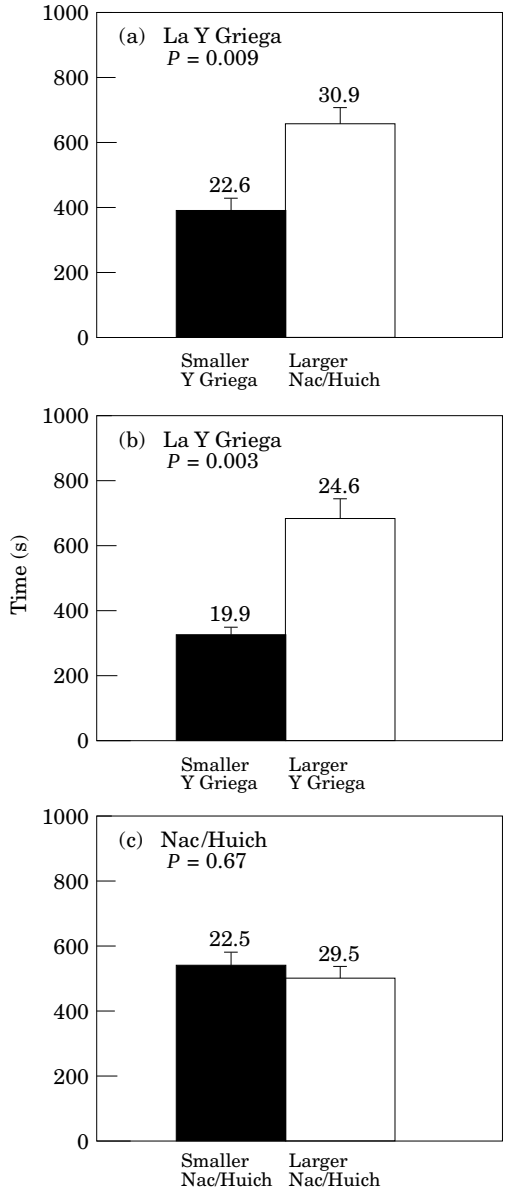
We compared the difference in time spent with the larger versus smaller males between the populations. The difference in the time La Y Griega Vieja females spent with the larger compared with the smaller La Y Griega Vieja males (mean ± SE = 370.2 ± 395.0 s) was significantly greater than the difference in time that Huichihuayan and El Nacimiento females spent with the larger compared with smaller males from their populations (mean ± SE = −41.4 ± 484.8 s; $t=2.5$, $df=34$; $P=0.02$). Females from the populations with no large males thus showed a stronger relative preference for larger males.

EXPERIMENT 2: PREFERENCES FOR LARGER MALES (1993)

Methods

Prior to 1990, large (>29 mm) males were not known to occur at La Y Griega Vieja. By 1990, however, some large males had been found at this site, and the overall size of males had increased. We determined whether the geographical variation in preference for male size across populations persisted over the 5 years we had been studying female preference in *X. pygmaeus* by testing for a preference for large male size again in 1993. Both males and females used in these tests were collected in the field in 1993. Table I summarizes the preference tests performed in 1993. First, females from La Y Griega Vieja were given a choice between large El Nacimiento males and smaller males from La Y Griega Vieja. Second, females from La Y Griega Vieja were also tested with pairs of males from La Y Griega Vieja to determine whether females from this population still preferred larger males from within their own population. Third, we tested females from each of the three populations (La Y Griega Vieja, Huichihuayan and El Nacimiento) with pairs of larger and smaller males from El Nacimiento. Females from Huichihuayan and El Nacimiento were tested separately, because the analysis of male size distribution (Morris & Ryan 1995) suggested that significantly fewer large males were at this site than at El Nacimiento. By testing the

same 10 pairs of males with females from all three populations, we controlled for differences in preferences due to differences between males other than size and therefore strengthened the comparison of preference differences between populations. The order in which the populations were tested was alternated to control for biases due to the number of times a pair of males had been previously tested. Finally, we compared the mean time differences between time spent with larger and smaller males across populations with a one-way analysis of variance.



Results

The negative relationship between preference for larger male body size and the presence of large males in populations of *X. pygmaeus* detected in 1988 was also detected in 1993. Females from the population with the lowest percentage of large males (La Y Griega Vieja) showed a significant preference for larger El Nacimiento males (mean ± SD, 1400.0 ± 153.6 s) over their own smaller males (517 ± 87 s; one-tailed paired $t=4.0$, $df=9$, $P=0.003$; Fig. 3a). La Y Griega Vieja females did not, however, show a significant preference for larger males (1158 ± 591 s) compared with smaller males from within their own population (948.0 ± 508.4 s; one-tailed paired $t=0.61$, $df=9$, $P=0.28$; Fig. 3a). When females from all three populations were given a choice between pairs of El Nacimiento males, La Y Griega Vieja females showed a significant preference for the larger males (1312 ± 456.0 s) over the smaller males (822 ± 425 s; one-tailed paired $t=1.8$, $df=9$, $P=0.05$; Fig. 3b), and females from the population with the highest percentage of large males (El Nacimiento) showed no significant preference for larger males (1033.0 ± 427.2 s) over smaller males (1072.0 ± 496.9 s; paired $t=0.13$, $df=9$, $P=0.90$; Fig. 3b). Females from the Huichihuayan population, with an intermediate percentage of large males as compared to La Y Griega Vieja and El Nacimiento populations, showed an intermediate preference for the larger El Nacimiento males (1177 ± 352.4 s) over the smaller El Nacimiento males (853 ± 309 s; one-tailed paired $t=1.59$,

Figure 2. Female preference tests conducted in 1988. Black bars indicate mean ± SE time with the smaller males; white bars indicate mean ± SE time spent with the larger males. Mean sizes of males tested (mm) are given within the bars (see Table I). (a) Preferences of females from La Y Griega Vieja for smaller La Y Griega Vieja males (Y Griega) versus El Nacimiento and Huichihuayan (Nac/Huich) larger males; (b) preferences of females from La Y Griega Vieja for smaller La Y Griega Vieja males versus larger La Y Griega Vieja males; (c) preferences of females from El Nacimiento and Huichihuayan for smaller conspecific males versus larger conspecific males.

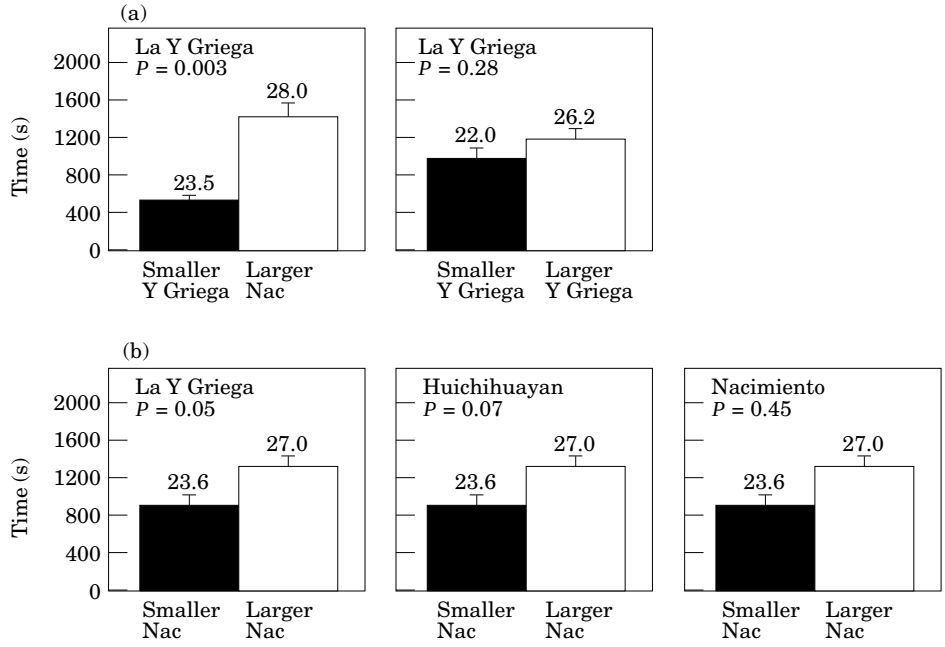


Figure 3. Female preference tests conducted in 1993. Black bars indicate mean ± SE time with the smaller males and the white bars indicate mean ± SE time with the larger males. Mean sizes of males tested (mm) are given above the bars (see Table I). (a) Preferences of females from La Y Griega Vieja for large El Nacimiento males (Nac) versus small conspecific males (Y Griega), and preferences of females from La Y Griega Vieja for large conspecific males versus small conspecifics. (b) Preferences of females from all three populations were tested with the same pairs of El Nacimiento males (Nac): preferences of La Y Griega Vieja females for smaller versus larger Nacimiento males; preferences of Huichihuayan females (Huich) for smaller versus larger Nacimiento males; preferences of El Nacimiento females for smaller versus larger Nacimiento males.

$df=9$, $P=0.07$; Fig. 3b). The mean difference in time spent with larger males compared with smaller males, however, was not significantly different between populations ($F_{2,26}=0.77$; $P=0.47$).

EXPERIMENT 3: PREFERENCE FOR COURTSHIP

Methods

Females from La Y Griega Vieja had shown a preference for large courting *X. nigrensis* males over their own smaller non-courting males in a previous study (Ryan & Wagner 1987). We compared the preference for courtship in La Y Griega Vieja and El Nacimiento females. In 1994, 10 females each from El Nacimiento and from La Y Griega Vieja received a choice between the same 10 pairs of males, each consisting of one large courting *X. nigrensis* male and one smaller non-courting *X. pygmaeus* male. The average ± SE size difference was 6.6 ± 2.40 mm (range=2.8–9.7). We determined whether there was a difference in preference between the populations by comparing the average differences in time females spent with larger heterospecifics and smaller conspecifics with a paired *t*-test. Ten females from El Nacimiento were also given a choice between 10 pairs of *X. nigrensis* and *X. pygmaeus* males matched for size. All *X. pygmaeus* males, including the large males, use sneak-chase behaviour rather than courtship (Ryan & Causey 1989; Morris & Ryan 1995). Although other differences between *X. nigrensis* and *X. pygmaeus* males may influence preference, this comparison separates any confounding preferences for male size and courtship. Average ± SE size difference between males in these tests was 0.1 ± 0.60 mm (range=0.0–1.2).

Results

In 1994, females from La Y Griega Vieja showed a significant preference for larger courting *X. nigrensis* males (mean ± SD = 1733.0 ± 512.8 s) over their own smaller males (620.0 ± 384.6 s; one-tailed paired $t = 2.99$, $df = 9$, $P = 0.02$; Fig. 4a) as had been shown for this population in 1986 (Ryan & Wagner 1987). This same preference was not shown by females from El Nacimiento (mean ± SD time with *X. nigrensis* males = 1170.0 ± 430.1 s; mean time with conspecific males 916.0 ± 314.6 s; paired $t = 1.11$, $df = 9$, $P = 0.3$; Fig. 4b). The mean ± SE differences in the time spent with the *X. nigrensis* males compared to the *X. pygmaeus* males were not significantly different between the two populations (La Y Griega Vieja: 757.0 ± 800.4 s; El Nacimiento: 253.0 ± 723.7; paired $t = 1.55$, $df = 9$, $P = 0.20$). Therefore, although it is clear that preference for larger, courting males has persisted in La Y Griega Vieja population over several years, it is not clear whether preference for courting males is lacking in El Nacimiento females or whether the lack of preference for larger males by females from this site (see experiment 1 and 2) confounds these results. Results from the test designed to control for differences in body size further support the lack of female preference for courtship in El Nacimiento. El Nacimiento females did not show a preference for the courting *X. nigrensis* males (1208 ± 501 s) over their own large males (matched in size) that do not court (966.0 ± 498.8 s; paired $t = 0.67$, $df = 9$, $P = 0.5$; Fig. 4c).

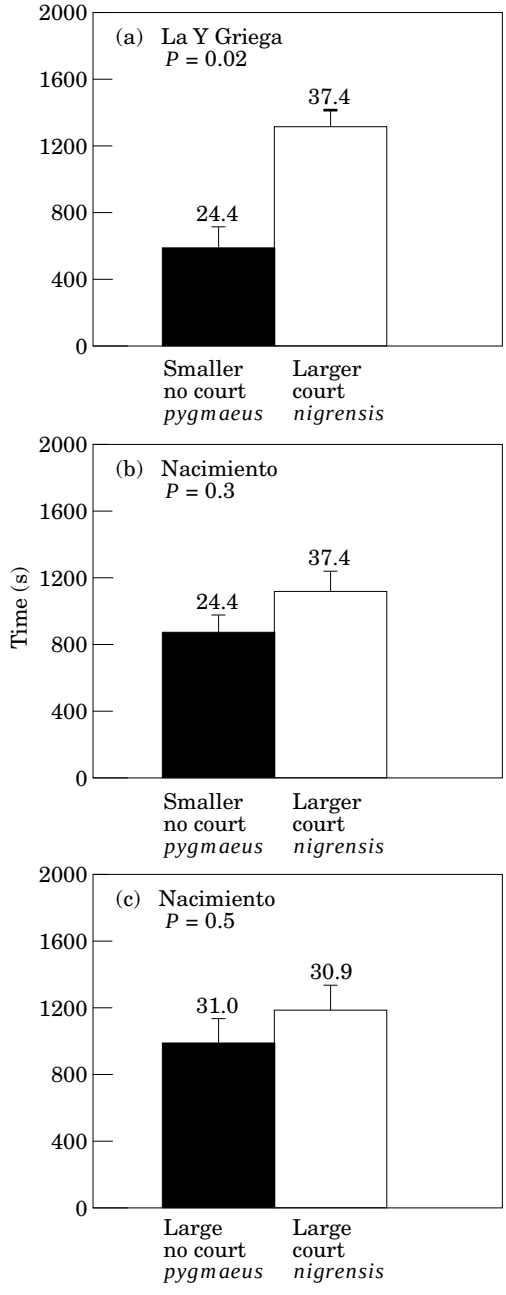


Figure 4. Female preference tests for courting heterospecifics over non-courting conspecifics. Black bars indicate mean time spent with conspecific males and white bars indicate mean ± SE time with heterospecifics. Mean sizes of males tested (mm) are given within the bars (see text). (a) Preferences of La Y Griega Vieja females for smaller conspecific males versus larger heterospecific males. (b) Preferences of El Nacimiento females for smaller, non-courting conspecific males versus larger, courting heterospecific males. (c) Preferences of females from El Nacimiento for non-courting, conspecific males versus courting heterospecific males matched in size. Females from La Y Griega Vieja (a) and from El Nacimiento (b) were tested with the same pairs of large *X. nigrensis* and small *X. pygmaeus* males.

DISCUSSION

Because *X. pygmaeus* females preferred larger, courting *X. nigrensis* males over their own smaller non-courting males, Ryan & Wagner (1987) predicted that if large size were to evolve in *X. pygmaeus*, it would be favoured by sexual

selection through female choice. Subsequently, non-courting *X. pygmaeus* males that were larger than previously reported for this species (>29 mm), some as large as the 'large' size class in *X. nigrensis* (>31 mm, Kallman 1984, 1989), were discovered in the Río Huichihuayan at El Nacimiento and in the town of Huichihuayan (Morris & Ryan 1995). In support of Ryan & Wagner's (1987) prediction, *X. pygmaeus* females from the site that lacked large males until the end of the study (La Y Griega Vieja) preferred large males from El Nacimiento and Huichihuayan over their own smaller males, as well as the slightly larger males from within their own population. Females from the site with the largest percentage of large males (El Nacimiento), however, did not show a preference for larger males over smaller males. This negative relationship between preference for large male size and male size was apparently present over the 5 years of this study.

Indirect selection models for the evolution of female preference are based on genetic correlations between preference and the male trait (Kirkpatrick & Ryan 1991). Selection experiments can be used to identify the existence of genetic correlations between mating preferences and preferred traits. The indirect selection models also predict that preference and trait should coevolve. This prediction can be tested by determining whether preference and trait are positively correlated across populations or closely related species (Hill 1994; Basolo 1995). Based on the assumption that there is a genetic basis to female preference for larger males and large male size in *X. pygmaeus*, our results are not consistent with the predictions of correlated evolution or indirect selection.

Results of some studies have shown a positive relationship between female preferences and male traits across populations. For example, preference for a greater degree of orange body coloration in guppies is correlated with a greater degree of orange body coloration in males (Houde & Endler 1990; Endler & Houde 1995). Positive relationships between female mating preference and male trait across populations are predicted by several different models for the evolution of female preference and therefore do not allow us to distinguish between the different models (Houde 1993). The negative relationship that we detected is not predicted, however, by indirect selection models. In addition, female preference for large male size did not increase when the male trait increased, but appears to have been lost in El Nacimiento population, where large males were first discovered. The direction of the change in female preference can be inferred from a comparison of the preference for large males found in other species of *Xiphophorus* (e.g. *X. nigrensis*, Ryan et al. 1990b), as well as other poeciliids (e.g. *P. latipinna*, Schlupp et al. 1994). The most parsimonious explanation is that preference for this character is ancestral to the northern swordtail clade, the clade that contains *X. pygmaeus* (Rauchenberger et al. 1990; Meyer et al. 1994). Because female preference appears to have been lost in El Nacimiento, some cost may be associated with female preference for large males in this population.

The large male phenotype appeared to be spreading over the 5 years of this study (Morris & Ryan 1995), and we were interested in how this change in male phenotype might influence female preferences for large size. Although the negative relationship between female preference and male size was detected at both the beginning and the end of the 5-year study, the results from the later preference tests differed from those of the earlier tests in two ways. First, although significant differences were detected between the populations in the strength of preference in the earlier tests, the differences between populations in strength of preference in the later tests were not significant. Second, we did not detect a preference for larger males by La Y Griega Vieja females within their own populations in the later tests, a preference that was detected in this population in the earlier tests. We present several possible explanations for the apparent decrease in the strength of preference over the study; further study is necessary to determine whether this trend of decreasing preference is significant and what factors could explain this change.

The mean size difference between the pairs of males was not as great in the later tests compared with the earlier tests. If preference for larger males increases in strength with greater size difference between males, then the smaller mean size difference in the later tests could explain why differences between populations were not detected. Mean size differences were similar in the 1988 and 1993 test that compared the preference of La Y Griega Vieja females for larger males within their

own population, and therefore probably do not explain why this preference was not detected in 1993. Earlier tests also differed from later ones in the duration that females were observed (10-min period in 1988 tests, versus 20-min period in 1993 tests). This protocol difference would account for the apparent decrease in the strength of preference only if preference were more difficult to detect the longer the females were observed (i.e. random movement of females increased after their initial exposure to the males). Females that showed a preference, however, often assessed males during the initial portion of the observation period and then tended to associate with one male. Therefore, we suggest that the longer observation times in later tests probably biased the results in the opposite direction of our conclusion, or should have made it easier to detect a preference in the later tests. Finally, although the mean size differences were similar in the tests of La Y Griega Vieja females for larger males within their own population, the average size of the males used was slightly larger in the later tests compared to the earlier tests. This difference could be important if the mechanism of female preference has a threshold/absolute nature and if most of the smaller males used in later tests were above the threshold/absolute size. Further tests are needed to examine this final possibility.

If we assume that the decrease in preference over the 5 years of this study is real and not due to the variables described above, then female preference may have decreased as the trait increased over the study period. Again, this hypothesis is interesting in light of the prediction from several selection models that preference and trait will coevolve. At least one explanation for this decrease in preference would not require selection: the females who lack preference may have spread downstream along with the larger male size. Another possibility is that the increase in male size could promote increased selection against female preference for large males downstream, especially if there is a cost to preferring larger males that directly relates to mating with larger males.

In summary, we have examined variation in female preference for large male body size across populations of *X. pygmaeus* and have detected a negative relationship between the preference and the trait. This relationship, which was still detectable 5 years after the initial tests, is not predicted by the indirect selection models for the evolution of female preference. A negative relationship between female mating preferences and the preferred male traits suggests that either direct selection or drift has played a more important role than indirect selection in the evolution of female preference. Although female preference for large courting males was apparently lost in the population with large males, female preference for large courting males was present in a population where there were no large males and no courtship. This creates a situation in which sensory exploitation could act, because female preference for large males at La Y Griega Vieja has the potential to select for larger males. Finally, the apparent decrease in the strength of the preference correlated with the increase in male body size at La Y Griega Vieja population over the 5 years of this study is not predicted by most models of sexual selection and warrants further investigation.

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