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Ontogeny and Phylogeny in the Northern Swordtail Clade of *Xiphophorus*

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Abstract.— It has been hypothesized that morphological diversity within clades can be generated by simple alterations of shared developmental programs. However, few studies have examined changes in heterochrony, the rate and timing of developmental events, in an explicitly phylogenetic context. We studied how developmental patterns have changed phylogenetically in the northern swordtail clade of *Xiphophorus*. We reared individuals of an outgroup and seven of nine species in the clade and followed their development for ~300 days. For each individual, we used nonlinear regression to estimate three growth parameters: growth rate, adult body size, and age of cessation of growth. We estimated sword growth rates in males by linear regression. We then used the means of these growth parameters to construct standard growth curves for each species and to study growth patterns in a phylogenetic context. A combined phylogeny was constructed from both phenotypic and DNA sequence data. The phenotypic data set, compiled from the literature, consisted of 86 morphological, pigmentation, behavioral, and random amplified polymorphic DNA characters, many of which had not been used before for phylogenetic analysis. DNA sequence data from three genes for a total of 1284 bases were also obtained from the literature and included in the analysis. Relationships between growth parameters were examined by phylogenetically independent contrasts in relation to seven different phylogenies based on the most–parsimonious trees generated from the phenotypic, DNA sequence, and combined data sets; this allowed us to identify relationships between variables that were not sensitive to ambiguities in *Xiphophorus* phylogeny. Our analysis revealed statistically significant correlations between female body size and male body size, and between female growth rate and male sword growth rate, for all seven phylogenies. Marginally statistically significant relationships were also identified between female body size and female growth rate, and between female growth rate and male body size. We relate these relationships to what is known about the ecology, genetics, and behavior of *Xiphophorus* to better understand the evolution of growth patterns of both the body as a whole and the sword in particular. The relationship of these data to the evolution of swords is discussed. [Development; evolution; growth; heterochrony; independent contrasts; sword; *Xiphophorus*.]

Heterochrony, or changes in the timing of developmental events, has been shown to be a pervasive force in the evolution of morphology. Changes in onset, offset, and the rate of development of morphological characters have been the basis for much of the observed morphological variation among many types of organisms (Gould, 1977; Alberch et al., 1979; McKinney and McNamara, 1991). However, only when heterochronic changes are examined in a phylogenetic context can the evolutionary patterns and processes of heterochrony be understood (Fink, 1982).

The fish genus *Xiphophorus* (Poeciliidae), comprising 22 described species of swordtails and platyfishes (Fig. 1), lends itself particularly well to the study of heterochrony within a phylogenetic context (Strauss, 1990, 1992). Considerable phylogenetic information about the genus is already available (Rosen, 1979; Heinrich and Schroder, 1986; Rauchenberger et al., 1990; Haas, 1993; Meyer et al., 1994), and *Xiphophorus* are easily raised in the laboratory for developmental study (Kallman, 1975; Campton, 1992). Here we focus on the ontogenies of species in the northern swordtail clade (Rosen, 1960; Rauchenberger et al., 1990) to identify changes in developmental rates and timing that have occurred during the evolution of the group. The northern swordtail clade is of particular interest because its members span most of the generic range in adult body size and in the elaboration of an extension of the ventral fin rays of the caudal fin into the "sword" found in males of some

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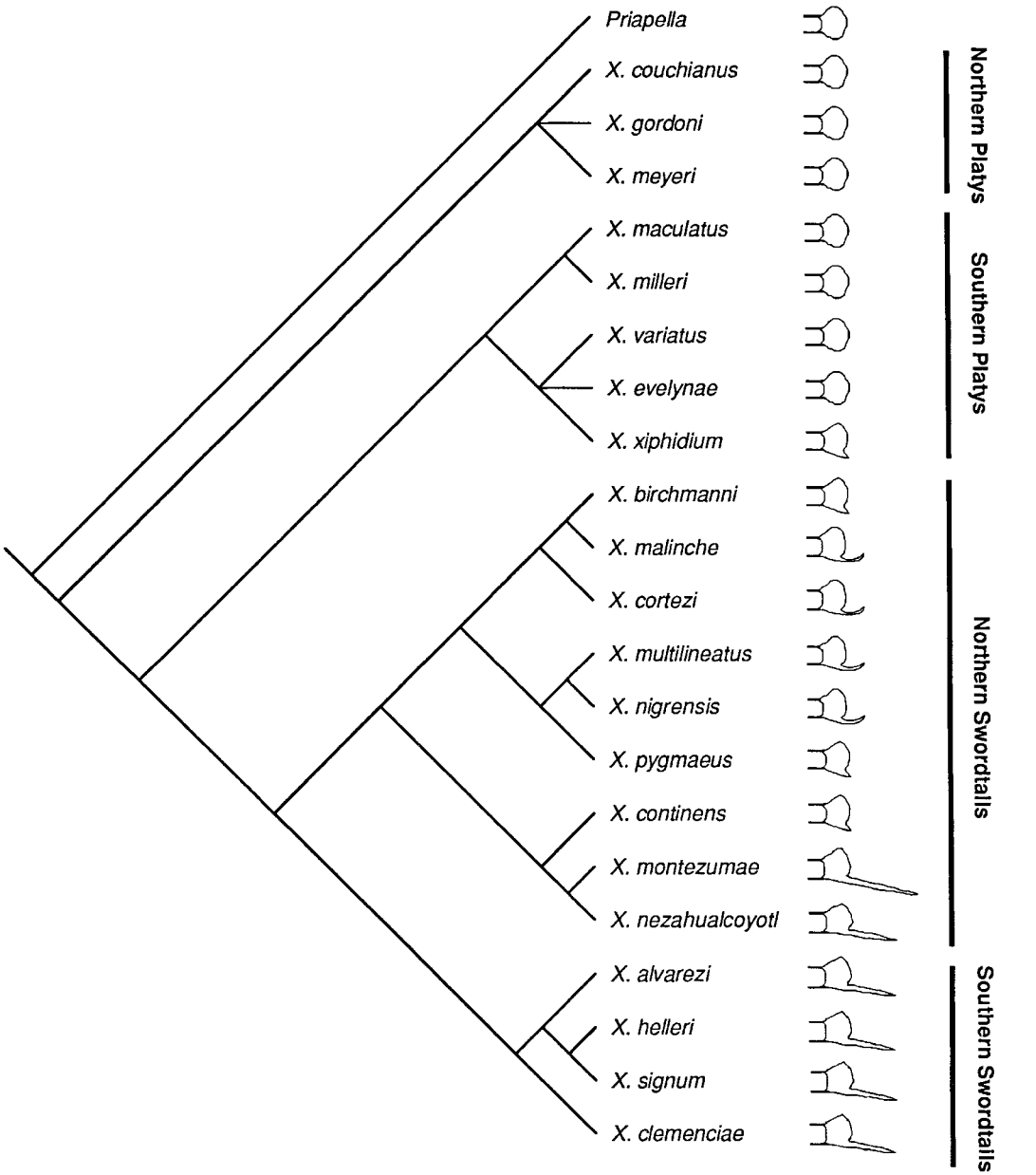

FIGURE 1. Phylogenetic hypothesis for species of the genus *Xiphophorus* as originally presented by Basolo (1991) and based on Rosen's (1979) phylogeny for the entire genus (morphology and pigmentation characters) and the phylogeny of the northern swordtail clade by Rauchenberger et al. (1990) (morphology, pigmentation, and electrophoretic characters). The phylogenies from these two sources were spliced together; the pictured hypothesis of phylogeny is not the result of an analysis of the combined phenotypic data sets. *X. andersi* is not included because it was not in either source phylogeny.

species in the genus (Rauchenberger et al., 1990; Basolo, 1996).

Understanding how growth patterns have evolved requires that they be examined in the context of a phylogeny. However, the available hypotheses of phylogeny (e.g., Rauchenberger et al., 1990; Meyer et al., 1994; Borowsky et al., 1995) appear to differ considerably for the species included in this study. This ambiguity has already contributed to a debate regarding the evolution of swords in males, the evolution of a preference for swords in females, and the validity of the preexisting sensory bias hypothesis of sexual selection (Meyer et al., 1994; Basolo, 1995a, 1995b, 1996; Shaw, 1995; Wiens and Morris, 1996).

Here we explore the evolution of swords in *Xiphophorus* by relating the growth of swords in males to the rates and timing of other features of *Xiphophorus* development. We interpret these developmental data in the context of three different phylogenies: a reanalysis of an augmented phenotypic data set (Rauchenberger et al., 1990), a molecular data set (Meyer et al., 1994), and a combined analysis including both data sets. We then used these three phylogenies as the basis for interpreting developmental data for overall body growth and sword growth rate by means of phylogenetically independent contrasts (Felsenstein, 1985). These contrasts reveal which growth parameters are related to one another and provide new suggestions for mechanisms by which swords may have evolved.

Methods

Ontogenetic Experiments

We obtained breeding stock for each of the nine species within the northern swordtail clade plus presumed outgroups, *X. helleri* and *X. clemenciae*, members of the southern swordtail clade identified as the sister group to northern swordtails (Rauchenberger et al., 1990; Meyer et al., 1994). Specimens were obtained from laboratory stocks generated from wild-caught specimens with known, well-documented locality information (Appendix 1). Because some of the species examined are genetically polymorphic for size (Kallman, 1989; Morris and Ryan, 1995), we used the largest available morphs within a species because these morphs take the longest amount of time to mature and therefore may be the most likely to include all developmental stages present in each species. We were not able to obtain males of the largest genotype of *X. multilineatus* during our breeding efforts, so males of the smallest morph of this species are included in our analyses. Breeding pairs were housed in 40-liter glass aquaria in an animal care facility at 22°C and 14 hr of illumination daily. All fish were fed ad libitum twice daily—in the morning with Tetramin Basic Flake Food, and in the evening with a frozen food composed of cooked shrimp, zucchini, canned tuna fish, human infant food, and gelatin (recipe available upon request). Tanks were checked twice a day for fry and when fry were produced, the adults were transferred to another aquarium, thus allowing the fry to remain in their natal tank for 30 days. Modal brood size was ~10 fish. At age 30 days, fry were removed from the tank and anesthetized with sodium bicarbonate solution according to the methods of Booke et al. (1978), except that the concentration of sodium bicarbonate used was 25 mg/liter. Standard length, total length, and the length of the caudal appendage (hypural plate to the tip of the sword), if present, were measured on anesthetized fish by placing them in a glass petri dish in a thin film of anesthetizing solution and measuring them with a steel ruler under a Wild M5 dissecting microscope to the nearest 0.5 mm.

After anesthetization, individual fish were placed in separate compartments (volume, 20 liters) of aquaria, to reduce possible crowding effects on growth rates (Campton, 1992). Fish could not see one another though the opaque divider, but water circulated between compartments through many small holes. Further measurements on anesthetized fish were taken at 30–day intervals until the fry were 300 days old or until 15 August 1995. Attempts were made to use this method to measure fry on their date of birth, but the stress associated with the procedure killed three fry of *X. nezahualcoyotl* from a brood of eight and appeared to have

severe effects on the further growth and development of the surviving fish. For this reason, later broods either were not measured on day 0 or were measured without being anesthetized, for these very young fish will remain motionless long enough to be measured without anesthesia.

When measured, fish were also sexed according to the development of the anal fin. In male poeciliid fish, the modified anal fin forms a gonopodium used for internal fertilization (Turner, 1941). Growth of the gonopodium by terminal addition of fin ray segments until sexual maturity (Turner, 1941) allowed recognition and staging of males, even at relatively early ages (Turner, 1941; Campton, 1992). Sexual maturity in females was recognized by the development of a gravid spot just anterior to the anus. Data were separated by sex for analyses of growth patterns.

Growth trajectories of individuals were analyzed by nonlinear regression with the NONLIN module of SYSTAT (Wilkinson, 1988). In general, *Xiphophorus* has nearly determinant growth; that is, the growth rate is nearly zero after sexual maturity is reached for all of the species in our laboratory. Given this pattern of growth, we estimated growth parameters for the initial growth rate (gr), final body size (bs), and the age of cessation of growth (ac), analyzing each individual separately. The model we fitted is given below:

$$\text{size} = \begin{cases} (\text{bs}) - (\text{gr})\ (\text{ac} - \text{age}), & \text{if age} < \text{ac} \\ \text{bs}, & \text{if age} \geq \text{ac} \end{cases}$$

Parameter estimates from these individual analyses were then used as the dependent variables in one-way ANOVAs with MiniTab for Windows (version 9.2, 1993).

Rates of sword growth in males of each species were analyzed by linear regression with MiniTab before interspecific comparison by one-way ANOVA. Rates of sword growth were analyzed rather than maximum sword size because the sword reaches its maximum size very late in development and because, given the time constraints on our experiments, we could not be certain that the sword of each individual reached what would have been its final length. The variance for maximum sword length is extremely large for most of the species in our study because the estimate of maximum sword length depends on only a single measurement for each individual. The variance for the rate of sword growth, which takes into account sword size over a longer period of time, is much lower, and gives better estimates to be used for independent contrasts, which cannot take variance around a mean into account (Felsenstein, 1985).

Means of parameter estimates were used to create standard growth curves for each species. When one-way ANOVA indicated significant interspecific differences between growth parameters, means were compared by the sequential variant of the Q method of multiple comparison (Snedecor and Cochran, 1967) to identify homogeneous sets of species with similar parameter means.

Phylogenetic Framework

The genus *Xiphophorus* has been the topic of several phylogenetic studies using a variety of methods and data sets. Analyses have included an evolutionary–taxonomical consideration of morphological data (Rosen, 1960); a cladistic analysis of morphological and pigmentation characters (Rosen, 1979); a cladistic analysis of morphological, pigmentation, and allozyme data for a portion of the genus (Rauchenberger et al., 1990); a cladistic analysis of morphological and behavioral characters (Haas, 1993); an analysis of mitochondrial and nuclear DNA sequences (Meyer et al., 1994); and an analysis of DNA fingerprinting patterns (Borowsky et al., 1995). However, no attempt has been made previously to use all of these data for analysis.

In an attempt to produce a robust estimate of phylogeny, we conducted three sets of analyses. Our first set of analyses included phylogenetically informative characters gleaned from the literature that were not DNA sequence characters. This group of characters were of four types: morphology, growth, and sex-determination characters; pigmentation characters; behavioral characters; and random amplified polymorphic DNA (RAPD) and restriction fragment

length polymorphism (RFLP) characters. All of these characters involve levels of biological organization above the level of individual nucleotides within a DNA sequence, so we refer to these characters collectively as the phenotypic data set. Our second set of analyses was a reanalysis of DNA sequence data from three genes first described by Meyer et al. (1994), which we refer to as the DNA sequence data set. Finally, we combined the phenotypic and DNA sequence data sets into a single analysis. All phylogenetic analyses were performed by the computer program PAUP* (test version 4.0d64 provided by D. L. Swofford), using the heuristic search algorithm along with the tree bisection and reconnection branch swapping algorithm. Ten replicate analyses with random number seeds were performed for each phylogenetic analysis in an effort to find all the most–parsimonious trees. In addition, 100 bootstrap replicates were performed for each analysis with 10 replicate random addition heuristic searches at each replicate. Groups compatible with 50% majority–rule consensus were retained in the final consensus tree because collapsing these nodes would result in a loss of power in the phylogenetically independent contrast analysis discussed below (Grafen, 1989; Purvis and Rambaut, 1995).

Because synonyms for the *Xiphophorus* species considered here have been discussed recently (Rauchenberger et al., 1990), they require little further discussion except to note that *X. marmoratus* (Obregon-Barbarosa and Contreras-Balderas, 1988) is probably synonymous with *X. meyeri* (Schartl and Schroder, 1987) according to collection locality data, morphological descriptions, and opinions of experts on *Xiphophorus* taxonomy (K. D. Kallman and R. L. Borowsky, pers. comm.).

Phenotypic analysis.—We collected a total of 86 phenotypic characters for 22 species of *Xiphophorus* and representatives of the other three genera in the tribe Poecilini (sensu Rosen and Bailey, 1963). The core of our phenotypic data set was 23 characters (excluding allozyme characters) for the species of the northern swordtail clade from Rauchenberger et al. (1990). The allozyme data from Rauchenberger et al. (1990) are being augmented and analyzed separately by D. Morizot (pers. comm.). To the Rauchenberger et al. (1990) data matrix we added another 63 characters for northern swordtails as well as for other *Xiphophorus* species and representatives of the other three genera in the tribe Poecilini (sensu Rosen and Bailey, 1963). The majority of the additional characters are from Rosen (1979), Haas (1993), Borowsky et al. (1995), or Basolo (1996). The total phenotypic data set of 86 characters for 28 taxa includes 17 morphological, growth, and sex-determining characters; 27 pigmentation characters; 13 behavioral characters; and 29 RAPD and RFLP characters. Descriptions of each character are given in Appendix 2. We also reanalyzed Rauchenberger et al.'s (1990) original data set, because it dealt specifically with the northern swordtail clade taxa that were included in our ontogenetic experiments.

The data matrix for the phenotypic data set is shown in Appendix 3. Of the character states in the phenotypic data set, 32.4% are unknown and scored as missing. A disproportionate amount of data is missing for the outgroups; within the genus *Xiphophorus* 15.6% of the data are missing. Most phenotypic characters in the data set either were binary or had character states that were not obviously ordered and so were analyzed as unordered characters. Other characters showed logical arrangements of character states; these arrangements are described in Appendix 2.

DNA sequence analysis.—The DNA sequence data used here originate from Meyer et al. (1994), who sequenced a total of 1,284 bases from each species, including 762 bp from two mitochondrial genes (cytochrome *b* and d-loop) and 522 bp from a nuclear gene (*X-src*). The smaller 16S ribosomal RNA data set of Bisazza et al. (1997), which samples a larger number of Poeciliid outgroups, was not included in our analysis because it includes only one *Xiphophorus* species and would introduce large amounts of missing data into our phylogenetic analysis. The original, aligned DNA sequence data set used by Meyer et al. (1994) was kindly provided by A. Meyer and is available as

a NEXUS file on the Systematic Biology Web site (www.utexas.edu/depts/systbiol). Gaps, transition–to–transversion ratios, and regions of ambiguous sequence alignment were treated as by Meyer et al. (1994) unless otherwise noted. Multiple individuals from the same species were merged before analysis by using MacClade (Maddison and Maddison, 1992); when individuals of the same species differed at specific basepair positions, the positions were coded as being polymorphic, as defined by Swofford (1993:95). This data set was then reanalyzed to ensure that merging the taxa would not significantly alter the topology of the tree produced by bootstrap analysis.

Combined analysis.—Sequence data and phenotypic data were combined for a combined analysis of phylogeny (Kluge and Wolf, 1993). In several different taxonomic groups, combining different types of data in a single analysis has been shown to increase the resolution of estimates of phylogeny—compared with using the different data sets independently to estimate phylogeny and then using consensus methods to resolve differences between the estimates (Vane-Wright et al., 1992; Eernisse and Kluge, 1993; Jones et al., 1993; Wheeler et al., 1993). For our combined analyses, each character received an equal weight.

Tests of Incongruence among Phylogenetic Trees

We used two methods to test for incongruence among phylogenetic trees. First, we conducted Templeton tests (Larson, 1994) between sets of most–parsimonious trees obtained from each of the three phylogenetic analyses. This procedure can be used to test whether alternative, suboptimal trees are a significantly poorer fit to a data set than the shortest tree for that data set. The suboptimal trees used in the Templeton test for each phylogenetic tree are the most–parsimonious trees obtained from the analysis of the other two data sets. Each Templeton test was performed twice with use of the Kishino–Hasegawa pairwise test as implemented in PAUP* (test version 4.0d64), once for each of the three data sets on the reciprocal tree, for a total of six tests. However, because Templeton tests do not always give a symmetrical result and because Templeton tests are extremely conservative (sometimes detecting highly significant incongruence even though combining data sets results in an increase in phylogenetic accuracy [Cunningham, 1997]), we also conducted a second type of test, the Incongruence Length Difference (ILD) test. The ILD is the difference between the number of steps required by the individual partitions to generate a tree topology and the number of steps it takes for the combined partitions to generate the same topology. The ILD test compares the ILD statistic of the specified partitions of informative characters with the ILD for a series of randomized partitions of the same sizes as the original partitions, but representing a mixture of characters from each partition (Cunningham, 1997). This test, as implemented in PAUP* (test version 4.0d64) as the partition–homogeneity test (100 replicates, with simple taxon sequence addition for each replicate, and a "maxtrees" setting of 100 trees per replicate to reduce analysis time), was used on the combined data set with the phenotypic data set and DNA sequence data set as the partitions after uninformative characters were removed. An additional variation of the ILD test was also implemented as part of our analyses. This variation, which we will refer to as the ILD jackknife partition test, involves the sequential removal of each of the seven component data partitions (four categories of phenotypic data and three sets of DNA sequence data) individually, and in pairs, followed by an ILD test of the remaining partitions to identify which partitions of phylogenetically informative characters are responsible for a disproportionate amount of phylogenetic incongruence. When data partitions responsible for incongruence are removed, the ILD test can reveal a decrease in the incongruence among the remaining data partitions.

Phylogenetic Mapping of Growth Parameters

We used phylogenetically independent contrasts (Felsenstein, 1985; Martins and Garland, 1991) to determine whether any of the parameters estimated from our onto-

genetic experiments correlate with one another with respect to each of the most–parsimonious trees obtained from our three phylogenetic analyses. Phylogenetically independent contrast methods do not allow missing data, so species without developmental data available were pruned from the trees. We used the DNADIST program of PHYLIP version 3.572c (Felsenstein, 1993) to calculate distances according to the Kimura (1980) model between each of the remaining taxa for which ontogenetic data were available, plus *X. maculatus* as an additional outgroup. The molecular sequence data gathered by Meyer et al. (1994) and used in the phylogenetic analyses described previously were used to calculate these distances. We then used the FITCH program of PHYLIP (Felsenstein, 1993) to calculate branch lengths by the Fitch–Margoliash method (Fitch and Margoliash, 1967) for the phenotypic, molecular, and combined trees for the northern swordtail clade determined by our phylogenetic analyses, with *X. helleri* and *X. maculatus* as outgroups. Fitch distances were calculated independently for males and females because the analyses involved different numbers of species. Fitch distances (FD) were then transformed so that adjusted branch lengths = 10^{FD} to standardize them for analysis by contrasts (Garland et al., 1992). The three phylogenies with branch lengths and the average parameter values for the ontogenetic data for each species were used to calculate independent contrasts (Felsenstein, 1985) with use of the CONTRAST program of PHYLIP (Felsenstein, 1993).

Results

Ontogenetic Experiments

Growth data were obtained for seven species—*X. birchmanni*, *X. continens*, *X. cortezi*, *X. montezumae*, *X. multilineatus*, *X. nigrensis*, and *X. pygmaeus*, all in the northern swordtail clade—and for the outgroup, *X. helleri* (Table 1). We were not able to obtain

TABLE 1. Estimates of *Xiphophorus* growth parameters. Values given as mean (SD).

Species	*n*	Growth rate (mm/day)	Adult body size (mm)	Age of cessation of growth (days)	Male sword growth rate (mm/day)
Male					
X. birchmanni	10	0.32 (0.078)	40.7 (5.07)	105 (17.1)	0.0351 (0.0168)
X. continens	1	0.11 (0.000)	30.4 (0.00)	140 (0.00)	0.0104 (0.0000)
X. cortezi	4	0.19 (0.057)	36.1 (5.78)	140 (2.93)	0.0400 (0.0151)
X. montezumae	3	0.21 (0.034)	45.7 (4.81)	160 (1.95)	0.0984 (0.0306)
X. multilineatus	3	0.15 (0.035)	25.2 (1.53)	107 (5.28)	0.0369 (0.0033)
X. nigrensisa[a]	0				
X. pygmaeus	6	0.22 (0.071)	27.0 (3.11)	109 (9.21)	0.0165 (0.0056)
X. helleri[b]	8	0.29 (0.031)	47.0 (3.78)	118 (14.8)	0.1933 (0.0372)
Female					
X. birchmanni	6	0.23 (0.066)	39.8 (3.33)	139 (27.1)	
X. continens	4	0.15 (0.029)	36.9 (0.80)	158 (26.4)	
X. cortezi	5	0.25 (0.042)	39.8 (3.33)	134 (12.3)	
X. montezumae	2	0.26 (0.020)	53.8 (4.87)	176 (24.4)	
X. multilineatus	6	0.17 (0.018)	25.2 (1.69)	96 (12.6)	
X. nigrensis	3	0.21 (0.067)	36.9 (4.71)	128 (16.9)	
X. pygmaeus	6	0.21 (0.044)	32.5 (3.99)	123 (6.06)	
X. helleri[b]	13	0.39 (0.074)	54.5 (3.95)	128 (21.7)	

[a] No male *X. nigrensis* were included in the ontogenetic portion of this study.
[b] *X. helleri* was the outgroup used for interpretation of the ontogenetic data.

growth data for *X. clemenciae*, *X. malinche*, or *X. nezahualcoyotl*.

Standard growth curves for both sexes of each species, computed from means of growth parameters estimated from individual growth data for all individuals of a species (as described above), are pictured in Figure 2. In general, the male *Xiphophorus* reared in the laboratory grew linearly until they approached sexual maturity, at which time they stopped growing. Females showed a similar pattern, although they continued growing at a very low rate after becoming sexually mature. The female age at cessation of growth approximates the time at which the transition from the higher juvenile growth rate to lower adult growth rate occurs. Females generally grew to larger maximum body sizes than males of the same species except that there appeared to be no size difference between adult males and females of *X. birchmanni* and *X. multilineatus*. One-way ANOVA for each of the four estimated parameters (initial growth rate, final size, age of cessation of growth, and male sword growth rate), with values for each individual fish used as replicates, show significant differences among species for both sexes (Table 2).

Changes in both growth rate and age of cessation of growth seem to have influenced adult body size in *Xiphophorus* (Table 1). The males of the three largest species of *Xiphophorus* included in this study fall into two categories, based on the results of the multiple comparisons test (Table 3). Two species, *X. birchmanni* and *X. helleri*, are characterized by males that grow very quickly for a relatively short period, while males of the third, *X. montezumae*, grow at a significantly lower rate but for a significantly longer time. The results of the multiple comparisons tests showed that males of the smallest species also differ in growth pattern (Table 3). *X. continens* males grow very slowly for about the median duration of growth, and *X. pygmaeus* males grow at the median growth rate (significantly greater than that of *X. continens*) but for the second shortest duration of any of the species examined and significantly shorter than *X. continens*. The male *X. multilineatus* of the small genotype included in our experiments grew both very slowly and for a relatively short time.

The multiple comparisons test also revealed differences in female growth patterns (Table 3). The large *X. helleri* grow very rapidly but for a moderate length of time. *X. montezumae* grow significantly less rapidly (though this is the second fastest growing species in the genus) but for the longest period of the species studied. *X. multilineatus*, the species with the smallest females among those examined, grow at a low growth rate for the shortest time for any species of *Xiphophorus* included in this study. The slightly larger *X. pygmaeus* females grow both somewhat faster (but not significantly faster) and significantly longer than *X. multilineatus*.

One–way ANOVA of rates of sword growth showed significant differences between species (Table 2). The multiple comparisons test revealed that *X. helleri*, a species with a large sword, had a sword growth rate significantly greater than all other *Xiphophorus* species examined (Table 3). *X. montezumae*, which also grows large swords, had the second highest sword growth rate, but it was not significantly greater than that of the remaining species examined (Table 3). Even though differences between the sword growth rates of the remaining species were not statistically significant, there is a trend of decreasing sword growth rates associated with smaller sword sizes. *X. montezumae* have a more rapid sword growth rate than do species with shorter and less elaborate swords (e.g., *X. cortezi* and *X. multilineatus*), which in turn have greater sword growth rates than species that rarely produce swords larger than 3 mm (e.g., *X. continens*, *X. birchmanni*, and *X. pygmaeus*). Sword growth rate may therefore be an important determinant of sword size in *Xiphophorus*.

Multiple comparison analysis of the species means for each of the growth parameters showed at least two homogeneous subsets for each growth parameter (Table 3). In all but two cases, species in the northern swordtail clade were included in more than one homogeneous subset. This indicates there are statistically significant dif-

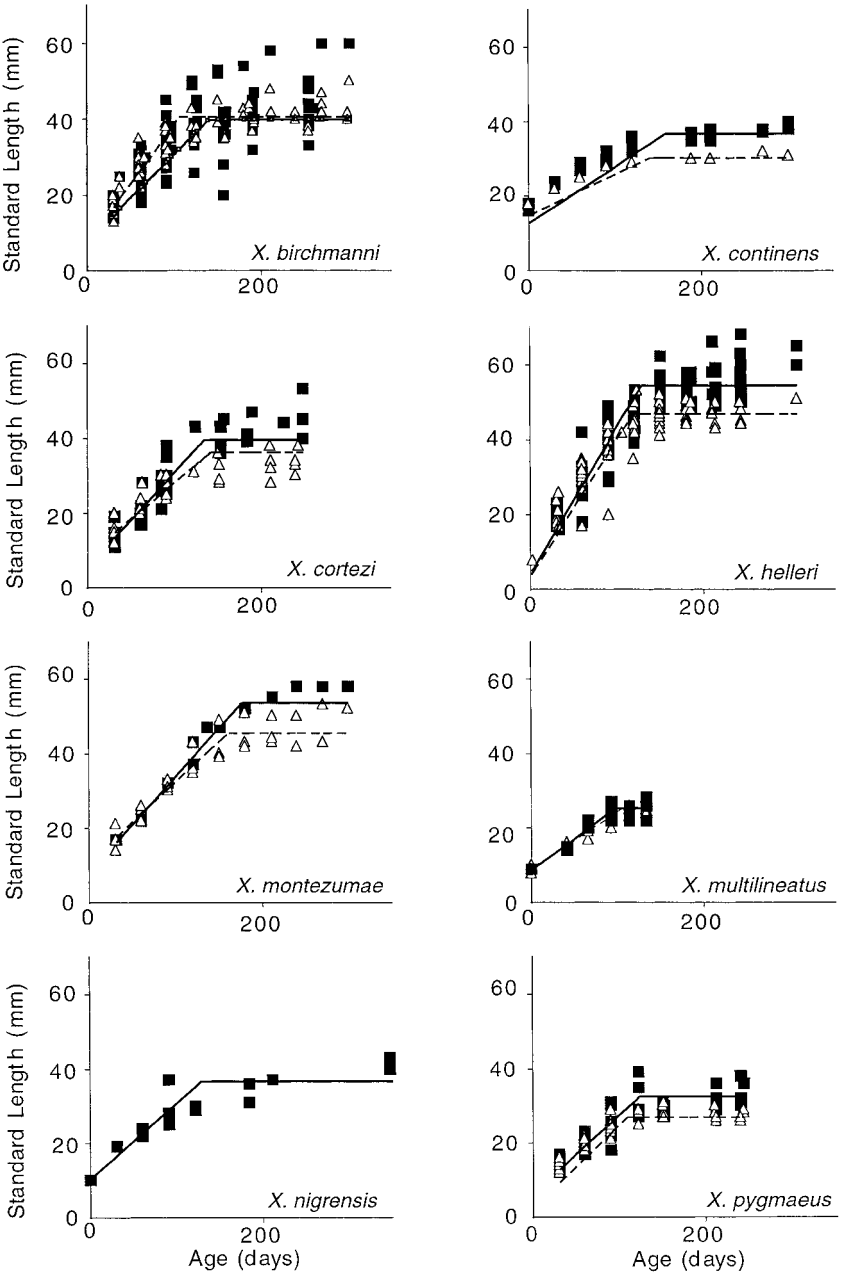

FIGURE 2. Growth curves of eight species of *Xiphophorus*. ■ = females; △ = males. Individual fish were measured every 30 days during development and the means of individual growth parameters were used to estimate standard curves for each sex of each species. In each graph, the solid line is the female standard curve and the dotted line is the male standard curve, except for *X. nigrensis*, for which no males were measured.

ferences in values between species within the northern swordtail clade, and the significant results obtained from an ANOVA of each growth parameter (Table 2) cannot

TABLE 2. One-way ANOVA tables for interspecific differences in growth parameters for male and female swordtails.

Growth parameter	df	MS error	*F*	*P*
Males				
Initial growth rate	6	0.00351	6.92	< 0.001
Adult body size	6	18.3	20.40	< 0.001
Age of cessation of growth	6	161	10.94	< 0.001
Rate of sword growth	6	0.000545	55.55	< 0.001
Females				
Initial growth rate	7	0.00344	13.97	< 0.001
Adult body size	7	16.8	40.77	< 0.001
Age of cessation of growth	7	458	4.90	< 0.001

be entirely the result of differences between *X. helleri*, the outgroup, and members of the clade. We therefore performed independent contrast analysis on our ontogenetic data set without including ontogenetic characters in the phylogenetic analyses.

Phylogenetic Analyses

Phylogenetic analyses included separate analyses of phenotypic data and DNA sequence data, as well as analysis of these data sets combined. Analysis of the complete phenotypic data set produced the majority–rule consensus tree of 702 equally most–parsimonious trees shown in Figure 3. Many of these trees differ only in the placement of the outgroups relative to one another and represent only 99 different topologies for the *Xiphophorus* genus itself. Within the northern swordtail clade, only 3 different topologies are represented among these most–parsimonious trees. The results of the analysis of the phenotypic data are somewhat different from those of Rauchenberger et al. (1990). In particular, the relationships among *X. multilineatus*, *X. nigrensis*, and *X. pygmaeus* are unclear, and all three possible species pairs are represented among the

TABLE 3. Homogeneous subsets of parameter values as determined by the studentized range (Q) method of multiple comparison (Snedecor and Cochran, 1967). For each parameter, species were ordered, left to right, from greatest value to smallest value, and species with parameter values that are not significantly different ($P < 0.05$) from each other are included on the same line of the table. Full species names are given in Table 1.

	Groups of species with homogeneous subsets of parameter values															
Growth parameter	Males							Females								
Initial growth rate	*bir*	*hel*						*hel*								
		hel	*pyg*	*mon*	*cor*	*mul*			*mon*	*cor*	*bir*	*pyg*	*nig*	*con*	*mul*	
						mul	*con*									
Adult body size	*hel*	*mon*	*bir*	*cor*				*hel*	*mon*							
			bir	*cor*	*con*					*bir*	*cor*	*con*	*mul*	*nig*	*pyg*	
				cor	*con*	*pyg*	*mul*									
Age of cessation	*mon*	*cor*	*con*					*mon*	*con*							
of growth		*cor*	*con*	*hel*					*con*	*bir*						
				hel	*pyg*	*mul*	*bir*			*bir*	*cor*	*hel*	*nig*	*pyg*		
															mul	
Sword growth rate	*hel*															
		mon	*cor*	*bir*	*mul*	*pyg*	*con*									

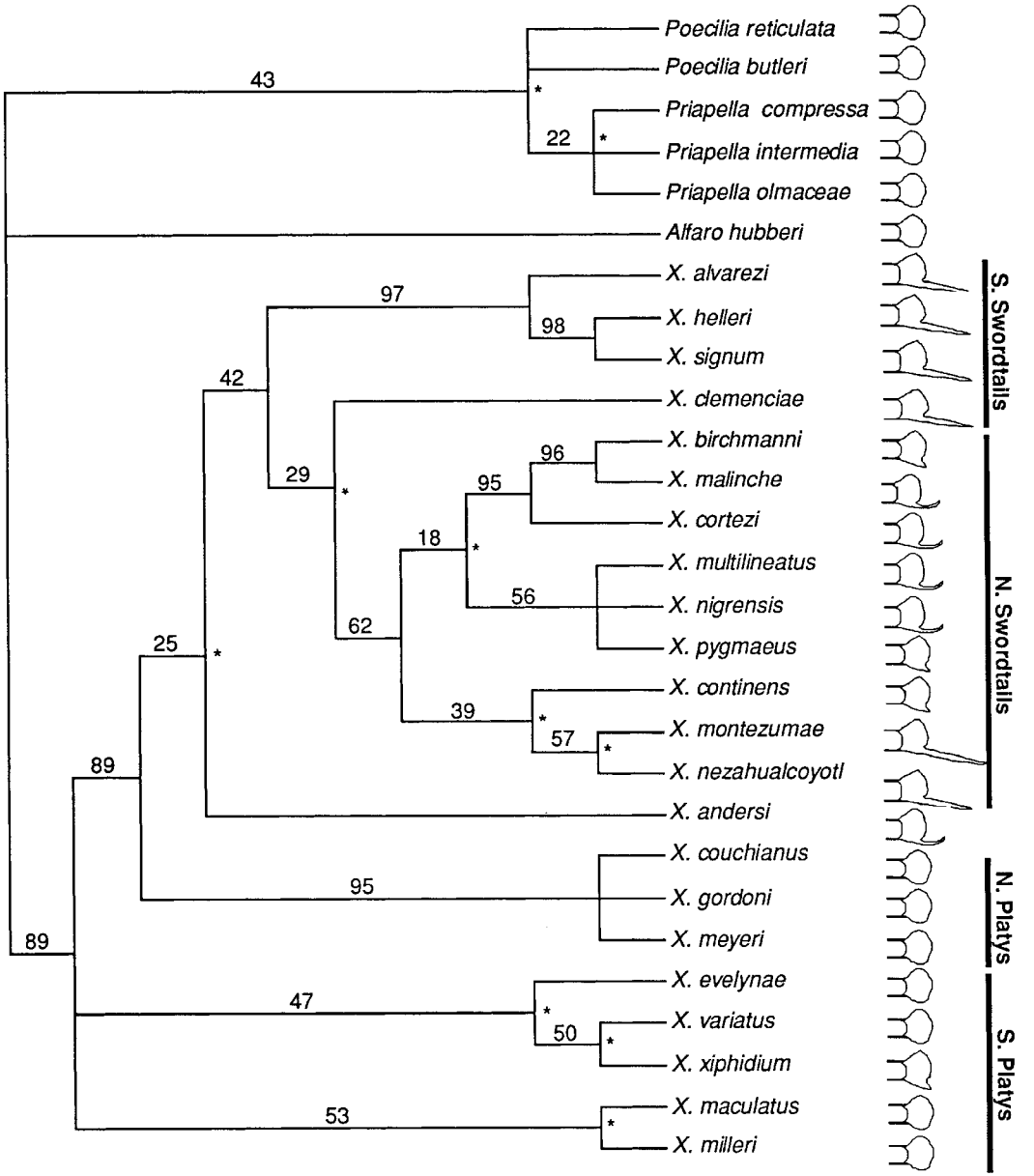

FIGURE 3. *Xiphophorus* phylogeny calculated by a maximum parsimony analysis of the phenotypic data set of 86 morphological, pigmentation, behavioral, and RAPD characters (see Appendix 2) for all 22 described *Xiphophorus* species and 3 outgroups. A heuristic search (10 replicates) found 702 shortest trees of length 212, consistency index = 0.632, and retention index = 0.740. A 50% majority–rule consensus of these trees is shown; * indicates node not supported by all most–parsimonious trees; numbers above branches indicate bootstrap support from 100 heuristic search replicates. The position of *X. clemenciae* differs between most–parsimonious trees, with different trees either supporting monophyly of the southern swordtail clade (33% of 702 trees), or placing *X. clemenciae* as the sister group to the northern swordtail clade and making the southern swordtails paraphyletic (67% of 702 trees).

equally most–parsimonious trees, including the topologies presented by Rauchenberger et al. (1990) and Borowsky et al. (1995). The relationships among *X. continens*, *X. montezumae*, and *X. nezahualcoyotl* are also not entirely resolved in this analysis. However, even though Rauchenberger et al. (1990) present the clade as being completely resolved, our reanalysis of their original data set produced two trees, one with *X. montezumae* and *X. nezahualcoyotl* as sister taxa (as presented by Rauchenberger et al.), and the other with the node unresolved.

Overall, our phenotypic phylogeny largely resembles the phylogenetic hypothesis presented in Figure 1 (Basolo, 1991), with high bootstrap values (≥89%) for the basal node of the genus and for two of the nodes in the southern swordtail clade. Our analysis also supports the northern swordtail clade but with a lower bootstrap value (62%). However, our phylogeny also differs in several respects from the Basolo (1991) tree. Our analysis places *X. andersi*, a species that had never been examined in a phylogenetic context by using phenotypic characters, in an intermediate position between swordtails and platyfish. Our phylogeny also does not place the northern platys in a basal position within the genus but instead places it as a sister group to the swordtails. Finally, the southern platyfish, which are placed basally in our analysis, have a completely unresolved basal node. The remaining nodes among the southern platys, which are shared by Basolo's (1991) phylogeny (Fig. 1) and our phenotypic data set (Fig. 3), do not have large bootstrap values associated with them.

Analysis of the DNA sequence data produced three most–parsimonious trees; a 50% majority–rule consensus of these trees is shown in Figure 4. A 50% majority–rule bootstrap tree (not shown) reveals the same interspecific relationships that Meyer et al. (1994:Fig. 2) presented. The 50% majority–rule consensus of the three most–parsimonious trees groups *X. maculatus*, a species generally considered to be a southern platy (Figs. 1, 3), with the southern swordtails. This unconventional placement is not revealed by the 50% majority–rule bootstrap tree presented by Meyer et al. (1994:Fig. 2). However, the equally puzzling placement of *X. clemenciae*, a southern swordtail (Fig. 1), among the platys is shown by both the trees of Meyer et al. (1994) and our Figure 4. In our analyses of DNA sequence data, the northern swordtails appear as the most basal clade of *Xiphophorus*, whereas the traditional phylogeny (Fig. 1) and the phenotypic analysis (Fig. 3) indicate the northern swordtails to be more recently derived than the platyfish. With the exception of *X. maculatus*, the platyfish are monophyletic in the molecular phylogeny and deeply nested within the genus *Xiphophorus* (Fig. 4), whereas in our phenotypic analysis they are basal and paraphyletic (Fig. 3). Finally, the arrangement of taxa within the northern swordtails and the southern platys are also different from those hypothesized by both Basolo's (1991) phylogeny (Fig. 1) and our analysis of the phenotypic data set (Fig. 3).

Analysis of the combined data sets produced the 50% majority–rule consensus tree of two most–parsimonious trees shown in Figure 5. Within the northern swordtail clade, only one tree topology is represented among these two most–parsimonious trees. In the arrangement of the major clades, the combined analysis is very similar to that of the molecular analysis, although the details of relationships within those clades are quite different. One notable difference is that in the combined phylogeny, *X. clemenciae* is not nested among the platyfish (as it is in molecular phylogeny), but rather is the basal member of the southern swordtail clades, as it is in Basolo's (1991) phylogeny (Fig. 1). This node is only weakly supported, so the placement of *X. clemenciae* is still ambiguous. There are also a few differences between molecular and combined analyses at various nodes among the southern platyfish, but generally the bootstrap values for these nodes in the combined analysis are low. Finally, within the northern swordtail clade, except for the sister taxa *X. multilineatus* and *X. nigrensis*, the relationships among the taxa within the clade differ considerably between the DNA sequence and the combined data analyses (Figs. 4, 5).

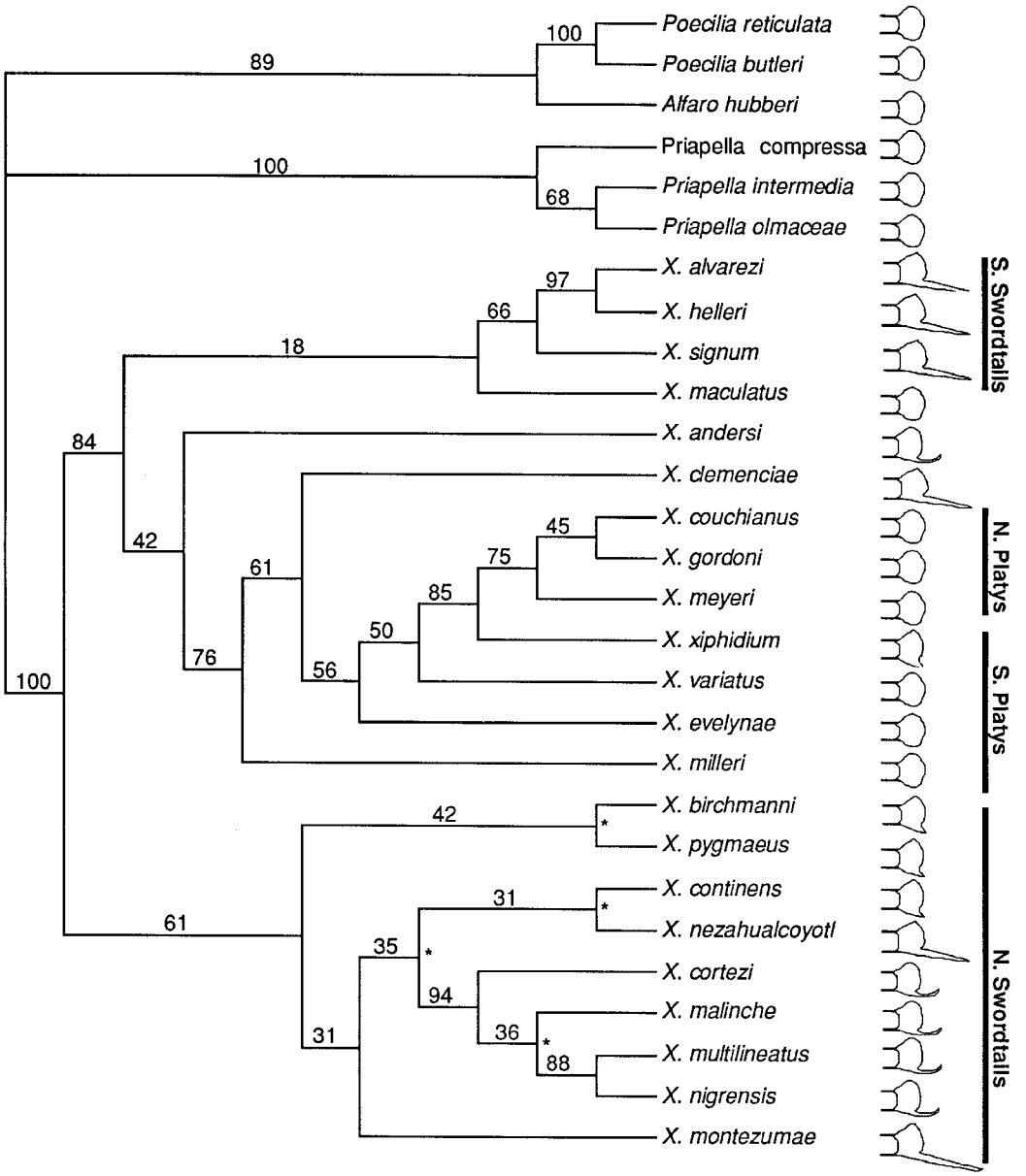

FIGURE 4. *Xiphophorus* phylogeny calculated by a maximum parsimony analysis of the DNA sequence data set consisting of 1,284 aligned bases (1,230 bases when gaps are excluded) from d-loop, cytochrome *b*, and *X-src* gene fragments for all 22 described *Xiphophorus* species and 6 outgroup species. D-loop sequences were analyzed with a 2:1 transversion:transition ratio. Transversions and transitions were weighted equally for the other genes. Three shortest trees of length 2,480, consistency index = 0.841, and retention index = 0.657 were found in 10 replicate heuristic searches. A 50% majority–rule consensus of these trees is shown; * indicates node not supported by all most–parsimonious trees; numbers above branches indicate bootstrap support from 100 heuristic search replicates. The position of *X. clemenciae*, usually considered a southern swordtail, and the position of *X. maculatus*, usually considered a southern platy, are grouped with the platys and the southern swordtails, respectively.

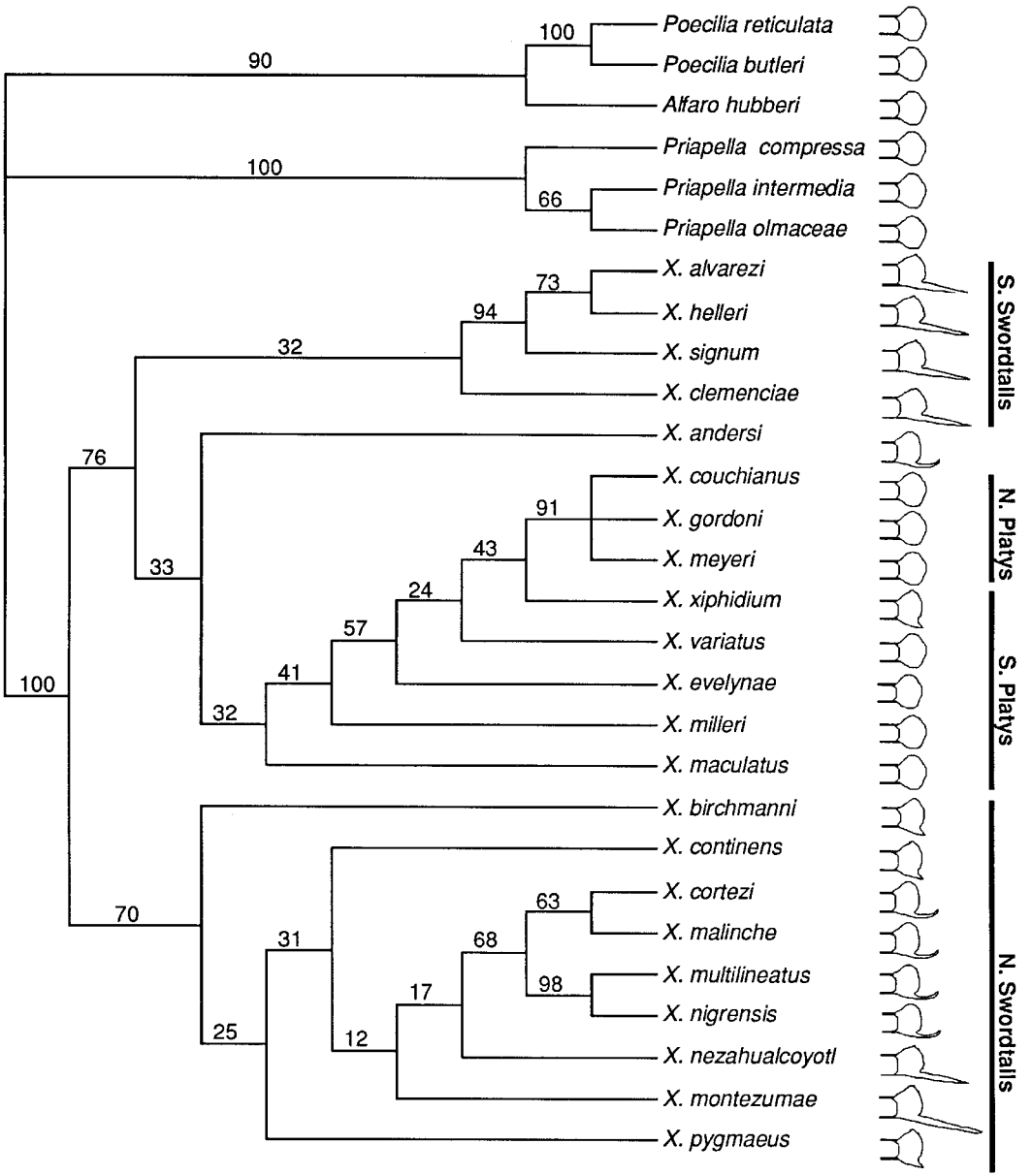

FIGURE 5. *Xiphophorus* phylogeny calculated by a maximum parsimony analysis of the combined data set containing all of the characters in both the phenotypic and DNA sequence data sets. D-loop sequences were analyzed with at 2:1 transversion:transition ratio for this analysis. All characters were weighted equally. Two shortest trees of length 2,737, consistency index = 0.811, and retention index = 0.643 were found in 10 replicate heuristic searches. A 50% majority–rule consensus of these trees is shown; numbers above branches indicate bootstrap support from 100 heuristic search replicates.

Tests of Incongruence among Phylogenetic Trees

The results of the Templeton tests revealed symmetrical significant differences between the most–parsimonious trees from the phylogenetic analyses of the phenotypic and DNA sequence data sets ($t = 5.59–8.93$, $P < 0.0001$). The Templeton tests revealed similar significant reciprocal differences between the phenotypic and combined data sets ($t = 3.91–9.27$, $P < 0.0002$). However, the Templeton tests showed a nonreciprocal relationship between the trees from the DNA sequence and combined data sets. For the sequence data set, the most–parsimonious combined trees were significantly worse than optimal ($t = 2.00–2.14$, $P = 0.032–0.046$), but for the combined data set, the most–parsimonious DNA sequence trees were not significantly worse than optimal ($t = 0.95–1.36$, $P = 0.17–0.34$).

The ILD test also revealed a significant incongruity between the phenotypic and DNA sequence data sets ($P = 0.01$). Removals of single partitions in the ILD jack-knife partition test did not remove any significant incongruity (all $P = 0.01$), and most removals of pairs of partitions also failed to remove any significant incongruity. However, removal of the pigmentation and *X-src* data partitions or the d-loop and *X-src* data partitions left the remaining data set without significant incongruence remaining ($P = 0.07$ and 0.09, respectively). Removal of all three data sets that were indicated at introducing incongruence into the data set (pigmentation, d-loop, and *X-src*) resulted in a further decrease in the incongruence of the remaining data partitions ($P = 0.23$). Ten replicate analyses with random number seeds were performed for each of the three data sets with the partitions removed, in an effort to find all the most–parsimonious trees. Only a single most–parsimonious tree was found for the data set with pigmentation and *X-src* removed (length 2300, consistency index = 0.808, retention index = 0.649; Fig. 6A); 8 most–parsimonious trees were found for the data set with d-loop and *X-src* removed (length 1717, consistency index = 0.883, retention index = 0.679; Fig. 6B); and 32 most–parsimonious trees were found with all three partitions removed (length 1637, consistency index = 0.897, retention index = 0.683; Fig. 6C). Although these analyses produced trees with lower levels of incongruence, they did not produce trees with higher average levels of bootstrap support for the nodes of the tree, nor did they reduce the number of most–parsimonious trees with respect to the northern swordtail clade, the clade of principal interest here; therefore, these trees were not included in the independent contrast analysis discussed below.

Phylogenetic Patterns of Growth

The results of the phylogenetically independent contrasts are reported in Table 4. Seven different phylogenies were used to perform these contrasts, representing three topologies derived from the phenotypic data set, three topologies derived from the DNA sequence data set, and one topology from the combined data set. Only correlations that were significant at $P < 0.1$ for all seven phylogenies were considered robust with respect to the phylogenetic ambiguity present in the *Xiphophorus* data set. Male body size and female body size were correlated at $P < 0.005$; male sword growth rate and female growth rate were correlated at $P < 0.008$; female body size and female growth rate were correlated at $P < 0.054$; and male body size and female growth rate were correlated at $P < 0.078$. Even though our ability to reconstruct the phylogeny of the northern swordtails is imperfect, we think that this set of relationships, because they are at least marginally statistically significant for all seven phylogenetic trees, appears to represent real relationships between the developmental traits we studied.

Discussion

Phylogenetic Framework

Evolutionary interpretations of the ontogenetic data described above depend on the phylogenetic framework used. Some argue that given multiple data sets, even those as different as morphological and DNA sequence data, all available evidence should always be used to construct the phylogeny

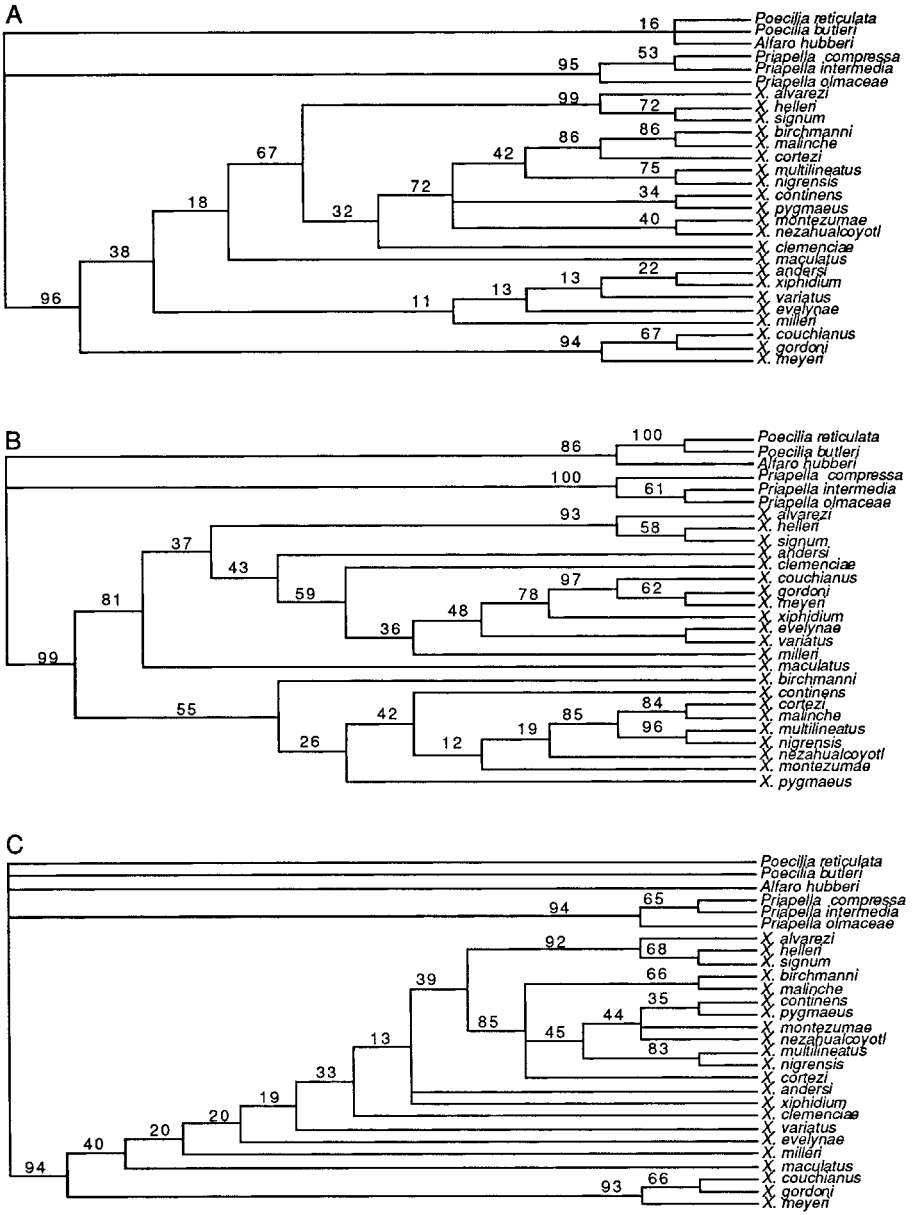

FIGURE 6. Fifty percent majority–rule consensus trees based on ILD jackknife partition test results. Numbers associated with nodes indicate the bootstrap value associated with that node. (A) Consensus of eight equally most–parsimonious trees generated from a heuristic search of the combined data set with d-loop and *X-src* DNA sequence partitions removed. (B) The single most–parsimonious tree generated from a heuristic search of the combined data set with pigmentation and *X-src* DNA sequence partitions removed. (C) Consensus of 32 equally most–parsimonious trees generated from a heuristic search of the combined data set with pigmentation, d-loop, and *X-src* data partitions removed.

(e.g., Kluge and Wolf, 1993). In contrast, others point out that the effectiveness of combining data is highly case-dependent and, under certain circumstances, may not even

TABLE 4. Correlation coefficients (r) and significance values (P) for phylogenetically independent contrasts for three *Xiphophorus* phylogenies; df = 5 except where noted. FGR = female growth rate; FBS = female body size; FAC = female age of cessation of growth; MGR = male growth rate; MBS = male body size; MAC = male age of cessation of growth; and MSGR = male sword growth rate.

Growth parameter	Phylogeny	r (and P)					
		FGR	FBS	FAC	MGR	MBS	MAC
FBS	Phenotypic 1	0.84 (0.008)[a]					
	Phenotypic 2	0.85 (0.007)[a]					
	Phenotypic 3	0.70 (0.054)[a]					
	Sequence 1	0.78 (0.022)[a]					
	Sequence 2	0.76 (0.027)[a]					
	Sequence 3	0.86 (0.006)[a]					
	Combined	0.79 (0.020)[a]					
FAC	Phenotypic 1	0.18 (0.866)[a]	0.67 (0.052)[a]				
	Phenotypic 2	0.06 (0.990)[a]	0.55 (0.070)[a]				
	Phenotypic 3	–0.19 (0.647)[a]	0.54 (0.057)[a]				
	Sequence 1	0.13 (0.764)[a]	0.70 (0.072)[a]				
	Sequence 2	0.05 (0.905)[a]	0.67 (0.162)[a]				
	Sequence 3	0.24 (0.559)[a]	0.69 (0.164)[a]				
	Combined	0.14 (0.742)[a]	0.71 (0.050)[a]				
MGR	Phenotypic 1	0.58 (0.173)	0.54 (0.208)	0.24 (0.603)			
	Phenotypic 2	0.65 (0.115)	0.62 (0.134)	0.22 (0.627)			
	Phenotypic 3	0.64 (0.121)	0.51 (0.242)	0.03 (0.948)			
	Sequence 1	0.50 (0.250)	0.33 (0.464)	0.03 (0.951)			
	Sequence 2	0.66 (0.108)	0.49 (0.265)	0.04 (0.926)			
	Sequence 3	0.71 (0.074)	0.67 (0.096)	0.31 (0.491)			
	Combined	0.71 (0.074)	0.67 (0.096)	0.31 (0.491)			
MBS	Phenotypic 1	0.81 (0.027)	0.94 (0.001)	0.58 (0.176)	0.68 (0.092)		
	Phenotypic 2	0.82 (0.024)	0.94 (0.001)	0.48 (0.276)	0.75 (0.053)		
	Phenotypic 3	0.70 (0.078)	0.91 (0.005)	0.36 (0.428)	0.69 (0.084)		
	Sequence 1	0.75 (0.051)	0.95 (0.001)	0.67 (0.098)	0.54 (0.206)		
	Sequence 2	0.75 (0.050)	0.93 (0.002)	0.60 (0.535)	0.71 (0.073)		
	Sequence 3	0.83 (0.022)	0.95 (0.001)	0.66 (0.104)	0.82 (0.023)		
	Combined	0.83 (0.022)	0.95 (0.001)	0.66 (0.103)	0.83 (0.023)		
MAC	Phenotypic 1	0.23 (0.623)	0.51 (0.246)	0.62 (0.139)	–0.36 (0.434)	0.34 (0.452)	
	Phenotypic 2	0.12 (0.801)	0.41 (0.362)	0.58 (0.168)	–0.38 (0.402)	0.23 (0.616)	
	Phenotypic 3	–0.18 (0.706)	0.34 (0.462)	0.66 (0.107)	–0.57 (0.184)	0.09 (0.842)	
	Sequence 1	0.41 (0.355)	0.83 (0.022)	0.88 (0.008)	–0.10 (0.837)	0.71 (0.075)	
	Sequence 2	0.24 (0.593)	0.72 (0.070)	0.87 (0.009)	–0.13 (0.777)	0.53 (0.223)	
	Sequence 3	0.40 (0.371)	0.70 (0.081)	0.85 (0.015)	0.07 (0.874)	0.55 (0.202)	
	Combined	0.40 (0.371)	0.70 (0.081)	0.85 (0.015)	0.07 (0.874)	0.55 (0.201)	
MSGR	Phenotypic 1	0.92 (0.004)	0.80 (0.033)	0.11 (0.807)	0.44 (0.321)	0.68 (0.091)	0.21 (0.644)
	Phenotypic 2	0.92 (0.004)	0.78 (0.038)	0.02 (0.968)	0.52 (0.228)	0.68 (0.093)	0.10 (0.838)
	Phenotypic 3	0.89 (0.007)	0.62 (0.139)	–0.30 (0.512)	0.49 (0.270)	0.74 (0.057)	–0.18 (0.707)
	Sequence 1	0.88 (0.008)	0.74 (0.057)	0.13 (0.778)	0.26 (0.574)	0.79 (0.033)	0.38 (0.402)
	Sequence 2	0.88 (0.008)	0.71 (0.076)	0.05 (0.919)	0.49 (0.263)	0.79 (0.035)	0.17 (0.716)
	Sequence 3	0.91 (0.005)	0.78 (0.037)	0.18 (0.702)	0.59 (0.165)	0.67 (0.101)	0.26 (0.572)
	Combined	0.91 (0.005)	0.06 (0.0057)	0.18 (0.702)	0.59 (0.165)	0.74 (0.056)	0.26 (0.571)

[a] df = 6.

be appropriate (Bull et al. 1993; de Queiroz, 1996). In our analyses, both the Templeton and ILD tests indicate that phenotypic and DNA sequence data sets are incongruent with one another, indicating that combining these data sets will not improve phylogenetic resolution. In spite of all of the work by many researchers over the last 50 years (Basolo, 1996), we apparently still do not have enough information to produce

a robust phylogeny of *Xiphophorus*. It is therefore premature to map character–state changes onto any *Xiphophorus* phylogeny without taking into account the inherent ambiguities of the phylogenetic reconstruction. Our requirement that independent contrast analysis identify statistically significant correlations at the $P < 0.10$ level between ontogenetic characters for seven different phylogenetic hypotheses (three phenotypic, three DNA sequence, and one combined topology) takes this ambiguity into account and makes our independent contrast results robust with respect to the phylogenetic ambiguity. The inclusion or exclusion of the combined topology in the independent contrast analysis does not influence the results of that analysis.

Ontogeny Interpreted in a Phylogenetic Framework

Given the considerable ambiguity in the phylogeny of *Xiphophorus*, what we need to analyze our growth data is a method of studying character evolution that will work in spite of such ambiguity. Standard analysis by phylogenetically independent contrasts relies on a knowledge of the "true" phylogeny of the group being considered (Felsenstein, 1985). Rather than taking a consensus of the three phylogenies, which would result in the loss of most of the resolution and therefore the power of the independent contrasts analysis (Grafen, 1989; Purvis and Rambaut, 1995), we chose to calculate phylogenetically independent contrasts separately for each phylogeny and then to discuss those relationships that have at least marginally statistically significant results for all seven phylogenies, as suggested by Harvey and Pagel (1991:121).

Analyses of phylogenetically independent contrasts that include more than two continuous characters are further complicated by issues of multiple comparison. As the number of characters being compared increases, so does the number of correlation coefficients being calculated and the possibility for an increase in the type I error rate. This issue has not, to our knowledge, been discussed in the context of phylogenetically independent contrasts, but it has been discussed for other statistical analyses by Rice (1989). Correction for multiple comparisons is certainly not standard for phylogenetically independent contrasts (e.g., Sessions and Larson, 1987; Pyron, 1996), but the sequential Bonferroni method suggested by Rice (1989) or the slightly less conservative Dunn–Sidak method described by Sokal and Rohlf (1995:241) could be used when large numbers of independent contrasts are being performed. Our justification for not using a Bonferroni–type correction is that seven independent correlations must be significant at $P < 0.10$ before we accept a relationship between two growth parameters. The probability of a single independent contrast to be significantly correlated for all seven phylogenetic topologies by chance alone is 1×10^{-7}, and the probability of this sort of type I error occurring anywhere in our entire independent contrast data set is 2.1×10^{-6}, an error rate low enough to make a Bonferroni-type correction unnecessary.

When two developmental characters are shown to be correlated with one another, there are at least two possible explanations. The first possibility is that the two characters are controlled by the same gene or set of genes in both organisms (pleiotropy) and that those genes are regulated in a similar manner in the species being considered. Alternatively, perhaps both traits respond to selection under the same environmental conditions and have evolved in parallel. Because the classical genetics of *Xiphophorus* have been studied for many years (e.g., Gordon, 1928; Kallman, 1975, 1989), there is a real possibility of differentiating between these two alternatives for some of the correlations presented here.

One of the most interesting results of our analysis is that male and female adult body size correlate with one another very strongly, even though neither growth rate nor age at cessation of growth correlate strongly with one another between the sexes in the species we examined (Table 4; Fig. 7). One possible explanation for this is that the mechanism by which adult body size is determined is the same in both sexes, but growth rate and cessation of growth are controlled independently of body size.

Alternatively, perhaps selection on body size has been similar in both males and females. However, neither of these hypotheses account for the observed difference in body size between males and females in most species of *Xiphophorus*. The prediction of both of these hypotheses is that males and females of any given species of *Xiphophorus* would have the same adult body size—but that was generally not the case for the taxa included in our ontogenetic study.

Another hypothesis is that there has been selection for males and females to maintain the same relative body sizes, even if the absolute body sizes of both sexes have changed considerably. In particular, there may have been selection for a constant relative body size to facilitate internal fertilization. Before fertilization can take place, the male must orient behind the female, bring the gonopodium to a forward position, and swim forward, inserting the gonopodial tip into the female's gonopore (Farr, 1989). A male that is slightly smaller than the female he is trying to fertilize may be better able to maneuver around the female and inseminate her than is a larger male. Large interspecific differences in body size may therefore be a contributing prezygotic barrier to hybridization between species. Prezygotic barriers to hybridization are likely to be very important in preventing hybridization in *Xiphophorus*. Hybridization in sympatric natural populations of different species of *Xiphophorus* is extremely rare (Rauchenberger et al., 1990), although there are few significant postzygotic barriers to hybridization (Morizot et al., 1991; Coyne, 1992; Schartl et al., 1995). Even under laboratory conditions, species of very different sizes will often not hybridize without the assistance of artificial insemination (K. Kallman, pers. comm.). This hypothesis that body size contributes to prezygotic barriers to interspecific fertilization predicts that sympatric species should have very different body sizes, whereas allopatric species should have more similar body sizes. At least to some degree, sympatric species do appear to differ in body size more than allopatric species do (Gordon, 1953).

According to analysis of independent contrasts, body size and growth rate are significantly correlated with one another within females, but not in males (although the correlation in males would be specific except for the influence of one of the DNA sequence data sets). However, body size is more strongly correlated with growth rate than age of cessation of growth in both sexes (Table 4; Fig. 7). This suggests that overall changes in adult body size between species in this clade of *Xiphophorus* are more strongly affected by accelerations and decelerations of a common growth trajectory

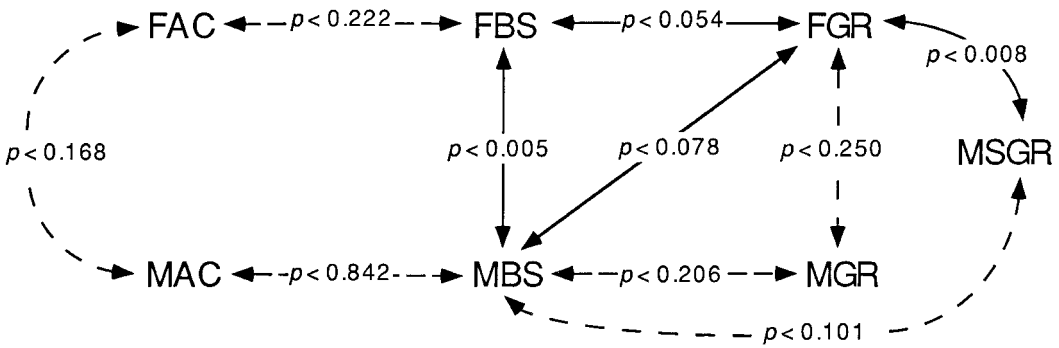

FIGURE 7. Summary diagram of relationships between growth characteristics. Pairs of parameters that showed significant ($P < 0.05$) or nearly significant ($P < 0.10$) correlations for phenotypic, DNA sequence, and combined phylogenies are indicated by solid lines. Pairs of parameters that did not show significant or nearly significant correlations for all three types of phylogenies, but are discussed in the text, are indicated by dashed lines. See Table 4 for abbreviations.

than by abbreviations or elongations of that trajectory. If this is the case, then neoteny and acceleration have been more important than progenesis and hypermorphosis in determining differences in adult body size between species (McKinney and McNamara, 1991:17).

These interspecific differences in growth pattern are very different from known patterns and control of growth within species. In male *Xiphophorus* of a given species, where the genetics of adult body size is relatively well understood, much of the difference in size between males can be related to specific alleles at a single locus, the *p* locus, that alter the age at cessation of growth but generally do not change the rate of growth between males of different genotypes (Sohn and Crews, 1977; Kallman and Borkoski, 1978; McKenzie et al., 1983; Kallman, 1989). In only two species of *Xiphophorus*, *X. nigrensis* and *X. multilineatus*, does the genotype at the *p* locus or at a very closely linked second locus alter growth rates (Appendix 2, character 11; Rauchenberger et al., 1990). Apparently, therefore, body size variation within populations of a given species is due primarily to polymorphism in the age at cessation of growth, whereas size differences between species are due to differences in growth rate. Although the mechanism, presumably genetic, by which growth rate is determined in *Xiphophorus* is unknown, it is probably not controlled by the *p* locus. If all growth rate variation were due entirely to *p* locus variation, we would probably observe more growth rate variation within species associated with the observed variation in the age of cessation of growth.

The significant relationship found by phylogenetically independent contrasts between male body size and female growth rate is probably the result of the highly significant correlation between male and female adult body size and the significant correlation between female body size and female growth rate (Table 4; Fig. 7) rather than a description of a statistically independent relationship.

In independent contrast analysis, male sword growth rate was significantly correlated with female growth rate but not with male growth rate (Table 4; Fig. 7). Why sword growth rate should be significantly correlated with an aspect of female development, and not an aspect of male development, is not clear. One possibility is that the same sets of genes influence female body growth rate and male sword growth rate. All of the structural genes required to produce a sword are autosomal because both hormone–treated females (Kallman and Bao, 1987) and reproductively senile females of some species of *Xiphophorus* (Kallman, 1984) can exhibit many aspects of male morphology, including the growth of a sword. Therefore, because females have all of the structural genes necessary to produce a sword, it is at least possible that these same genes play a role in determining female growth rate. Another hypothesis is that the selective environments that have favored high rates of female body growth in these species of *Xiphophorus* also favor high rates of sword growth in males. Not enough is currently known about the ecology of *Xiphophorus* to support or refute this hypothesis.

Male sword growth rate was also marginally correlated with male body size (Table 4; Fig. 7), suggesting that, overall, species with larger male body sizes have greater sword growth rates. Except for *X. nigrensis* and *X. multilineatus*, the same exceptional species discussed in reference to growth rates above, there is no evidence of a pleiotropic effect between intraspecific adult body size and male sword growth rate (Appendix 2, character 12; Rauchenberger et al., 1990). Genetic data have shown that the introduction of a large (*L*) allele at the *p* locus by introgression into *X. continens* and *X. pygmaeus* (both of which lack swords) results in adult animals with a larger adult body size but still lacking swords (Rauchenberger et al., 1990:21). A possible explanation for these results is that the genes that determine adult body size in males and females are genetically dissociable from genes that determine high sword growth rates. Or perhaps the presence or absence of a sword is controlled by a genetic mechanism independent of what controls the size and growth rate of a sword, which in turn may

be linked to the genetic mechanism that controls adult body size.

Selectionist explanations for the correlation between adult body size and sword growth rate (and therefore large swords) are also possible. Large body size is associated with greater fecundity in females (e.g., Moyle and Cech, 1988; Nichol and Pikitch, 1994). In males, large body size is associated with social dominance (Borowsky, 1987; Campton, 1992, Morris et al., 1992), greater mating success (Borowsky, 1981; Ryan and Wagner, 1987; Zimmerer and Kallman, 1989; Ryan et al., 1990), and greater swimming endurance (Ryan, 1988). Large, colored swords seem to increase male mating success (Basolo 1990a, 1990b, 1995a, 1995b). Because social dominance in males also contributes to increased mating success (Morris et al., 1992), differences in male reproductive fitness alone could result in selection for both large body size and large swords. A component of this increased male reproductive fitness may simply be the increased conspicuousness of large males with large swords (Halnes and Gould, 1994). Under conditions in which the appropriate genetic variation exists and the benefits of high visibility exceed the costs of high visibility, one might predict both large body size and large swords to be under positive selection.

The visibility hypothesis is supported by the fact that genetically determined body size variation (Kallman, 1989), the ability to produce a sword under suitable hormone treatment (Zander and Dzwillo, 1969), and female preference for the presence of a sword (Basolo, 1995b) are all phylogenetically widespread in *Xiphophorus*. Therefore, under conditions in which high visibility is favored, large body size and large swords could be selected rather easily. These same conditions of high reward and low cost for being highly visible may also select for noncryptic pigmentation patterns. At least two different body pigmentation patterns have been shown to increase male *Xiphophorus* mating success (Borowsky and Khouri, 1976; Morris et al., 1995), and other pigmentation patterns found on the sword itself are likely to have a similar effect (Basolo, 1996). It is therefore not surprising that all swords or caudal protrusions of any appreciable length in the genus *Xiphophorus* are pigmented, and we conclude that sword length and sword coloration are likely to evolve in parallel.

Relevance of Ontogeny to the Evolution of Swords

The current debate about the evolution of swords in *Xiphophorus* focuses on the selective forces that contributed to the origin of these structures (e.g., Meyer et al., 1994; Basolo, 1995a, 1995b; Shaw, 1995; Wiens and Morris, 1996). However, this debate largely ignores what may be an equally interesting problem—that is, how these structures are lost (Meyer, 1997). In addition, the discussion has largely ignored problems associated with ambiguities in the phylogeny of these organisms and uncertainties in the reconstruction of ancestral states of the sword (Schluter et al., 1997). It is extremely likely that swords have been both gained and lost in the genus *Xiphophorus*; therefore, the selective factors that have contributed to the loss of these structures deserve more attention. If there is a preference among females for males with swords, what are the circumstances that result in the loss of swords in some taxa? The interspecific growth correlations we have demonstrated show how selection on correlated characters may influence both the gain and loss of sword morphology. For example, in all of the available phylogenies, the most–parsimonious reconstruction of sword evolution indicates that the absence of a sword in *X. continens* is a derived condition. We suggest that selection for small adult body size in males and selection for slow female growth rate in *X. continens* may have contributed to the complete loss of the sword in males, even though females may still prefer males with this structure. Only an understanding of the genetic and developmental mechanisms that produce swords, in combination with a better knowledge of the ecological and behavioral context in which these structures are used in these fish, will provide us with a complete picture of how swords can originate and be modified.

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Appendix 1. Origins and Pedigree Numbers of *Xiphophorus* Stocks.

Species	Parental stock origin	Stock center pedigree no.
X. birchmani	Chicontepic, Rio Calabozo System, Veracruz, Mexico, 1983	6604, 6609
X. clemenciae	Finca San Carlos, Rio Sarabia, Oaxaca, Mexico, 1968	6766, 6786
	Rio Grande, Oaxaca, Mexico, 1992	6452, 6501
X. continens	Ojo Frio, San Luis Potosi, Mexico, 1981	6540, 6764
X. cortezi	Rio Huichihuayan, Rio Axtla System, San Luis Potosi, Mexico, 1984	6532, 6541
X. helleri	Sarabia, Rio Sarabia, Oaxaca, Mexico, 1968	6702
X. malinche	Rio Canali, Hidalgo, Mexico, collection date not available	6560
X. montezumaea[a]	Rascon, Rio Axtla System, San Luis Potosi, Mexico, 1981	6586
	Rascon, Rio Gallinas System, San Luis Potosi, Mexico, collection date not available	6510, 6521
X. multilineatus	Rio Coy, San Luis Potosi, Mexico, collection date not available	not applicable
X. nezahualcoyotl	Ocampo, Rio Tamesi System, Tamaulipas, Mexico, 1984	6550, 6801
X. nigrensis	Rio Choy, San Luis Potosi, Mexico, collection date not available	6527
X. pygmaeus	Rio Huichihuayan, Rio Axtla System, San Luis Potosi, Mexico, 1972	6479, 6549, 6727

[a] All individuals of *X. montezumae* used in the growth pattern analyses were from the Rio Gallinas population.

Appendix 2. Descriptions of Phenotypic Characters

Morphology, Growth, and Sex-Determination Characters

1. *Sword.*—This character has received a great deal of attention over the years and there has been a great deal of controversy over how it should be coded (Rauchenberger et al., 1990: character 36 [discussed but not included in their data matrix]; Basolo, 1991, 1996; Meyer et al., 1994; Wiens and Morris, 1996; Meyer, 1997). We followed the ordered coding suggested by Meyer et al. (1994): Complete absence of a sword was coded as 0; caudal protrusion or small sword (0.1–0.3 times the length of the caudal fin, generally ≤ 3 mm; Basolo, 1996:character P) was coded as 1; large sword (0.7–6 times the length of the caudal fin, generally > 6 mm; Basolo, 1996:character E) was coded as 2. We justify this coding because we have considered sword pigmentation characters elsewhere (characters 19–24) and because pigmentation of the sword and caudal fin clearly is controlled by loci that are largely independent of the genes involved in sword growth. According to our definition, *X. andersi*, *X. birchmanni*, *X. continens*, *X. pygmaeus*, and *X. xiphidium* have protrusions, and *X. alvarezi*, *X. clemenciae*, *X. cortezi*, *X. helleri*, *X. malinche*, *X. montezumae*, *X. multilineatus*, *X. nezahualcoyotl*, *X. nigrensis*, and *X. signum* have large swords. Basolo (1996) reported that *X. continens* lacks a protrusion, but we have observed males of this species with small (~ 1 mm) protrusions. All the other species in the genus *Xiphophorus*, the species in the genera *Priapella* and *Alfaro*, and most of the species in the genus *Poecilia* lack swords or protrusions (Rosen and Bailey, 1963; Meyer et al., 1994). There has been considerable discussion about the nature of the sword-like structures in *Poecilia* without consensus yet about whether these structures are homologous to those found in *Xiphophorus* (Meyer et al., 1994; Basolo, 1996; Meyer, 1997). To avoid controversy, we have left the character state for the genus *Poecilia* as missing data for this character.
2. *Sword consisting exclusively of unbranched rays.*—The presence of branched rays in the sword was coded as 0; absence was coded as 1 (Rauchenberger et al., 1990:character 2).
3. *Upturned sword.*—Certain species in the genus have upturned swords (Rosen, 1979; Rauchenberger et al., 1990), whereas others either lack a sword entirely or have a relative straight caudal appendage. Straight swords were coded as 0, upturned swords were coded as 1, and the absence of a sword was coded as — and treated as missing for PAUP analysis (Rauchenberger et al., 1990:character 4).
4. *Distal serrae on ray 4p of the gonopodium.*—Within *Xiphophorus*, the distal serrae on ray 4p are quite variable, ranging from poorly developed (Rauchenberger et al., 1990:character 23) to well developed (Rosen, 1979), and in some species, well developed and fused (Rosen, 1979:character 9; Schartl and Schroder, 1987). These serrae are absent in the outgroups (Rosen and Bailey, 1963). This character was coded 0 when serrae

are absent; 1 when poorly developed; 2 when well developed; and 3 when well developed and fused. The character states were ordered for analysis.

5. *Well-formed hook on ray 5a of the gonopodium*.—All swordtails have a well-formed hook on ray 5a of the gonopodium, but in the southern swordtails (except for *X. clemenciae*) this hook is greatly enlarged (Rosen, 1979:character 35; Rauchenberger et al., 1990:character 35 [discussed, but not included in their data matrix]). The platyfishes and *X. andersi* lack this enlarged hook (Meyer and Schartl, 1979; Rosen, 1979). The absence of a large hook was coded as 0; the presence of a large hook was coded as 1.

6. *Granular tissue on the dorsal part of the hook on ray 3 of the gonopodium*.—*Alfaro* and *Poecilia* lack a hook and its associated granular tissue on ray 3 of the gonopodium in males (Rosen and Bailey, 1963). *Priapella* has a slightly decurved hook on ray 3 with a dorsal covering of dense membranous or cartilaginous tissue (Rodriguez, 1997). In species in the genus *Xiphophorus*, this covering forms an ossified blade on the dorsal aspect of the more highly decurved hook on ray 3 (Rosen, 1979:character 3). Rodriguez (1997) has argued that the ray 3 hook in *Priapella* is not homologous to the blades found on ray 3 in the *Xiphophorus* species because it differs in both shape and composition. However, the shape of the hook or blade on ray 3 (as will be discussed further below) is extremely labile, and the ossification of cartilaginous tissue is the normal course of events in formation of endochondral bone, so the argument for the homology of these structures (Rosen and Bailey, 1963; Rosen, 1979) seems reasonable. In *X. alvarezi* the hook on ray 3 is shortened and strongly decurved (Rosen, 1979:character 34). *Xiphophorus helleri* and *X. signum* share these characteristics and additionally have the blade of granular tissue modified so that it sharply points distally, with the overlying ramus of ray 4a strongly angular where it conforms with the pointed tip of the blade (Rosen, 1979:character 37). Finally, in many of the platyfish, the ray 3 hook has become greatly enlarged (Rosen, 1979). The absence of ray 3 hook and granular tissue was coded as 0; the presence of a short, slightly decurved hook on ray 3 was coded as 1; the presence of a short, highly decurved ray 3 hook was coded as 2; the presence of a short, highly decurved ray 3 hook with the blade pointed sharply distally was coded as 3; and the presence of a greatly enlarged ray 3 hook was coded as 4. This character was coded as a character-state tree, an ordered character type described by Swofford (1993:13); the possible state transformations are shown in Figure A1.

7. *Subdistal spines on ray 3 of the gonopodium*.—*Alfaro* and *Poecilia* do not have subdistal spines on ray 3. *Priapella* has a few elongate but peglike spines. All species of *Xiphophorus* have large, angular subdistal spines on gonopodial ray 3. *X. couchianus*, *X. meyeri*, and *X. gordoni* have spines with the distal tips scalloped or deeply indented (Rosen, 1979:character 8). Absence of subdistal spines was coded as 0; presence of elongate and peglike spines was coded as 1; presence of large and angular spines was coded as 2; presence of large, angular spines with scalloped or indented distal tips was coded as 3. This character was treated as ordered.

8. *Size of segments of the distal ramus of ray 4a of the gonopodium*.—In some members of the southern swordtails, the segments of the distal ramus of ray 4a are enlarged at the point of downward curvature over the blade of ray 3 (Rosen, 1979:character 34). ray 4a segments not enlarged was coded as 0; segments somewhat enlarged was coded as 1; and segments very enlarged and flared was coded as 2. This character was treated as ordered.

9. *Subdistal serrae on ray 4p of the gonopodium*.—Members of the genera *Poecilia*, *Priapella*, and *Xiphophorus* have a large number ($\geq$ 10) of well-developed subdistal serrae. Species in the genus Alfaro have only a few very poorly developed subdistal serrae (Rosen and Bailey, 1963). The presence of a few, small subdistal serrae was coded as 0; the presence of $>$ 10 well-developed serrae was coded as 1.

10. *Head bump*.—This ridge of fatty tissue develops between the occiput and the dorsal fin in the males of *X. malinche* and *X. birchmanni*; it was discussed by Rauchenberger et al. (1990:characters 30). Species that possess this tissue were assigned a 1; species that lack a head bump were assigned a 0.

11. *Male pelvic fin fleshy in the distal third*.—A fleshy pelvic fin is characteristic of males in the genera *Xiphophorus* and *Poecilia*. This character is absent in *Alfaro* and *Priapella* (Rosen and Bailey, 1963; Rosen, 1979:character 1; Parenti and Rauchenberger, 1989) and scored as 0; presence of a fleshy pelvic fin was scored as 1.

12. *Teeth compressed, spatulate*.—The outgroups *Alfaro* and *Priapella* have large, conical teeth fixed in large, robust jaws and were scored as 0. The outgroup *Poecilia* and the genus *Xiphophorus* have compressed, spatulate teeth set in a more delicate set of jaws (Rosen and Bailey, 1963; Rosen, 1979:character 2) and were scored as 1.

13. *Elongate ventral caudal fin rays*.—In many species of *Xiphophorus* the ventral-most principal caudal fin rays of adult males are elongated (Meyer and Schartl, 1979; Rosen, 1979; Rauchenberger et al., 1990). This is distinct from the presence of a sword, for many species show this sort of caudal fin development without the presence of a sword. Presence of elongated fin rays was scored as 1; absence was scored as 0.

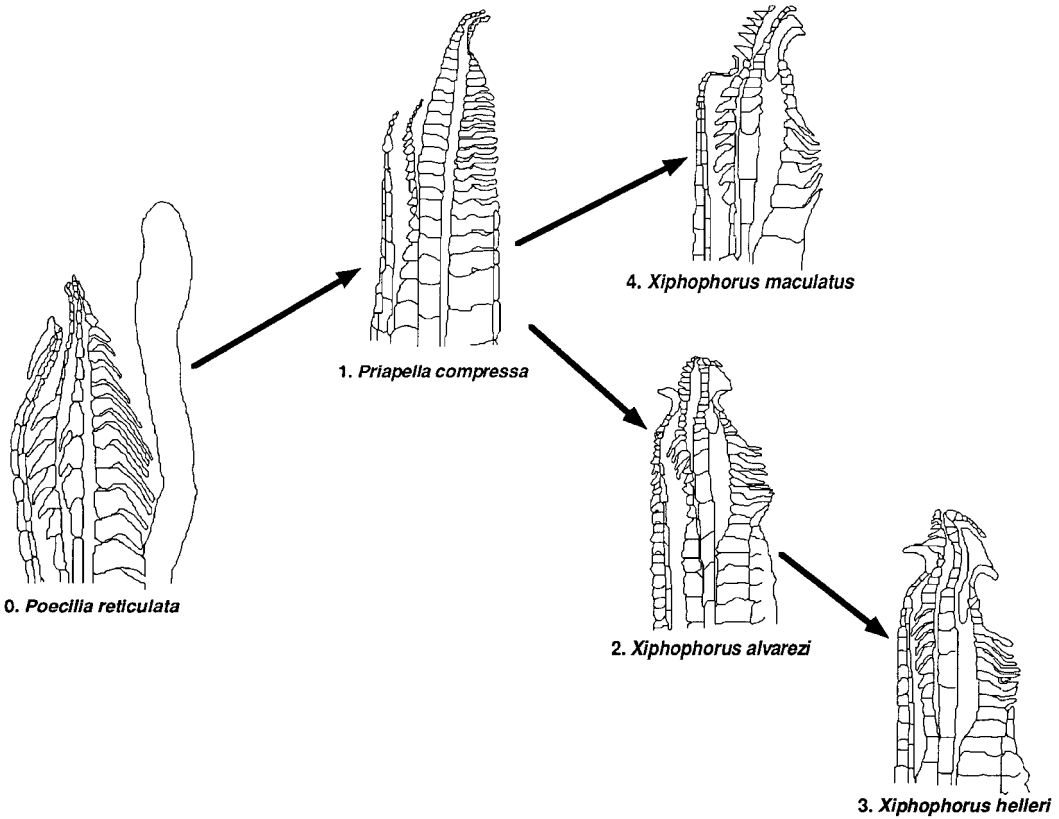

FIGURE A1. The character–state tree for character 27, granular tissue on the dorsal part of the hook on ray 3 of the gonopodium in males. Ray 3 hook absent and granular tissue absent were coded as 0; short, slightly decurved ray 3 hook was coded as 1; short, highly decurved hook was coded as 2; short, highly decurved hook with blade pointing sharply distally was coded as 3; and hook greatly enlarged was coded as 4. These descriptions were originally used by Rosen (1979) to infer phylogeny of this genus, but he used multiple nested binary characters. Using a character–state tree combines these data into a single character and eliminates complications resulting from nested data. *X. alvarezi* and *X. maculatus gonopodia* are after Rosen (1979); the other gonopodia are after Rosen and Bailey (1963).

14. *Width of the base of the dorsal fin*.—The southern platyfish have been traditionally divided into the species with short dorsal fin bases (*X. xiphidium*, *X. variatus*, *X. evelynae*; coded as 1) and those with long dorsal fin bases (*X. maculatus*, *X. milleri*; coded as 2). It is difficult to make this sort of comparison outside of the platyfish because among the swordtails, species vary quite widely in size. In coding this character, Rosen's (1979) character state descriptions were used for making state assignments, and species not assigned an unambiguous character state by Rosen were designated unknown.

15. *Growth rate*.—Differences in size and age at sexual maturation are controlled in many species of *Xiphophorus* by the sex-linked *p* locus (Kallman, 1989). Rauchenberger et al. (1990:character 24) discussed how in *X. nigrensis* and *X. multilineatus* genetically late-maturing fish grow significantly faster than early-maturing fish of the same species. In the remainder of the northern swordtail clade and in *X. helleri*, *X. maculatus*, *X. milleri*, *X. andersi*, *X. alvarezi*, and *X. couchianus*, fish of the same species with different alleles at the *p* locus grow at similar rates (K. D. Kallman, pers. comm.). Species in which all allelic variants at the *p* locus grow at similar rates were scored as 0; those with allelic variants that grow faster were scored as 1; and those species not tested were scored as missing.

16. *Allometric growth of sword*.—In most species of *Xiphophorus* with swords, the sword index or ratio of sword length to standard length is constant intraspecifically (Rauchenberger et al.,

1990:character 25), which was scored as 0. Two species are exceptions to this generalization, with the largest animals having proportionately larger swords for their body size, which was scored as 1.

17. *Sex-determining mechanism*.—A great many studies have examined the sex-determining mechanism of *Xiphophorus* (Peters, 1964; Kallman, 1983, 1984, 1989; Kallman and Bao, 1987; Tiersch et al., 1989). Most of these have examined the inheritance of sex-linked pigmentation patterns in crosses to determine the chromosomal basis for sex determination in each species. *X. nezahualcoyotl*, *X. cortezi*, *X. pygmaeus*, *X. nigrensis*, *X. multilineatus*, *X. andersi*, *X. couchianus*, *X. variatus*, and *X. evelynae* have been shown to have XX females and XY males. *X. alvarezi* has a WY female/YY male sex-determining mechanism. Reportedly *X. helleri* also has a WY-YY sex-determining mechanism (Tiersch et al., 1989), but no evidence for such a designation was presented, and this is contrary to data from genetic crosses that indicate *X. helleri* has a polyfactorial mode of sex determination (Peters, 1964). This ambiguity and the difficulty of coding a polyfactorial mode of sex determination forced us to code *X. helleri* as missing data. *X. maculatus* populations have been found with W, Y, and X chromosomes represented, and all combinations of chromosomes except WW have been found in the population. In these populations, WX, WY, and XX are female, and XY and YY are male (Kallman, 1984). Unfortunately, *Alfaro* and *Priapella* have not been examined for sex-determining mechanism. More work has been done with *Poecilia*, where genetic crosses with *P. reticulata* have indicated an XX-XY sex-determining mechanism (Kallman, 1984) and cytogenetic studies of *P. latipinna* and *P. sphenops* var. *melanistica* have shown a possible WY-YY sex-determining mechanism (Haaf and Schmid, 1984; Sola et al., 1990). Analysis of repetitive DNA sequences has confirmed these results in *Poecilia reticulata* and *P. sphenops* and have also shown that *P. velifera* has a WY-YY sex-determining mechanism (Nanda et al., 1990, 1992). *Poecilia butleri*, the species for which sequence data are available (Meyer et al., 1994) has not, to our knowledge, been examined for sex-determining mechanism, but we coded it as having a WY-YY sex-determining mechanism in the combined data analysis to represent the several species of *Poecilia* that do have such a mechanism but for which there are no sequence data. Species with an XX-XY sex-determining mechanism were coded as 0; species with a WY-YY sex-determining mechanism were coded as 2; species having a combination of both mechanisms were coded as 1. This was analyzed as an ordered character because any population undergoing a transition from an XX-XY sex-determining mechanism to a WY-YY sex-determining mechanism or vice versa must experience some period of time during that transition when W, X, and Y chromosomes are all found in the population. In other words, a transition between the two mechanisms must involve two steps: a gain of a new chromosome, followed by a loss of one of the older chromosomes.

Pigmentation Characters

18. *Dusky band continuous with dorsal pigment of sword*.—In the southern swordtails, there is a midlateral band of dusky pigment that is continuous with the dark dorsal pigment of the sword in males (Rosen, 1979:character 32). This condition was coded as 1; absence of this pattern was coded as 0.
19. *Proximal dorsal pigmentation of the sword*.—Black melanophores along the proximal upper margin of the sword are present in most species of swordtails (Basolo, 1996:character PU). Species that lack this pattern were scored as 0; species that have this pattern were scored as 1.
20. *Distal dorsal sword pigment*.—This is one of two distinct pigmentation patterns in the dorsal part of the sword, which is found in *Xiphophorus*. The other is the grave spot described in character 21. The absence of the distal pigment pattern was coded as 0; its presence was coded as 1 (Rauchenberger et al., 1990:character 3; Basolo, 1996:character DMU).
21. *Grave spot*.—The grave spot is a region of dark pigment on the ventral portion of the caudal fin in some species of *Xiphophorus* (Rauchenberger et al., 1990:character 33 [discussed but not included in their data matrix]). In other species, this is present in a modified form, where it extends to cover the entire dorsal margin of the sword (Rosen, 1979:character 32). In species with an extended grave spot, the grave spot pigmentation replaces the distal dorsal sword pigmentation described in character 20. Absence of any grave spot pigmentation was coded as 0; presence of the grave spot was coded as 1 (Basolo, 1996:character G); and presence of grave spot pigmentation along the dorsal surface of the sword was coded as 2 (Basolo, 1996:character MDU). This character was treated as ordered.
22. *Ventral margin of caudal fin and sword densely edged by melanophores*.—This character was discussed by Rauchenberger et al. (1990:character 34) but was not included in their matrix. Absence of the melanophores was coded as 0; presence was coded as 1 (Basolo, 1996:character L).
23. *Yellow and orange carotenoid sword pigmentation*.—The yellow and orange sword pigmentation present in many swordtail species has been discussed by Rauchenberger et al. (1990:character 20). These pigmentation patterns have also been described by Basolo (1996) and compose, in part, her character C along with our characters 24 and 28, green sword pigmentation. We justify subdividing Basolo's character because both green pigments (pteridines)

and orange and yellow pigments (carotenoids) are included in Basolo's (1996) character C, and these are the end products of two completely distinct biosynthetic pathways (Needham, 1974). The recruitment of two distinct biosynthetic pathways to produce sword pigmentation almost certainly represents two independent evolutionary events, and it seems appropriate to code them separately. The orange pigment in the swords of *Xiphophorus* has been identified as the carotenoid lutein (Kallman and Bao, 1987), whereas the yellow pigment has not, to our knowledge, been identified. The absorption spectra of carotenoid pigments can be altered either by chemical modifications of the pigment molecule itself (Karrer and Jucker, 1950) or by conjugation of the pigment molecule to various glycoproteins or lipoproteins (Needham, 1974). Since the relationship of the orange and yellow carotenoids found in the swords of *Xiphophorus* is unknown, we thought it best to consider them nonindependent and combine them into a single character. Absence of yellow or orange pigment pattern was coded as 0; presence of either color was coded as 1. There are two differences in our data for this character relative to those of Rauchenberger et al. (1990:Table 7). First, although they report that *X. montezumae* lacks a yellow sword (character 7), at least some males of that species have yellow swords (J. M. Marcus, pers. obs.), so we have coded the character as present. Second, although in their Table 7 *X. nezahualcoyotl* is indicated not to have a yellow sword, the text (Rauchenberger et al., 1990:18) records the yellow sword as present, in agreement with our observations (J. M. Marcus, pers. obs.), hence the coding in our analysis.

24. *Green pteridine sword pigmentation*.—Green swords are present in at least some males of *X. alvarezi*, *X. montezumae*, *X. signum*, and *X. helleri* (Kallman and Bao, 1987; Basolo, 1996:character C, in part, along with our characters 23 and 28); these species were coded as 1. Other species were coded as 0.

25. *Drosopterin*.—This is a red pigment found in many species of *Xiphophorus*. Its distribution in the northern swordtails has already been discussed (Rauchenberger et al., 1990:character 18). The enzymes necessary to produce drosopterin are also found in *X. alvarezi*, *X. clemenciae*, *X. couchianus*, *X. evelynae*, *X. gordoni*, *X. helleri*, *X. maculatus*, and *X. variatus* (K. D. Kallman, pers. comm.). Absence of the enzymes was scored as 0; presence was scored as 1. Information is not available for the other species in the genus or for the outgroups, so they were scored as missing.

26. *Sex-linked red and yellow patterns*.—Rosen (1979:character 14) described a specialized series of sex-linked red and yellow patterns in males of the southern platy group. The individual alleles at this presumptive locus have never been adequately characterized for most of the species in the genus, but a large number of them, including species not known to Rosen (1979), have sex-linked red and yellow color patterns (Rauchenberger et al., 1990). It is unclear that all of these color patterns can be traced to a single locus, but because they are caused by the presence of carotenoid pigments (Kallman, 1975; Kallman and Bao, 1987), one could argue that they are caused by a family of alleles at a single locus. For this reason, the presence of any of these patterns was coded as 1; the absence of these patterns was recorded as 0.

27–29. *Color patterns*.—The color patterns yellow caudal fin (character 27), yellow dorsal and ventral edges of the caudal fin (character 28), and solid yellow body coloration (character 29) are discussed for the northern swordtails in Rauchenberger et al. (1990:their characters 19, 21, 22). Yellow dorsal and ventral edges of the caudal fin have also been described by Basolo (1996) and compose, in part, her character C along with our characters 23 and 24. The yellow pigment patterns described here result from the presence of single alleles at a locus closely linked to the *p* locus on the Y chromosome (Kallman, 1989). In the remainder of the genus, species often have color patterns that are determined by a larger number of loci, making it difficult to determine if the particular alleles discussed here are present in the rest of the genus (Kallman, 1975). Very little genetic information has been collected for the outgroups. Absence of each pigment pattern was coded as 0; presence was coded as 1. There are two differences in our data for these characters relative to those of Rauchenberger et al. (1990:Table 7). First, although they report that *X. montezumae* lacks a yellow sword (character 7), at least some males of that species have yellow swords (J. M. Marcus, pers. obs.) and we have coded the character as present. Second, although their Table 7 indicates that *X. nezahualcoyotl* does not have a yellow sword, their text (Rauchenberger et al., 1990:18) records the yellow sword as present, in agreement with our observations (J. M. Marcus, pers. obs.), hence the coding in our analysis.

30. *Two or more rows of red lateral marks*.—Several swordtail species have two or more stripes of red pigment following the scale rows on the flanks of individuals of both sexes (Rosen, 1979:character 33). Linkage data for the genes responsible for these patterns are unavailable. Presence was coded as 1; absence was coded as 0.

31. *Multiple lateral stripes*.—Some species have two or three clearly visible zigzag lateral stripes, whereas in others these stripes are only faintly visible (Rauchenberger et al., 1990:character 26; Rosen, 1979:character 16). Absence of the pigmentation pattern was scored as 0; a faintly visible pigmentation pattern was scored as 1;

and a clearly visible pigmentation pattern was scored as 2. The character states were ordered for analysis.

32. *Solid midlateral stripe at birth.*—A solid midlateral stripe may be present at birth (Rauchenberger et al., 1990:character 27) or may develop by age 30 days (J. M. Marcus, unpub. data). Absence of the midlateral stripe at birth was scored as 0; presence of a solid midlateral stripe at birth was scored as 1.

33. *Vertical bars.*—Species of *Xiphophorus* may have thin vertical bars (Rosen and Kallman, 1969) or broad blotches (Rauchenberger et al., 1990:character 31) on their flanks. Absence of bars or blotches was scored as 0; thin vertical bars were scored as 1; and broad blotches were scored as 2.

34. *Body bicolored.*—Some species of *Xiphophorus* exhibit a distinctive pattern in which the dorsal aspect of the body is much darker than the ventral aspect. This pattern is most pronounced in *X. gordoni* (Schartl and Schroder, 1987). Absence of a distinct bicolor pattern was coded as 0; presence of a distinct bicolor pattern was coded as 1; and presence of a sharply bicolored pattern was coded as 2. This was treated as an ordered character.

35. *Dark subdermal dashes of pigment.*—Most pigment patterns in *Xiphophorus* are dermal, but *X. couchianus*, *X. meyeri*, and *X. gordoni* have deep subdermal blotches of black pigment. In *X. meyeri*, this appears to have developed into a three-dimensional invasive growth that enters the underlying muscle mass (Rosen, 1979:character 7; Schartl and Schroder, 1987). The ecological and physiological consequences of this unusual pigment type are unknown. Subdermal pigment absent was coded as 0; subdermal pigment present was coded as 1; and subdermal dashes of pigment elaborated into a three-dimensional growth was coded as 2. This character was analyzed as an ordered character.

36. *Two or more oblique lines behind pectoral base.*—In some species, there are two or more oblique black lines extending downward from the midlateral stripe just behind the pectoral base (Rosen, 1979:character 17). The presence of these oblique lines was coded as 1; absence of these lines was coded as 0.

37. *Middorsal spots.*—Males of some species have dark spots on the nodes in the reticulum along the uppermost scale rows, beginning at the level of the dorsal fin and extending onto the caudal fin (Rauchenberger et al., 1990:character 32). This pattern is not present in other species of *Xiphophorus* and for them was coded as 0; presence of middorsal spots was coded as 1.

38. *Dorsal fin with dark marginal pigment and a subbasal row of dark spots on the interradial membrane.*—Absence of this pattern was coded as 0; presence was coded as 1 (Rosen, 1979:character 24; J. M. Marcus, pers. obs.).

39. *Black or darkly pigmented gonopodium.*—Many species of *Xiphophorus* are polymorphic for the presence of dark pigment on the gonopodium of males (Rosen and Kallman, 1969; Meyer and Schartl, 1979; Borowsky, 1984; Obregon-Barbarosa and Contreras-Balderas, 1988); these were scored as 1. Species that lack black gonopodia were scored as 0.

40. *Caudal blotch (Cb).*—A micromelanophore pattern determined by a dominant allele at an autosomal locus (Kallman and Atz, 1966). This allele appears to cause an identical pattern in all of the species in which it is present. The distribution of Cb is discussed in Rauchenberger et al. (1990) for the northern swordtail clade and for *X. clemenciae*. Its absence has been noted in all other species of *Xiphophorus* (Rosen and Kallman, 1969; Kallman, 1975; Meyer and Schartl, 1979; Borowsky, 1984; Schartl and Schroder, 1987). The absence of Cb was coded as 0; its presence was coded as 1 (Rauchenberger et al., 1990:character 1).

41–42. *Spotted caudal (Sc, character 41) and Carbomaculatus (Cam, character 42).*—These characters were discussed at length by Rauchenberger et al. (1990:characters 28–29). However, an error in Rauchenberger et al.'s (1990) Table 7 shows *X. cortezi* as lacking Sc. This is inconsistent with the text. Sc was coded as present in *X. cortezi* for this analysis. Species expressing these patterns were assigned a 1; species that lacked these patterns were assigned a 0.

43. *Alleles at the tailspot locus.*—Many members of the traditional southern platy group are polymorphic at the tailspot locus, an autosomally linked micromelanophore pigmentation pattern locus that causes the development of darkly pigmented areas on the caudal fin (Kallman and Atz, 1967; Rosen and Kallman, 1969; Kallman, 1975; Rosen, 1979; Borowsky, 1984). Different alleles at this locus produce different patterns of pigmentation. Shared alleles at this locus have been described in several species. *X. maculatus* is polymorphic for at least nine alleles (*M, Mc, T, Co, Cc, C, O, D, +*), *X. variatus* has at least seven (*C, Ps, Ct, M, D, Cu, +*), *X. xiphidium* is polymorphic for at least four alleles (*C, Ps, Ct, +*), and *X. milleri* also appears to be polymorphic for four alleles (*Ss, Pt, B, +*), the first two of which may be homologous to *O* and *D* found in other species. The abbreviations used here were explained by Borowsky (1984). Others species lack alleles for pigmentation at this locus. Because different species share different specific alleles with one another, an estimate of the minimum number of gains and losses of alleles between each pair can be calculated by the use of a step matrix, with each gain and each loss counting as one step (Mabee and Humphries, 1993). A fixed wild-type allele was designated state 0; the alleles possessed by *X. milleri* was state 1; the alleles

possessed by *X. maculatus* was state 2; the alleles of *X. variatus* was state 3; and the alleles found in *X. xiphidium* was state 4. The following step matrix indicates the number of steps between each of the observed character states:

	0	1	2	3	4
0	.	3	8	5	3
1	3	.	7	7	6
2	8	7	.	7	9
3	5	7	7	.	3
4	3	6	9	3	.

44. *Sex-linked macromelanophore locus.*–Many species possess one or more alleles at this locus that produce pigment patterns made up of unusually large pigment cells called macromelanophores. Alleles differ in the placement and distribution of these patterns (Rosen, 1960; Kallman and Atz, 1967; Borowsky, 1984). The absence of sex-linked macromelanophore-producing alleles was coded as 0; the presence of such alleles was coded as 1.

Behavioral Characters

45. *Well-developed precopulatory behavior.*—Heinrich and Schroder (1986) divided the members of the genus *Xiphophorus* into two groups: those with well-developed precopulatory behavior (coded here as 1) and those without well-developed precopulatory behavior (coded here as 0).

46–57. *Behavioral characters.*—Nipping (character 46), gonopodial thrusting (character 47), slow approach (character 48), sneak chase (character 49), close transverse presenting behavior (character 50), side change over head behavior (character 51), stiff frontal presenting (character 52), circling behavior (character 53), figure-eight (character 54), open display behavior (character 55), arching display (character 56), and dancing display (character 57) have been described in detail by Haas (1993). Performance of a particular behavior was coded as 1; the absence of a particular behavior was coded as 0.

RAPD and RFLP Characters

58–85. *RAPD characters.*—We included 28 RAPD characters from the largest of the three data sets from Borowsky et al. (1995:Table 4), which included the largest number of *Xiphophorus* species. We chose not to include data from the two smaller data sets because to do so would introduce missing data for > 50% of the species included in our analysis and therefore may distort tree topology or give unresolved trees (W. Wheeler, pers. comm.). Absence of a RAPD band was coded as 0; presence of a RAPD band was coded as 1.

86. *INV-Xmrk EcoR1 restriction fragment length polymorphism.*—Schartl (1990) found the *Xmrk* gene associated with a 10-kb fragment in an *Eco*R1 restriction enzyme digest of genomic DNA in *X. gordoni*, *X. couchianus*, and *X. meyeri*. In all other species examined, the gene was associated with a 7-kb fragment; these were scored as 0. Association with a 10-kb fragment was scored as 1.

APPENDIX 3. PHENOTYPIC CHARACTER MATRIX USED FOR PARSIMONY ANALYSIS

Characters are listed in the order in which they are described in the text. Unless otherwise noted in the text, 0 = absent; 1 = present; ? = data are missing; – = character does not apply to a particular species. Both ? and – were treated as missing data in the PAUP (Swofford, 1993) analysis. Polymorphic RAPD characters are denoted by a *P* in the matrix.

```
                          10        20        30        40        50        60        70        80
                          .         .         .         .         .         .         .         .

P. reticulata     ?--000001?11???-0---0?--????????????????0????????????????????????????????????????????
P. butleri        ?--000001?11???-2---0?--????????????????0????????????????????????????????????????????
A. hubberi        0--000100?00???-?---0?--????????????????0????????????????????????????????????????????
P. compressa      0--001001?11???-?---0?--????????????????0????????????????????????????????????????????
P. intermedia     0--001001?11???-?---0?--????????????????0????????????????????????????????????????????
P. olmaceae       0--001001?11???-?---0?--????????????????0????????????????????????????????????????????
X. alvarezi       200222211011?002110211110???10000000000000011111100000?0?????????????????????????????
X. andersi        200204201011?000000000?????000?00?0??000??111111010000?????????????????????????????0
X. birchmanni     1--214201111?0-?-0-11--101100002000?0011?0?11111001110011P110101110P0100101101100110
X. clemenciae     201214201011?00?111111?10000100000000000000111111000001010110000001P1P10PP00011110100
X. continens      1--214201011?0-?-0-10--100000100000?0000?0?????????????1001??011101111101011?1011?
X. cortezi        211214201011?000011111?10110000100000011100111111100101001110011011100110011001PPP001101P0
X. couchianus     0--404301011?0-0---00--10???000111000100000111111100000?????????????????????????????1
X. evelynae       0--204201011111?-?---00--11???020100100000001011111100000?????????????????????????????0
X. gordoni        0--304301011??-?---01--10???000121000100000011111100000?????????????????????????????1
X. helleri        200223221011?002110211110???10010000000000011111100100110111000100011000110100111000 0
X. maculatus      0--204201011120-1---00--11???000100000100021011110000000001011011101100000000110101010
X. malinche       211214201111?00?011111?10??000020001?01???0?????????????11P110P01P100010010110110011?
X. meyeri         0--304301011??-?---00--?0???000?12?0?1000??011111100000?????????????????????????????1
X. milleri        0--204201011120-0---00--?1???000100000100011011111100000?????????????????????????????0
X. montezumae     211214201011?00?011111?10000020100000000000111111001110011011110111011111101011111110
X. multilineatus  211114201011?1100111117001110011000?0100?01?????????????000001011101110101011111111?
X. nezahualcoyotl 211214201011?000011111?100000201000?0100?1?111110011100111110101011111110101110?011?
X. nigrensis      211114201011?1100111117001100010000010100001111110011000107100101110117010101110?10
X. pygmaeus       1--114201011?0-0-0-00--00101001000001000000111111001000010000010111011101010101010110
X. signum         200223221011??0?1102171?0???000000000000000011111100100117?????????????????????????0
X. variatus       0--204201011111?-0---00--11???020100100102031111111100000000PP1000P1110P0010PP011101010
X. xiphidium      100204201011117-0-000000?1???020100100100041111111010000000110?000011000010000011011 0
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