Copeia, 2001(2), pp. 355–364

Why Are Some Male Pygmy Swordtails Large?

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Swordtails in the genus *Xiphophorus* exhibit substantial variation in male body size influenced by genetic variation at the pituitary (*P*) locus on the Y-chromosome. However, males of *Xiphophorus pygmaeus* have historically been classified as uniformly small; they were thought to possess one of two *P*-alleles, both for small size. In 1988, large male *X. pygmaeus* were discovered at two sites in the Río Huichihuayán, Mexico. Surprisingly, females from these sites have lost the ancestral preference for large males although females from adjacent sites have not. This study investigates the heritability of large body size in male *X. pygmaeus* to understand its role in the evolution of male mating strategies and female preference in this species. We conducted breeding experiments with *X. pygmaeus* from two populations to determine whether large size is influenced by the same *P*-allele system found in other swordtails. Small fathers had a significant effect on the size at maturity of their sons but large fathers did not. All sons were small in size regardless of paternal size. Large fathers were no more successful than small fathers in producing offspring, and we found no significant relationship between age at maturity and size at maturity of the sons. In some cases, there was a significant difference in prematuration growth rate of sons between populations and in postmaturation growth rates within and between populations, yet all sons remained small in size. These results suggest large size is not paternally heritable through a *P*-allele in this species but could be a result of changes in pre- or postmaturation growth rates. The evolutionary persistence and spread of large male size may not follow current models of sexual selection on *P*-alleles and the loss of female preference in one population may be explained by the apparent lack of heritability of this male trait.

SEXUAL selection can result in the evolution of sexual dimorphism with males exhibiting a variety of elaborate traits that increase their mating success. Typically, sexually selected traits affect mating success by increasing male attractiveness or their ability to gain direct access to females (Darwin, 1859; Andersson, 1994). Less often sexual selection has resulted in parallel dimorphism among males. In addition to the aforementioned male mating strategies, there are "alternative" ones typified by the absence of conspicuous display behaviors and morphologies. Males that engage in these alternative strategies obtain mates by relying on more subtle behavioral patterns usually referred to as sneaking or satellite (see reviews in Wilson, 1975; Andersson, 1994; Gross, 1996).

Alternative mating strategies can be part of an ontogenetic pattern in which older males engage in conspicuous mate attraction (e.g., bullfrogs), and smaller, younger males adopt strategies like sneaking copulations or mimicking female coloration (Rostker, 1983; Eberhard, 1991; Baird et al., 1996). A less common occurrence is a genetic polymorphism in mating strategy (reviewed in Gross, 1996), which has been documented in stomatopods (Shuster and Wade, 1991), ruffs (Lank, 1995), and swordtails (Kallman, 1989; Ryan and Causey, 1989; Zimmerer and Kallman, 1989).

Swordtails in the genus *Xiphophorus* are ideal for investigating genetically determined polymorphic mating strategies because they can exhibit substantial variation in male body size, which is correlated with mating strategy in some species (Ryan and Causey, 1989; Rauchenberger et al., 1990; Ryan, 1993). Much of the variation in body size is determined by allelic differences at the pituitary (*P*) locus on the Y-chromosome. This locus influences the timing of sexual maturity, which coincides with cessation or drastic decrease in growth (reviewed in Kallman, 1989). Early maturing males are smaller, and later maturing males are larger; thus, the effects of a *P*-allele are lifelong. Although body size at maturity can be influenced in the laboratory by extreme manipulations (Borowsky, 1978; McKenzie et al., 1983; Campton, 1992), body size tends to breed true (Kallman, 1989). For example, in *X. nigrensis*, more than 90% of the variation in size of sons is explained by size of the father (Ryan and Wagner, 1987).

In *X. nigrensis* and *X. multilineatus*, variation in body size is also correlated with variation in body shape, dorsal fin height, development of the caudal extension, or "sword," and presence

of courtship display (Zimmerer and Kallman, 1989; Ryan and Causey, 1989; Rauchenberger et al., 1990). In *X. nigrensis*, there are three alternative alleles at the *P*-locus, representing three male phenotypes, and four *P*-alleles in *X. multilineatus*, representing four phenotypes. Although there is some overlap between size classes of the *P*-genotypes, the smaller males are shorter in standard length, narrower, have less conspicuous dorsal fins, and lack well-developed swords and conspicuous courtship displays. Larger males are longer, deeper, usually possess well-developed swords, and have conspicuous dorsal fins that are exhibited during courtship (Ryan and Wagner, 1987; Ryan and Causey, 1989; Ryan et al., 1990).

Paternity analysis of offspring from field-caught, gravid females shows that instantaneous reproductive success is higher for larger males (Ryan et al., 1990), which is a result of their greater attractiveness to females (Ryan, 1988; Morris et al., 1992). Greater mating success of larger males is balanced by higher survivorship to sexual maturity of smaller males because smaller males mature over a much shorter time period (Kallman, 1989; Morris and Ryan, 1990; Ryan et al., 1992).

In *Xiphophorus pygmaeus*, males do not exhibit the same patterns of variation in size, morphology, and mating behavior as in its two closest relatives, *X. nigrensis* and *X. multilineatus* (Rauchenberger et al., 1990); they do not exhibit courtship display behavior (Ryan and Causey, 1989) and historically were thought to possess only two *P*-alleles, both for small size. They were never found to exceed a standard length of 29 mm; typical size is 20–24 mm (Kallman, 1989). Some female *X. pygmaeus*, however, are similar to *X. nigrensis* and *X. multilineatus* females in their response to large body size in males (Morris et al., 1996). Females at La Y Griega Vieja (formerly known as Chimalaco) on the Río Huichihuayán in Mexico, prefer large, courting *X. nigrensis* males to their own smaller, noncourting conspecifics (Ryan and Wagner, 1987).

In 1988, large male *X. pygmaeus* (> 29 mm SL) were found at two sites in the Río Huichihuayán (Morris and Ryan, 1995). To our knowledge, the Nacimiento site (the springhead), had not been sampled previously, and the downstream site near the town of Huichihuayán had been sampled but never yielded large males (K. Kallman and D. Morizot, pers. comm.). These males were larger than typical *X. pygmaeus* males and comprised a discrete size class from small *X. pygmaeus* males producing a bimodal male size distribution (Morris and Ryan, 1995). Although these large males are in the same size range as intermediate and large *X. nigrensis* and *X. multilineatus* males, they do not have well-developed swords and do not exhibit courtship behavior (Morris and Ryan, 1995). Females from the Nacimiento site, where these males are found, do not prefer them over smaller conspecifics (Morris et al., 1996). However, females at La Y Griega Vieja, which had previously shown a preference for large *X. nigrensis* males (Ryan and Wagner, 1987), prefer these larger conspecific males even though they are not present locally (Morris et al., 1996).

In this study, we investigated whether large body size in *X. pygmaeus* males is determined by the same Y-linked, *P*-allele mechanism that contributes to much of the variation in body size seen in other swordtails (Kallman, 1989). The presence or absence of a *P*-allele for large size could provide insight into the asymmetry of female preference in this species and the evolution of preference in this clade.

Materials and Methods

We collected *X. pygmaeus* adults by seine from two sites in 1992 and 1993, the Nacimiento (21°28′34″N, 98°58′37″W) and La Y Griega Vieja (21°27′09″N, 98°56′19″W) of the Río Huichihuayán in the state of San Luis Potosí, Mexico. We also collected adult *X. nigrensis* by seine from a single site at the Nacimiento of the Río Choy (21°59′18″N, 98°53′02″W) in San Luis Potosí, Mexico. We maintained all field-caught fish in outdoor 4000-liter breeding tanks at the Brackenridge Field Laboratory at the University of Texas at Austin (see Morris and Ryan, 1990).

We placed fry born to field-caught adults of each species from each site in separate outdoor ponds to rear virgin females. The ponds were lined with netting of 0.158 cm^2 mesh to facilitate removal of fry during sampling. We removed fry from each pond weekly and visually inspected them for signs of sexual maturity. As a male approaches sexual maturity, the anal fin becomes modified into a tubular intromittant organ, the gonopodium (Rosen, 1960). The process of modification spans several weeks during which males cannot inseminate females (Grobstein, 1940; Kallman and Schreibman, 1973; Snelson, 1984). We removed males from the ponds when the initial stages of modification were visually apparent and, thus, before sexual maturation was complete, ensuring the virginity of females. In many female livebearing fishes, a gravidity spot appears when developing eggs cause the black exterior lining of the ovarian organ to become visible through the skin (Constanz, 1989). We classified females as sex-

ually mature when this gravidity spot appeared on the abdomen, and these virgin females were brought to the lab for breeding.

Crosses.—We crossed *X. pygmaeus* in a nested breeding design to determine patterns of inheritance of large size. Large males are found at only one of the collection sites, the Nacimiento of the Río Huichihuayán, whereas small males are found at both. We crossed each field-caught male with two virgin females to detect possible maternal and population effects. Seven large and six small males from the Nacimiento were each crossed with one virgin female from the same site as well as one virgin female from the second site, La Y Griega Vieja. Eight small males from La Y Griega Vieja were also crossed with females from both sites.

We crossed eight large and six small *X. nigrensis* field-caught males with virgin *X. nigrensis* females following the same protocols outlined for *X. pygmaeus*. The *X. nigrensis* crosses served as controls to detect any laboratory effects on the expression of *P*-alleles for large size.

We measured the standard length of each male and female and placed each pair in a 19-liter aquarium equipped with gravel, an undergravel filter, and a single submerged, plastic plant. Fish were fed Tetramin ad libidum twice daily. Males remained with females for two months. We monitored aquaria twice daily for the presence of fry; if present, fry were removed from the aquarium immediately to prevent cannibalism by the female. The number of days from the pairing of the male and female and the birth of the first fry was calculated for each cross. Offspring born within 30 days of pairing were considered evidence the female was not virgin. These females and all of their offspring were removed from further analysis.

Previous studies have shown social environment (visual and full contact) can affect size at maturity in other *Xiphophorus* species (Borowsky and Diffley, 1981; Campton, 1992). Consequently, we isolated each fry in a 0.9-liter glass jar covered with 0.318 cm^2 mesh netting and surrounded by cardboard partitions. We fed fry similar amounts of live brine shrimp in solution by pipette once daily to excess until they reached adulthood. We changed water and cleaned jars every 10 days.

We visually inspected fry for signs of sexual maturity at least once per week. Females were considered mature when the gravidity spot appeared; males were considered mature when the gonopodium was fully formed (third, fourth, and fifth anal fin rays elongated and fused) and pectoral fins fully pointed (Constanz, 1989). We estimated size at maturity by computing the average of three successive measurements of standard length in millimeters. Although microscopic inspection of the fish would have enhanced the precision of the date of maturation, it would have required anaesthetizing the fish once a week. The effects of repeated use of anaesthesia on fish growth are unknown (Snelson, 1989); therefore, we chose not to use microscopic evaluation of the fish. In addition, the precision of our method of classification would not have biased our estimation of sexual maturity toward a particular father, mother, cross, or population.

Size of each mature male was measured every 30 days following maturation until the end of the study (1995) or death of the fish. We examined the pre- and postmaturation growth trajectories of each male for linearity using simple bivariate plots. We found no evidence of curvilinearity, which is consistent with the growth trajectories documented by Marcus and McCune (1999) for several *Xiphophorus* species. We estimated prematuration growth rate as the arithmetic average of total growth over the number of days from birth to sexual maturation. We estimated postmaturation growth rate as growth over the first six months following maturation.

We used the total number of fry born to a single female as one brood. We estimated juvenile male mortality rate as the number of mature male offspring that died prior to the end of the study, and sex ratio within broods in the same manner; we counted the number of mature females per mature male within a brood. We recognize these are not the ideal methods to determine juvenile mortality and sex ratio, but gender is not morphologically obvious prior to the onset of sexual maturation. We chose to avoid using closer examination methods because they could introduce undue stress that might affect growth and mortality of the offspring.

We used nested analyses of variance and linear regressions to evaluate the effects of father size and mother size on son size. Ideally, we would have used sizes of laboratory-reared fathers and laboratory-reared sons in our regressions to control for environmental effects, but this was not possible because we cannot rear large *X. pygmaeus* males in the laboratory. However, the different environments of fathers and sons are unlikely to drastically affect the expression of *P*-alleles because large *X. nigrensis* males that were not laboratory-reared, produced large, laboratory-reared sons (see Results). We examined the effects of source population, cross success, age at maturity, prematuration

TABLE 1. THE POPULATIONS OF ORIGIN, SIZE CLASSES, AND SIZES OF *Xiphophorus pygmaeus* FATHERS AND SEXUALLY MATURE SONS ARE LISTED BELOW. Size is given in standard length (SL), which is snout to hypural plate. Standard deviation (SD) is given in parentheses following size, and standard error (SE) is given in the subsequent column. All measurements are in millimeter units. *n* is the number of individuals. An asterisk (*) indicates the group of sons significantly smaller than other groups of sons at the $P < 0.05$ level in a Student's *t*-test.

	Fathers					Sons				
Population	Size class	*n*	Mean SL ± (SD) mm	SE	Range	Size class	*n*	Mean SL ± (SD)	SE	Range
Nacimiento	Large	4	32.0 (0.9)	0.1	30.0–32.7	Small	40	19.4 (0.7)	0.1	18.0–21.3
	Small	4	23.9 (1.1)	0.1	22.5–25.0	Small	34	18.9 (0.7)*	0.1	17.7–21.3
La Y Griega Vieja	Small	4	23.0 (1.5)	0.3	21.5–25.0	Small	48	19.2 (0.8)	0.1	17.5–21.3

growth, and postmaturation growth using Student's *t*-tests (two-tailed), and nested analyses of variance. We used the nonparametric Kruskal-Wallis and Mann-Whitney *U*-tests to compare brood sizes, juvenile mortality rates and brood sex ratios because these data do not meet the assumptions of an analysis of variance. All statistical analyses were performed using SPSS (SPSS, Inc., 1994, unpubl.) and Statview (Abacus Concepts, Inc., 1996, unpubl.).

Otoliths.—To determine pattern of growth in small and large *X. pygmaeus* males, we estimated daily growth using otolith rings. In *X. nigrensis*, the number of otolith rings can be used as a measure of age at maturity in days (Morris and Ryan, 1990). To determine whether this relationship was also true for *X. pygmaeus*, we measured standard lengths and removed otoliths from mature, field-caught *X. pygmaeus* females ($n = 85$: 58 La Y Griega Vieja, 27 Nacimiento) and small males ($n = 20$: 10 La Y Griega Vieja, 10 Nacimiento). Standard lengths were measured for females ($n = 8$: 3 La Y Griega Vieja, 5 Nacimiento) and males ($n = 6$: 2 La Y Griega Vieja, 4 Nacimiento) of known age and otoliths removed to calibrate the results from field-caught individuals. We mounted otoliths on slides in microscope immersion oil, or Permount®, then covered and examined asterisci using a Nikon phase contrast microscope under 10× objective and 10× eyepiece. We counted the number of rings three times from an image of the asteriscus captured with a CCD video camera and computer enhanced on a Macintosh computer using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available at http://rsb.info.nih.gov/nih-image/). The number of rings was averaged across all three counts. We conducted simple linear regressions to determine the relationship between standard length and number of otolith rings then compared this relationship among populations.

RESULTS

Crosses.—*Xiphophorus pygmaeus*: Six of the 42 crosses resulted in only female offspring. One cross resulted in two sons born 28 days after initiation of the cross, casting doubt on the virginity of their mother. They and their mother were removed from further analysis. Sixteen crosses were unsuccessful because the male or female died before offspring were born; none of the dead females were carrying embryos. The mean gestation interval among all other females was 63.9 days (± 28.4 SD). Four large fathers produced 40 sons (with five mothers) and eight small fathers produced 82 sons (with 14 mothers) resulting in 19 families. All sons were small in size, and large fathers did not sire larger sons than did small fathers (Table 1). There is a statistically significant relationship between father size and son size among families (nested ANOVA: $F_{11,9} = 3.697$, $P = 0.011$) but no such relationship between mother and son (nested ANOVA: $F_{9,101} = 1.11$, $P = 0.366$ (Table 2). Size of mothers mated to small fathers and those mated to large fathers had no significant contribution to the total variance in the size of sons (nested ANOVA: small: $F_{8,66} = 1.11$, $P = 0.368$; large: $F_{1,35} = 0.63$, $P = 0.433$). Because our hypothesis is that large size in this species is determined by a Y-linked *P*-allele, we expect all sons carrying Y-chromosomes whose fathers were large to be large. Any effect of mother is expected to contribute to variation within this size class, as is typical of other swordtail species (Kallman, 1989). Therefore we used linear regressions to evaluate further the contribution of father size to son size using all of the data and family means. There is a weak but statistically significant relationship between father size and

TABLE 2. A NESTED ANALYSIS OF VARIANCE OF THE EFFECTS OF FATHER SIZE AND MOTHER SIZE ON SON SIZE IS SUMMARIZED BELOW. The source of variation, degrees of freedom (df), Sums of squares (SS), Mean Squares (MS), *F*-values, and probability values (*P*-values) are given for each variable.

Source of variation	Nested ANOVA			*F*-value	*P*-value
	df	SS	MS		
Father	11	12.77	1.16	2.39	0.011
Mother within Father	9	4.83	0.54	1.11	0.366
Within Mother + Residual Error	101	48.99	0.49		
Model	20	17.60	0.88	1.81	0.029
Total	121	66.59	0.55		

son size (all data: $R^2 = 0.068$, $F_{1,120} = 7.201$, $P = 0.004$; family means: $R^2 = 0.179$, $F_{1,17} = 3.697$, $P = 0.0714$; Fig. 1). Separate analyses of large and small fathers show that small fathers have a significant effect on the size of their sons (all data: $R^2 = 0.083$, $F_{1,80} = 7.201$, $P = 0.0089$; family means: $R^2 = 0.280$, $F_{1,12} = 4.66$, $P = 0.0518$), whereas the relationship between large fathers

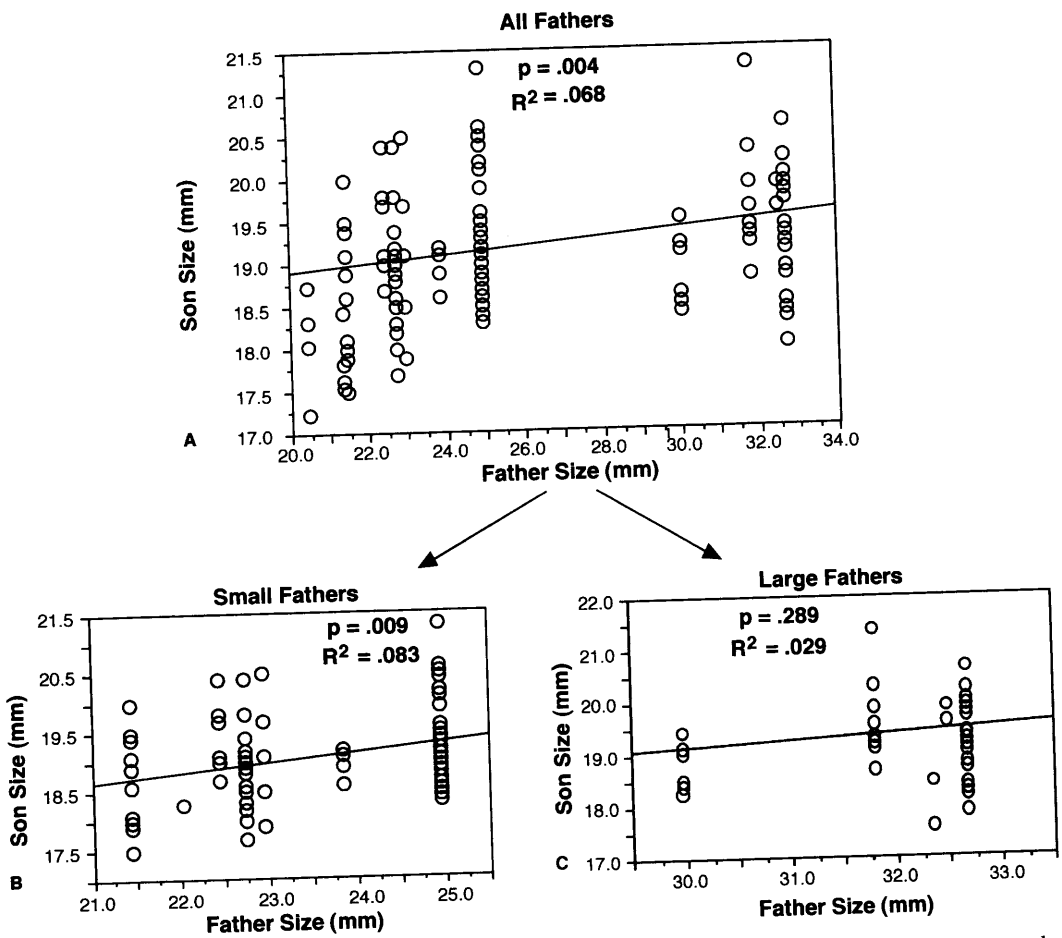

Fig. 1. Linear regression plots of father size on son size for *Xiphophorus pygmaeus*. Graph A shows the relationship between the sizes of all fathers and sons from both sites. Graph B shows the relationship between small fathers from both sites and their sons. Graph C shows that between large fathers and their sons. The significance of the relationship between father size and son size is a result of the influence of small fathers on their sons.

TABLE 3. BELOW ARE MEANS AND STANDARD ERRORS OF BROOD SIZES, AND TOTAL NUMBER, SEX RATIOS (NUMBER OF MATURE FEMALES/MALE), AND MORTALITY RATES OF OFFSPRING OF *Xiphophorus pygmaeus* AND *Xiphophorus nigrensis* FATHERS. *n* is the number of individuals. An asterisk (*) indicates a significant difference at $P < 0.05$ in a Kruskal-Wallis test. Large *X. nigrensis* fathers produced significantly fewer numbers of offspring and smaller broods than any of the other groups of fathers. There are no significant differences in any of the remaining variables between groups of fathers.

Species	Sire size class	*n*	Brood size			Total # offspring			Sex ratio (# females/male)			Offspring mortality rate		
			Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
X. pygmaeus	Large	4	22.3	15.1	5.7	36.8	12.5	6.3	0.56	0.5	0.3	0.315	0.26	0.13
	Small	12	15.8	9.9	2.3	25.0	19.8	5.7	0.87	0.4	0.2	0.236	0.30	0.09
X. nigrensis	Large	4	5.0*	3.0	1.7	5.0*	3.0	1.7	0.78	0.7	0.4	0.489	0.32	0.18
	Small	12	19.8	10.1	4.5	24.7	23.8	11.9	0.51	0.1	0.1	0.247	0.26	0.13

and their sons is not significant (all data: $R^2 = 0.289$, $F_{1,38} = 1.12$, $P = 0.296$; family means: $R^2 = 0.280$, $F_{1,3} = 2.49$, $P = 0.280$; Nested ANOVA: $F_{3,1} = 3.88$, $P = 0.121$). The critical point is that large fathers (greater than 29 mm) did not produce large sons (Table 1), as would be expected if a Y-linked, *P*-allele were present. This is consistent with previous work on the genetics of male size in *X. pygmaeus* (Kallman, 1989; Morris and Ryan, 1995) and suggests any influence of large fathers on the size of their sons is too weak for us to detect in this study.

Population of origin of mother or father did not affect the size of sons (nested ANOVA mother: $F_{17,102} = 0.22$, $P = 0.640$; father: $F_{1,11} = 1.02$, $P = 0.320$) nor did the interaction of maternal and paternal population (nested ANOVA $F_{11,17} = 0.07$, $P = 0.790$).

Large fathers produced neither greater nor fewer numbers of offspring than small fathers [large: mean = 22.3 ± 15.1, small: mean = 15.8 ± 9.9, $t_{(26)} = -0.990$, $P = 0.34$] even with mother size as a covariate (ANCOVA father: $F_{1,11} = 1.01$, $P = 0.340$; mother: $F_{1,20} = 0.070$, $P = 0.796$; father × mother interaction: $F_{1,20} = 0.0179$, $P = 0.680$). Population of origin of either parent also had no effect on the number of offspring [$t_{(26)} = 0.669$, $P = 0.510$; nested ANOVA male effect: $F_{1,11} = 1.266$, $P = 0.304$; female: $F_{11,20} = 1.695$, $P = 0.210$]. Brood sizes did not differ significantly between large and small fathers ($U = 17.00$, $P = 0.514$; Table 3). Juvenile mortality rates within broods of large and small males did not differ significantly ($U = 16.00$, $P = 0.433$; Table 3), nor did sex ratios of mature offspring ($U = 10.00$, $P = 0.308$), suggesting the lack of large sons is not a result of differential mortality. In addition, sex ratios were often male biased rather than female biased, the opposite of the expected ratio if males experienced differential mortality.

Although no *X. pygmaeus* sons attained large size in this study, analyses of age at maturity, prematuration growth, and postmaturation growth were conducted for all sons. We sought to examine the possibility that large size in field-caught males is a result of a difference in growth rate before or after sexual maturity. Simple linear regression revealed no significant relationship between age at maturity and size at maturity among all sons, and almost none of the variation in size was explained by a difference in age at maturity ($R^2 = 0.0004$, $F_{1,122} = 0.055$, $P = 0.820$). A comparison of age at maturity among sons of large and small Nacimiento fathers revealed no significant difference [$t_{(62)} = -0.875$, $P = 0.380$]. Likewise, there was no significant difference between sons of La Y Griega Vieja fathers and of large Nacimiento fathers [$t_{(86)} = ;-1.591$, $P = 0.120$]. However, there was a significant difference in age at maturity between sons of La Y Griega Vieja fathers and sons of small Nacimiento fathers [$t_{(82)} = -2.437$, $P = 0.017$]. At sexual maturation sons of small Nacimiento fathers were an average of 21 days older than sons of La Y Griega Vieja fathers (Table 4). Delay in maturation is correlated with larger size in other swordtails (Kallman, 1989), but sons of small Nacimiento fathers were not significantly larger than sons of any other group of *X. pygmaeus* fathers. In fact, they were significantly *smaller* than all other sons [$t_{(120)} = 2.954$, $P = 0.0038$; Table 1].

A comparison of prematuration growth rates indicated that sons of all Nacimiento fathers grew significantly more slowly than sons of La Y Griega Vieja fathers [$t_{(120)} = -1.921$, $P = 0.057$]. But, there was no significant difference in growth rates between sons of small and large Nacimiento fathers [$t_{(62)} = 0.998$, $P = 0.320$].

We explored the possibility of heritable differences in postmaturation growth by comparing mean growth rates of all sons. We used the mean daily growth rate over six months after

TABLE 4. BELOW ARE THE MEAN, STANDARD DEVIATION (SD), AND STANDARD ERROR (SE) IN TIME TO MATURITY AND PRE- AND POSTMATURATION GROWTH RATES OF *Xiphophorus pygmaeus* SONS OF SMALL AND LARGE FATHERS FROM TWO POPULATIONS. Time to maturity is represented as number of days from birth to sexual maturity. Pre- and postmaturation growth are represented as the change in standard length in millimeters per day prior to and after maturity, respectively. Postmaturation growth rate is the increase in standard length/day over six months. An asterisk (*) indicates a significant difference between populations and a dagger (†) a significant difference from all other groups at $P < 0.05$, in a Student's *t*-test.

		Age at maturity (days)			Prematuration (mm/day)			Postmaturation (mm/day)		
Population	Size class	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
Nacimiento	Large	206.7	50.4	7.9	0.099*	0.027	0.004	0.003	0.0028	0.0005
	Small	217.7†	58.5	10.0	0.093*	0.024	0.004	0.005*	0.0034	0.0006
La Y Griega Vieja	Small	191.4	39.2	5.7	0.105	0.024	0.003	0.004	0.0029	0.0004

sexual maturation as a measure of postmaturation growth rate because there was a linear relationship between time since maturation and growth for all sons in this study and in another recent study (Marcus and McCune, 1999). There was a significant difference in postmaturation growth within the Nacimiento population between sons of small and large fathers [$t_{(62)} = -2.798$, $P = 0.0066$; Table 4]. There was a marginal difference between sons of small Nacimiento fathers and La Y Griega Vieja fathers [$t_{(82)} = -1.894$, $P = 0.062$] and no significant difference between sons of large Nacimiento fathers and of La Y Griega Vieja fathers [$t_{(86)} = 1.234$, $P = 0.220$]. Sons of small Nacimiento fathers grew at a greater rate after maturity than those of large Nacimiento or La Y Griega Vieja fathers. This increase in postmaturation growth rate suggests sons of small Nacimiento fathers might have the potential to reach greater size than sons of either La Y Griega Vieja fathers or large Nacimiento fathers despite their smaller size, increased age at maturity, and slower prematuration growth.

Crosses.—Xiphophorus nigrensis: We examined the relationships between father, mother, and son sizes among seven families of *X. nigrensis*. No father or mother is represented twice because males produced sons with only one of the females with which they were crossed. In this species, male size class is determined by Y-linked *P* alleles, and our intent is simply to show that the alleles for male size are expressed as expected in offspring reared under our laboratory conditions even though their fathers were not reared in the laboratory. Three large fathers (all $\geq$ 34.0 mm) produced four sons, all large (31.4, 37.5, 39.3, 39.8 mm SL). Four small fathers (all $<$ 30 mm) produced a total of 25 sons, all small (17.7–21.3 mm SL, mean = 18.9), and the size of sons differed significantly between large and small fathers [$t_{(26)} = 22.011$, $P = 0.0001$. Size of father had a significant influence on the size of sons ($R^2 = 0.926$, $F_{1,27} = 336.9$, $P < 0.0001$), as did the size of mother and father together ($R^2 = 0.929$, $F_{1,27} = 170.9$, $P < 0.0001$), whereas size of mother alone did not significantly influence the size of sons (mother: $R^2 = 0.115$, $F_{1,27} = 3.49$, $P = 0.072$). Sex ratio among mature offspring and juvenile mortality rates did not differ between broods of large and small *X. nigrensis* fathers (sex ratio: $U = 3.00$, $P = 0.513$; juvenile mortality: $U = 3.00$, $P = 0.289$; Table 3). These variables also did not differ between large *X. nigrensis* and large *X. pygmaeus* fathers (sex ratio: $U = 5.00$, $P = 0.724$; juvenile mortality: $U = 3.00$, $P = 0.287$; Table 3), or between small *X. nigrensis* and small *X. pygmaeus* fathers (U = sex ratio: $U = 5.00$, $P = 0.153$; juvenile mortality: $U = 20.00$, $P = 0.628$; Table 3). Furthermore, there were no significant differences in these variables among offspring of large *X. nigrensis*, large *X. pygmaeus*, small *X. nigrensis* and small *X. pygmaeus* fathers (brood size: $H_3 = 6.65$, $P = 0.0845$; sex ratio: $H_3 = 2.10$, $P = 0.554$; juvenile mortality: $H_3 = 2.93$, $P = 0.403$). Brood sizes of large *X. nigrensis* fathers were significantly smaller than those of small *X. nigrensis* fathers ($U = 0.500$, $P = 0.0269$; Table 3); however, all sons produced by large fathers attained large size, and all sons produced by small fathers attained small size.

Otoliths.—In *X. nigrensis* otolith rings are added daily until sexual maturation is reached but not thereafter. Thus, the number of rings predicts age at maturity and, along with body size, can be used to calculate prematuration growth rate (Morris and Ryan, 1990). Simple linear regressions indicated a significant relationship between size and number of otolith rings in small *X. pygmaeus* males from La Y Griega Vieja ($R^2 = 0.436$, $P = 0.038$, $F_{1,8} = 6.17$) and a marginally

insignificant relationship in small Nacimiento males (R^2 = 0.352, P = 0.071, $F_{1,8}$ = 4.34). These results suggest that the number of rings roughly predicts age at maturity in small field-caught males of this species also.

We compared the relationship between number of rings and size of a large Nacimiento male with the regression plot for small Nacimiento males. We used the regression equation for Nacimiento small males to predict the size of this large Nacimiento male. We predicted his size would be 22.45 mm if his growth pattern were similar to that of small males. Because his actual size was 30.5 mm, it suggests this large male may have experienced different growth rate patterns either before or after maturation. However, additional otolith data from large males are necessary to make any inferences about the mechanism of large size. Unfortunately large males are rare, but we hope to examine additional otoliths in the future.

Simple linear regressions among females revealed very weak relationships between size and number of rings (La Y Griega Vieja: $F_{1,56}$ = 3.664, P = 0.061, R^2 = 0.061; Nacimiento: $F_{1,25}$ = 4.769, P = 0.039, R^2 = 0.160). Despite these significance values, the low R^2-values and visual inspection of the regression plot suggest the relationship are very weak. These analyses indicate that the addition of rings in mature *X. pygmaeus* females does not occur in a predictable manner and may not be useful for inference of age in sexually mature females.

Discussion

In this study, large *X. pygmaeus* males did not produce large sons, unlike the large male *X. nigrensis*. These results indicate large size in *X. pygmaeus* males is not determined by Y-linked, *P*-alleles. This is consistent with the conclusions of a previous study that also found no evidence of patroclinous inheritance of large size in this species (Morris and Ryan, 1995). The results of the *X. nigrensis* crosses also clearly indicate that the allele for large size, if present, is transmitted from father to son and expressed under the conditions of this study.

Nevertheless, large males are still present in the El Nacimiento population and have been reported recently in the La Y Griega Vieja population (M. Ryan and G. Rosenthal, unpubl.). How large males are produced in this species is still not clear. Some of the more obvious alternative hypotheses are that large size is determined by autosomal genes (paternal or maternal), environmental factors, maternal genes, or interactions among any or all of these factors.

It has been suggested that hybridization of *X. pygmaeus* females with males of another swordtail species could introduce an allele for large size into the *X. pygmaeus* population. Kallman (1989) showed that a *P*-allele can be experimentally introduced into *X. pygmaeus* from *X. nigrensis* or *X. multilineatus* and is expressed. However, *X. pygmaeus* is allopatric from both of these close relatives and cannot naturally hybridize with them. Although *X. pygmaeus* is sympatric with *X. cortezi*, and could acquire an allele for large size through introgression, protein electrophoretic analysis does not support this explanation (G. Rosenthal and D. Morizot, unpubl.). However, our results indicate that, if an allele for large size is present in *X. pygmaeus* it is no longer associated with the *P*-locus and its expression pattern is no longer characteristic of Y-linked traits.

It has been shown in other swordtail species that social environment influences final size at maturity (Borowsky, 1978; Campton, 1992). The presence of larger males results in slight changes in size at maturity in *X. helleri*; some males delay final maturation until they reach a size larger than the largest male present (Campton, 1992), but their size does not exceed the size range associated with their *P*-allele. It has also been shown in *X. cortezi* that social context is critical for the attainment of large size regardless of the *P*-allele (Kallman, 1989); all males reared in isolation were small. We cannot address the possibility that social context has a similar influence on male size in *X. pygmaeus* because we reared all offspring in isolation. However, in a previous study of *X. pygmaeus*, where fry of each brood were raised together, no large sons were produced (Morris and Ryan, 1995). Therefore, we do not believe social influence is an important determinant of large size in males of this species.

Large size in *X. pygmaeus* could be influenced by an interaction between paternal and maternal genes within a population. Our data do not support this hypothesis because we detected no significant effects of mother size or population on the size of their sons. This is consistent with previous studies showing there is little, if any, maternal influence on size at maturity in males of closely related *Xiphophorus* species (Kallman, 1989; Morris and Ryan, 1990). However, strictly maternal effects could determine large male size through autosomal or X-linked alleles, but our study was not designed to detect them. These hypotheses are certainly worthy of further investigation.

Large male *X. pygmaeus* also could have attained their size through changes in maturation schedule. Differences in prematuration growth

rates of males of different size classes have been documented in the closely related *X. nigrensis*; males with the allele for large size grow to maturity at a slightly faster rate than small males (Kallman, 1989). However, in this study, growth rate and age at maturity among sons of large Nacimiento fathers and small La Y Griega Vieja fathers were similar, and sons of small Nacimiento fathers grew significantly more slowly and matured at significantly older ages than sons of large Nacimiento or small La Y Griega Vieja fathers. We also detected a small but significant elevation of postmaturation growth rate in sons of small Nacimiento fathers. These results suggest sons of small Nacimiento fathers might have the potential to reach greater size than sons of either La Y Griega Vieja fathers or large Nacimiento fathers despite their smaller size and increased age at maturity, and slower prematuration growth. However, the important point for this study is that the altered maturation schedules and growth are not a direct result of a Y-linked, *P*-allele for large size, because they occurred only in the sons of small Nacimiento fathers. None of our results indicate unequivocally the cause of the appearance of large *X. pygmaeus* males in the Río Huichihuayán. However, we can reject the hypothesis that large size is the result of a Y-linked *P*-allele. This confirms a previous study suggesting large size in male *X. pygmaeus* is not heritable from father to son as in other swordtail species (Morris and Ryan, 1995).

Under indirect models of sexual selection the heritability of large size is intimately tied to the fate of female preference for the trait. Under direct models, preference can be lost if females incur a cost when they consort with males possessing the preferred trait. Our results indicate no obvious reproductive costs to females who mate with large males. In *X. pygmaeus*, the variation in female preference for large males across populations is a bit mysterious. Clear knowledge of the basis of large size in males, as well as additional direct effects of this trait on females, would allow us to understand why the preference for large males has been lost in the Nacimiento, the population where large males occur, and why the preference persists in females of La Y Griega Vieja, a population from which large males are absent.

Acknowledgments

We thank B. Trainor, G. Rosenthal, T. Prude, C. Knight for their assistance with fish care and collection; K. Agnew, D. Cannatella, K. Kichler, M. Brauer, T. Wilcox, and J. Bull for useful comments on the manuscript; and D. Morizot for access to unpublished data. We acknowledge the resources provided by the Brackenridge Field Laboratory of the University of Texas at Austin. This research was funded by NSF grants IBN 92-21703 and 96-30230 to MJR. We thank the Government of Mexico for permission to conduct this research. All animals used were maintained according to Institutional Animal Care and Use Committee approval A-1496. Animals were returned to stock populations at Brackenridge Field Laboratory, University of Texas at Austin, after their use in this study.

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