

*Copeia*, 2003(2), pp. 299–307

## Dissolution of Sexual Signal Complexes in a Hybrid Zone between the Swordtails *Xiphophorus birchmanni* and *Xiphophorus malinche* (Poeciliidae)

GIL G. ROSENTHAL, XOCHITL F. DE LA ROSA REYNA, STEVEN KAZIANIS,  
MATTHEW J. STEPHENS, DONALD C. MORIZOT, MICHAEL J. RYAN,  
AND FRANCISCO J. GARCÍA DE LEÓN

The evolution of sexual signaling systems is influenced by natural and sexual selection acting on complex interactions among traits. Natural hybrid zones are excellent systems for assessing fitness effects on sexual phenotypes. Most documented hybrid zones, however, show little variation in sexual signals. A hybrid zone between the swordtails *Xiphophorus birchmanni* and *Xiphophorus malinche* is characterized by numerous recombinants for male sexual traits. Analyses of geographic variation in morphological and isozyme traits in the Río Calnali, Hidalgo, Mexico, reveal an upstream-to-downstream gradient from *X. malinche*- to *X. birchmanni*-type traits. A second hybrid zone, likely isolated from the R. Calnali, occurs in the nearby Arroyo Pochutla. Although the presumed female preference for swords predicts the introgression of swords from *X. malinche*-like populations into hybrid populations, the opposite pattern was observed. Swords are reduced in populations otherwise characterized by *X. malinche* traits. Sexually dimorphic traits were poorly correlated within individuals, indicating that sexual selection does not act against recombinant phenotypes. Hybrid males also exhibit trait values outside the range of parental variation. These patterns are consistent with predictions that females are permissive, preferring generally conspicuous males without attending to specific features.

CONSPICUOUS sexual signals often involve suites of traits that interact in complex ways to influence receiver perception. Both multivariate statistical analysis (Endler and Houde, 1995) and experimental manipulation of phenotypes (Sinervo and Basolo, 1996) have taken a combinatorial approach, evaluating multiple trait combinations to ascertain their effect on female mating preferences. Yet controlled studies such as these can only suggest the maximum theoretical evolutionary effect of sexual selection, since other aspects of the trait complex being analyzed are unconstrained by the biology of the system and by their fitness consequences in nature.

Natural hybrids, however, offer the opportunity to measure the overall evolutionary effects of natural and sexual selection on trait complexes. Traits can often interact in surprising ways (e.g., Nijhout and Emlen, 1998); hence natural hybrids can also indicate the range of trait combinations that are genetically and developmentally possible. This requires a hybrid zone involving parental species with pronounced, measurable differences in sexually selected traits. Documenting the distribution of recombinant phenotypes in a hybrid zone permits one to refine hypotheses as to how multiple-trait signals are maintained in nature and can provide insights into the genetic mecha-

nisms underlying trait-preference associations. Most studies to date on sexual signals in hybrid zones, however, have focused on variation in one or a few, functionally related, signal variables (e.g., Sanderson et al., 1992; Parsons et al., 1993; McDonald et al., 2001).

The swordtails *Xiphophorus birchmanni* (Fig. 1A) and *Xiphophorus malinche* (Fig. 1B) both inhabit shallow, rocky streams. The two species are members of the monophyletic Río Panuco basin, or northern, species group of swordtails. The phylogenetic hypotheses differ as to the relationship of the two species. Hypotheses based on behavioral, morphological, and allozyme traits (Rauchenberger et al., 1990), RAPDs (Borowsky et al., 1995), and a combined analysis of these (Marcus and McCune, 1999) place the two as sister species. The strict and semistrict consensus trees in Morris et al.'s (2001) allozyme study place both species in a polytomy with *Xiphophorus cortezi*. Meyer et al.'s (1994) hypothesis, based on DNA sequence data, shows weak support for pairings of *X. birchmanni* with *Xiphophorus pygmaeus* and *X. malinche* with *X. cortezi*. Marcus and McCune's (1999) total-evidence tree based on morphological, behavioral, RAPD, and sequence data shows weak support for *X. birchmanni* at the base of the Río Panuco basin swordtails, and pairs *X. malinche* and *X. cortezi*.

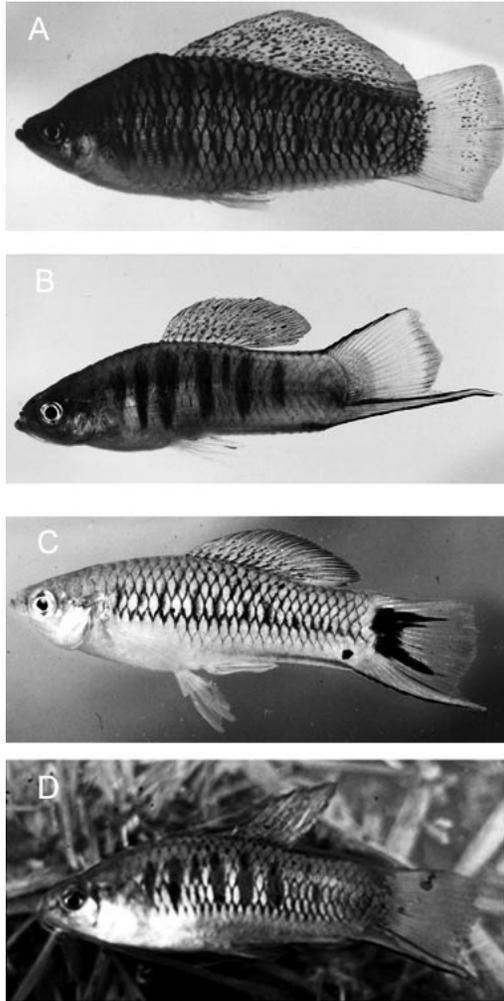


Fig. 1. Representative males from parental and hybrid populations. (A) *Xiphophorus birchmanni* collected at Huiznopal, Río Conzintla. Note nuchal hump. (B) *Xiphophorus malinche* from Río Claro stock. (C) hybrid from Calnali-low. *Spotted caudal* macromelanophore pattern, diagnostic of *X. birchmanni*, shows evidence of melanosis. The sword is diagnostic of *X. malinche*, whereas the regular vertical bars are diagnostic of *X. birchmanni*. (D) Hybrid collected at Calnali-mid. Note irregular bars, diagnostic of *X. malinche*, and deep body and reduced sword, diagnostic of *X. birchmanni*.

*Xiphophorus malinche* has a strictly highland distribution, whereas *X. birchmanni* is generally found at lower elevation, in warmer water (Rauchenberger et al., 1990). The species are characterized by dramatically different complexes of sexually dimorphic traits. Male *X. malinche* are "typical" northern swordtails, with a pronounced, pigmented elongation of the lower rays of the caudal fin ("sword") and a moderately sized dorsal fin. *Xiphophorus malinche*

males also express an irregular array of oval, flanking vertical bars. *Xiphophorus birchmanni*, in contrast, lack swords or bear short swords, have an elongate dorsal fin, and express vertical bars in a regular series (Rauchenberger et al., 1990). In addition to sexually dimorphic traits, the species are distinguished by the number of rows of middorsal spots (3–4 in *X. malinche* vs 1–2 in *X. birchmanni*; Rauchenberger et al., 1990) and by different isozyme alleles at two loci (see below).

Females in other swordtail species choose males on the basis of swords (Basolo, 1990, 1995a; Rosenthal and Evans, 1998), vertical bars (Morris et al., 1995), and body size (Ryan and Wagner, 1987; Ryan et al., 1990; Morris et al., 1996). The dorsal fin has been implicated in mate choice in another poeciliid, the sailfin molly *Poecilia latipinna* (Ptacek, 1998). In male *X. birchmanni*, *X. malinche* and other northern swordtails, the dorsal fin is only fully erect during courtship and agonistic interactions, strongly suggesting that it plays a role in signaling. In addition, the species differ in the expression of two independent, Mendelian melanophore pigment pattern polymorphisms: *Caudal blotch* (*Cb*) is polymorphic in *X. malinche* but absent in *X. birchmanni*, whereas the converse is true for *Spotted caudal* (*Sc*) [Rauchenberger et al., 1990].

The production of viable hybrid *Xiphophorus* is a routine laboratory practice; many species hybridize readily when housed in the same aquarium (Kazianis et al., 1996). Hybrid genetic crosses are especially valuable to the cancer research community, since melanistic pigment patterns derived from crosses of parental stocks are often phenotypically enhanced, resulting in extreme melanization and melanoma formation (Schartl, 1995; Fig. 1C). Nevertheless, there are no well-documented cases of natural hybridization in *Xiphophorus* (but for a possible instance, see Rosen, 1979). We report here genetic and morphological evidence of a hybrid zone in the Río Calnali, Hidalgo, Mexico, characterized by numerous individuals with recombinant suites of sexually dimorphic traits.

#### MATERIALS AND METHODS

*Isozyme electrophoresis*.—Fish were collected by seine and dip net in March 1999 from three sites on the Río Calnali, Hidalgo, Mexico (Fig. 2): Calnali-high (20°53'54"N, 98°37'39"W [GPS map datum: WGS 84]), Calnali-mid (20°53'34"N, 98°36'36"W), and Calnali-low (20°55'26"N, 98°34'35"W). We sampled apparent hybrids from a single locality on the nearby Arroyo Pochutla (20°51'39"N, 98°34'23"W). We also sampled allopatric populations of the two parent species. *Xiphopho-*

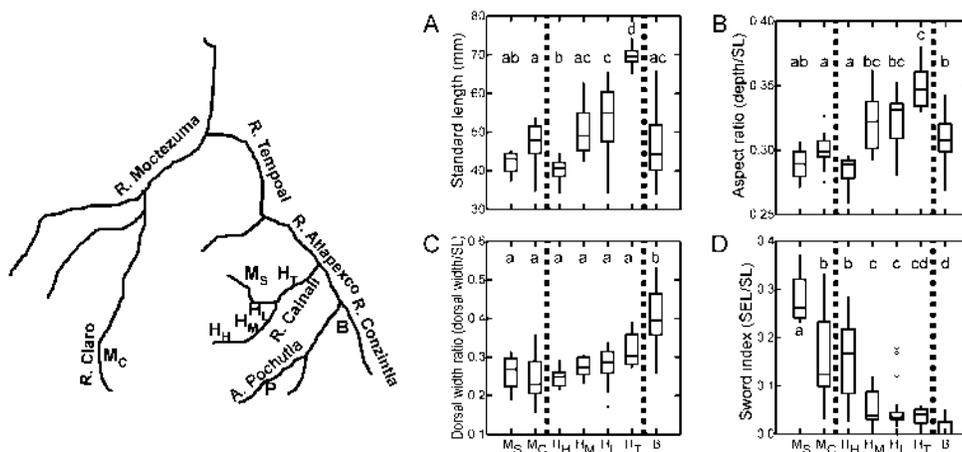


Fig. 2. Study sites and male morphometric trait distributions. Boxes and whiskers encompass 75% and 90% of trait variation respectively; horizontal line within box indicates median. Outside values and far outside values are indicated by asterisks and open circles, respectively. Lowercase letters indicate significant differences in ANOVA for standard length and in ANCOVA for depth, dorsal fin width, and sword extension length (see Materials and Methods). M<sub>C</sub>, *Xiphophorus malinche*, near Chicayotla; M<sub>S</sub>, *X. malinche*, Río Claro; H<sub>H</sub>, hybrid zone, Calnali-high; H<sub>M</sub>, hybrid zone, Calnali-mid; H<sub>L</sub>, hybrid zone, Calnali-low; H<sub>T</sub>, hybrid zone, Teocapán; P, hybrid zone, A, Pochutla; B, *Xiphophorus birchmanni*.



*rus malinche* were wild-caught or first-generation descendants of collections in 1998–1999 from the type locality, the Río Claro at Tlatzintla (20°52'51"N, 98°47'56"W), and *X. birchmanni* were wild-caught or F<sub>1</sub> descendants of collections in 1998–1999 from a population on the Río Conzintla (20°53'6"N, 98°29'16"W; Fig. 2).

Allozyme data were derived from analysis of 28 protein-coding loci from six *X. birchmanni* and two *X. malinche* populations collected previously (DCM, unpubl.; Morris et al., 2001). Two isozymes, coded by unlinked genes (Morizot et al., 1998; adenosine deaminase [*ADA*] and mannose-6-phosphate isomerase [*MPI*]) exhibited fixed differences between *X. malinche* and *X. birchmanni* (Rauchenberger et al., 1990). We also assayed three variable loci: peptidase S (PEP-S), muscle protein 5 (MP-5), and aspartate aminotransferase (AAT-1). Starch gel electrophoresis and histochemical staining followed methods previously used for *Xiphophorus* (Morizot and Schmidt, 1990). Presumptive genotypes were assigned from allozyme phenotypes using codominant inheritance models based on known enzyme subunit structures. Only individuals for which both *ADA* and *MPI* were scorable were used for assignment of hybrid identity.

We computed F<sub>ST</sub> values using all individuals genotyped at a minimum of three of the five loci studied ( $n = 60$ ). F<sub>ST</sub> measures the degree of differentiation between populations (Wright, 1969). Once it has been estimated, according to Weir and Cockerham (1984), it is possible to

test its departure from zero with a permutation procedure. All statistical treatments and calculations were performed using the randomization approach provided by the Genetix software package. An empirical null distribution simulating the random distribution of genotypes is obtained by permutation. The genotypes were permuted among samples. The obtained distributions enable us to then estimate the significance of the deviation of the real data from its expected value under the null hypothesis. We used 1000 permutations for all tests. We computed F<sub>ST</sub>, based on all five loci for each population relative to the total sample. Unscorable genotypes were coded as missing data. We obtained pairwise *P*-values for F<sub>ST</sub>.

**Morphometrics.**—Samples were taken at four localities on the Río Calnali in February 1998, going from upstream to downstream: Calnali-high, Calnali-mid, Calnali-low (see above), and Teocapán (20°53'54"N, 98°34'38"W). We also sampled allopatric populations of *X. birchmanni* on the Río Conzintla and of *X. malinche* at the type locality on the Río Claro at Tlatzintla; and a presumably parapatric population of *X. malinche* in an unnamed tributary of the Río Calnali, between the towns of Calnali and Chicayotla (20°55'26"N, 98°34'35"W). Locations are shown in Figure 2.

Mature males were identified by the presence of a fully developed gonopodium. We placed each male on a laminated sheet of graph paper

and videotaped the left and right sides of each fish for approximately 1 sec with a Hitachi VMH100-LA Hi-8 video camera. On the audio track of the videotape, we scored fish for regular or irregular vertical bars on the flank. Males not retained for study were released at point of capture after videotaping.

One frame of videotape from each side of each fish was digitized using a Targa 1000 video capture board (spatial resolution  $720 \times 486$  pixels). We used the UTHSCSA ImageTool program (developed at the University of Texas Health Sciences Center at San Antonio) to measure morphometric variables. Scale was standardized using the graph paper background. For each fish, we measured four morphometric traits likely to play a role in sexual signaling: standard length (Fig. 2A; snout to hypural plate); depth (Fig. 2B; anterior insertion of dorsal fin to anterior insertion of pelvic fin), dorsal fin width (Fig. 2C; anterior to posterior insertion of dorsal fin; this measure is highly correlated with dorsal fin height in swordtails [GGR, MJS, and MJR, unpubl. data] and is easier to measure without injuring the animal), and sword extension length (Fig. 2D; distance from the intersection of a line perpendicular to the axis of the ventral-most unextended caudal ray and crossing its tip with the ventral margin of the caudal fin, to the tip of the longest caudal ray). Measurements were taken for each side of the body and averaged for use in analyses. Continuous variables were transformed by  $\ln(x)$  ( $\ln[x + 1]$  for sword extension) for analysis.

To assign hybrid identity, we performed an unrotated principal components analysis on standard length, sword extension length, depth, and dorsal fin width for populations within the hybrid zone and for nonhybrid populations ( $n = 96$ ). The first two principal components explained 54% and 35% of variance, respectively. The first component was strongly associated with body size and dorsal width ratio, and the second with sword extension length. For the morphometric analysis, hybrids were defined as individuals falling outside the minimum convex polygon containing each parental species on a plot of the first two principal components.

We performed a one-way multivariate analysis of variance by population on standard length, sword extension length, depth, and dorsal fin width. The MANOVA was followed by a post hoc analysis of variance on standard length by population and by analysis of covariance on the other traits, with standard length as the covariate. We performed Fisher LSD tests for differences between individual populations. We examined associations between bar morphology and the

expression of continuous traits by performing separate two-way analyses of variance by population and bar morphology on each retained principal component. Because our analyses tested the null hypothesis of no recombination of parental phenotypes, we did not attempt to correct for Type I error inflation in statistical tests. This ensured that Type II error was minimized, the conservative alternative in this context.

## RESULTS

*Isozyme electrophoresis.*—Of 22 individuals typed at Calnali-high, -mid, and -low and Pochutla (Fig. 2), 11, or 50%, possessed allozymes of both species at *ADA* and *MPI*, and are, therefore, presumed to be hybrids (Calnali-high, one *X. birchmanni*, one *X. malinche*, two hybrids; Calnali-mid, one *X. birchmanni*, no *X. malinche*, four hybrids; Calnali-low, three *X. birchmanni*, no *X. malinche*, two hybrids; A. Pochutla, no *X. birchmanni*, five *X. malinche*, three hybrids; examples shown in Figs. 1C–D). It is important to note that for a hypothetical pair of hybrid parents, both heterozygous at both loci, one in eight offspring would be homozygous at both loci for alleles of one or the other parental species. Analysis of genotypes indicated that all hybrids were either the product of hybrid-hybrid crosses or backcrosses of hybrids to a parental phenotype; no  $F_1$  hybrids were detected (Table 1). Allele frequencies varied across populations, with *X. malinche* alleles more prevalent upstream and at the single Arroyo Pochutla site, which appears to be part of a second, separate hybrid zone (Tables 1–2). The preponderance of recombinant hybrids is further supported by broad variation in hybrid trait phenotypes (Fig. 3), congruent with the clinal variation in isozyme allele frequencies shown in Table 2.

The upstream-to-downstream transition from *X. malinche* to *X. birchmanni* alleles is also seen in pairwise comparisons of  $F_{ST}$  values (Table 3), where the downstream Calnali-low and -mid populations are not significantly different from *X. birchmanni*. The  $F_{ST}$  value for the Arroyo Pochutla differed significantly from both parental species, indicating a hybrid population. Among R. Calnali sites,  $F_{ST}$  values did not differ significantly, suggesting gene flow across the hybrid zone. The genetic similarity between Calnali-low and *X. birchmanni* may reflect the presence of nearby *X. birchmanni* populations farther downstream. Calnali-high is the upstream-most site on the Rio Calnali. Pure *X. malinche* populations are thus absent from the R. Calnali, although all individuals in the small tributary stream at Chicayotla were typed as *X. malinche*.

TABLE 1. ALLELE FREQUENCIES AND SAMPLE SIZES FOR EACH LOCUS BY POPULATION.

Locus	Allele	Population					
		<i>X. malinchei</i>	Calnali-high	Calnali-mid	Calnali-low	Arroyo Pochutla	<i>X. birchmanni</i>
Overall		n = 14	n = 9	n = 7	n = 11	n = 8	n = 8
MPI	100	0	0.4444	0.5	0.9091	0.125	1
	120	1	0.5556	0.5	0.0909	0.875	0
		n = 12	n = 9	n = 6	n = 11	n = 8	n = 7
PEP-S	100	0	0.1	0.0833	0.3571	0.0625	0.1667
	110	0.9615	0.7	0.9167	0.6429	0.5625	0.75
	120	0.0385	0.2	0	0	0.375	0.0833
		n = 13	n = 5	n = 6	n = 7	n = 8	n = 6
ADA	100	0	0.625	0.7	0.9	0.125	1
	120	1	0.375	0.3	0.1	0.875	0
		n = 13	n = 4	n = 5	n = 5	n = 8	n = 3
MP-5	100	0	0	0.4	0.75	0.0625	1
	120	1	1	0.6	0.25	0.9375	0
		n = 13	n = 2	n = 5	n = 6	n = 8	n = 2
AAT-1	100	0	1	0.6667	0.8571	0.375	0.75
	110	0	0	0	0	0	0.125
	120	1	0	0.3333	0.1429	0.625	0.125
		n = 6	n = 2	n = 3	n = 7	n = 4	n = 4

*Morphometrics.*—Parental *X. birchmanni* and *X. malinche* populations assorted as two distinct clusters on a principal components plot of four sexually dimorphic traits (body size, body depth, sword length, and dorsal fin size), but most hybrids fell outside the range of either parental species (Fig. 3). A similar pattern was obtained using only sword index and dorsal width ratio (Fig. 4). Swords and dorsal fins, which are conspicuously elongated in *X. malinche* and *X. birchmanni*, respectively, did not show a negative association (Figs. 2, 4). There was a nonsignificant *positive* correlation between sword index and dorsal fin width at Calnali-high ( $n = 11$ , Spearman rank correlation  $r = 0.34$ , NS) and Calnali-low ( $n = 25$ ,  $r = 0.14$ , NS), and a very weak, non significant negative correlation at Teocapán ( $n = 6$ ,  $r = -0.08$ , NS). At Calnali-mid, there was a significant negative correlation ( $n = 11$ ,  $r = -0.67$ ,  $P < 0.05$ ), but significance was lost if one individual morphologically typed as *X. malinche* was removed from the analysis.

Bar morphology, a qualitative trait diagnosing the parent species, was independent of other sexually dimorphic traits within the hybrid zone. Regular bars characteristic of *X. birchmanni* increased in frequency going downstream, along with allozyme and other morphological traits characteristic of that species (Table 2). Regular or irregular bars were not, however, significantly associated with either of the first two principal components (Fig. 3; two-way ANOVA

by population and bar morphology; PC1,  $df = 38$ ,  $F = 0.799$ ,  $P = 0.377$ ; PC2,  $df = 38$ ,  $F = 0.065$ ,  $P = 0.800$ ). There was no significant interaction between presence or absence of irreg-

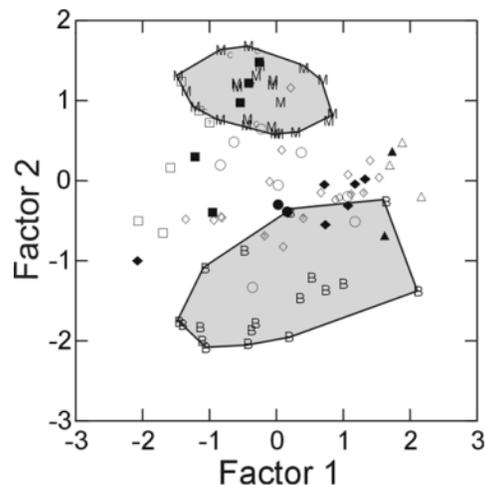


Fig. 3. Principal components plot of study populations. Shaded polygons indicate parental species ( $M_C$ , *Xiphophorus malinche*, Chicayotla;  $M$ , *X. malinche*, Río Claro;  $B$ , *Xiphophorus birchmanni*, Río Conzintla). Hybrids:  $\square$ , Calnali-high;  $\circ$ , Calnali-mid;  $\triangle$ , Calnali-low;  $\diamond$ , Teocapán. Filled symbols indicate regular bars, filled symbols irregular bars, question marks bar status undetermined. Parental species were fixed with respect to bar morphology.

TABLE 2. FREQUENCIES OF DIAGNOSTIC TRAITS BY POPULATION: *X. birchmanni* ALLELES OF ADENOSINE DEAMINASE (ADA) AND MANNOSE-6-PHOSPHATE ISOMERASE (MPI); Cb AND Sc CAUDAL PIGMENT POLYMORPHISMS; AND REGULAR BAR PHENOTYPE.

	ADA	MPI	Cb	Sc	Regular bars
<i>X. malinche</i>	0%	0%	20%	0%	0%
Calnali-high	44%	63%	18%	9%	50%
Calnali-mid	50%	70%	40%	10%	43%
Calnali-low	91%	90%	4%	69%	81%
Teocapán	—	—	100%	67%	67%
A. Pochutla	13%	13%	—	—	—
<i>X. birchmanni</i>	100%	100%	0%	37%	100%

ular bars and population; therefore, this cannot be accounted for by among-population differences. The caudal blotch (Cb) and spotted caudal (Sc) caudal pigment patterns which are polymorphic in *X. malinche* and *X. birchmanni* respectively, did not show clear geographic structure (Table 2). Five individuals at Calnali-low and four at Teocapán exhibited both pigment patterns, including the melanotic male in Figure 1C.

Although populations at Calnali-mid were characterized by high frequencies of *X. malinche* alleles (Table 2), male swords there and at Teocapán were substantially shorter than in pure *X. malinche* populations ( $P < 0.001$  for both sword index and sword length, all three populations) and were not significantly different from *X. birchmanni* (Fig. 2).

#### DISCUSSION

The proportion of  $F_2$  or later hybrids, and the population-genetic evidence for gene flow within the hybrid zone, makes it reasonable to assume that there is not strong natural selection against hybrids of *X. malinche* and *X. birchmanni* within the hybrid zone. There is a distinct, clinal shift from traits characteristic of *X. malinche* at

Calnali-high, furthest upstream, and *X. birchmanni* at Calnali-low, 6.03 km straight-line distance downstream. *Xiphophorus birchmanni* alleles and traits are relatively overrepresented throughout the hybrid zone. This may reflect weak selection against *X. birchmanni* phenotypes at upper elevations. Additionally, there may be differences between the species in dispersal, or in the propensity to mate with heterospecifics (Wirtz, 1999).

How should sexual selection act on hybrid phenotypes? One possibility is that recombinant males will be maintained. This could occur if hybrid females prefer hybrid males to males of either parental species, as is true in some other taxa (Doherty and Hoy, 1985). Such a preference could arise if females have a set of specific preferences for specific traits, and females recombinant for a set of preferences prefer males with the corresponding recombinant traits. Recombinant males would also be maintained if females were permissive with respect to broad ranges of trait combinations (Rosenthal and Evans, 1998).

Alternatively, hybrid females may prefer parental males (von Helversen and von Helversen, 1975). Recombinant phenotypes may be at a disadvantage if they disrupt functionally inte-

TABLE 3. PAIRWISE  $F_{ST}$  VALUES AND UNCORRECTED  $P$  VALUES FOR STUDY POPULATIONS

	Calnali-high	Calnali-mid	Calnali-low	A. Pochutla	<i>X. birchmanni</i>
<i>X. malinche</i>	0.7415 $P < 0.001$	0.6234 $P = 0.006$	0.8220 $P < 0.001$	0.2750 $P = 0.002$	0.9092 $P < 0.001$
Calnali-high		-0.1028 $P = 0.672$	0.2425 $P = 0.064$	0.2304 $P = 0.067$	0.4601 $P = 0.028$
Calnali-mid			0.0799 $P = 0.224$	0.2105 $P = 0.045$	0.1650 $P = 0.158$
Calnali-low				0.5336 $P = 0.001$	-0.0819 $P = 0.760$
A. Pochutla					0.6340 $P < 0.001$

grated trait complexes (Carson et al., 1989). The sword in *Xiphophorus*, for example, is a composite trait made up of a fin elongation plus melanin, pterin, and/or carotenoid pigmentation. Removal of any of these components can cause a reduction in female preference (Basolo, 1995b). Recombinant phenotypes are also selected against if females inherit preferences for suites of species-typical traits. If the traits serving as visual cues in mate choice constitute a specific mate recognition signal, females should eschew males displaying recombinant signals, and selection should favor the maintenance of genetic correlations for display traits.

The latter scenario can be ruled out at Calnali, because hybrid males display a broad range of recombinant sexually dimorphic phenotypes. Combinations of sexually dimorphic traits in most hybrid individuals are well outside the range of variation of either species (Table 2). Moreover, our electrophoretic data clearly indicate the presence of multiple genotypes arising from probable hybrid-hybrid matings. If species-specific complexes were favored, hybrid females should backcross to the parental species. Sexual selection against hybrid males should thus be relatively weak. Although it is difficult to estimate the age of the hybrid zone, it is unlikely to be the product of recent human intervention. Both species are commercially unimportant, and there is no reason to suspect human introduction. Waters in the Río Calnali are clear and comparable to swordtail habitat elsewhere in the Río Pánuco basin.

Female preference for swords is an ancestral trait in *Xiphophorus* (Basolo, 1995a). If females in the Río Calnali have retained the preference for swords, one would expect the sword to introgress throughout the hybrid zone, because sexually selected traits are known to do in other taxa (Parsons et al., 1993; McDonald et al., 2001) and certainly to be present in populations with a high frequency of *X. malinche* electrophoretic markers and other *X. malinche* traits. The opposite pattern, however, seems to be operating in this hybrid zone. Although the Calnali-mid population was characterized by relatively high frequencies of *X. malinche* alleles at *ADA* and *MPI*, and by a high frequency of the irregular bars characteristic of *X. malinche* (Table 2), male swords there, at Calnali-low, and at Teocapán were substantially shorter than in pure *X. malinche* populations and were not significantly different from *X. birchmanni*. This may reflect female permissiveness with respect to male traits that enhance apparent body size (Rosenthal and Evans, 1998). Quantitative traits exhibited a distinct gradient from *malinche*-typical traits

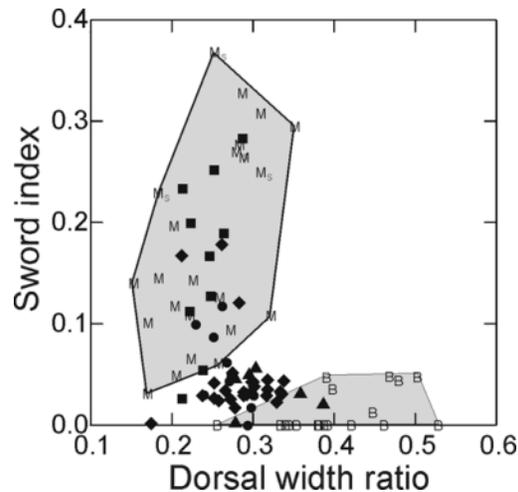


Fig. 4. Sword index and dorsal width ratio in study populations. Shaded polygons indicate parental species ( $M_C$ , *Xiphophorus malinche*, Chicayotla; M, *X. malinche*, Río Claro; B, *Xiphophorus birchmanni*). Hybrids:  $\bullet$ , Calnali-high;  $\circ$ , Calnali-mid;  $\square$ , Calnali-low;  $\triangle$ , Teocapán.

upstream to *birchmanni*-typical traits downstream, with a dramatic shift in trait values across the cline. The populations downstream expressed reduced sword lengths and body-size traits at mean values that exceeded both parental species (Fig. 2). All three downstream populations were significantly deeper-bodied than parentals, and both Calnali-low and Teocapán were larger-bodied (Fig. 2).

Female *Xiphophorus helleri* select males on the basis of large apparent size, whether from sword length or body size, and a general preference for large size is widespread (Rosenthal and Evans, 1998). Transgressive expression of body length and body depth may relax selection favoring swords. Alternatively, genes involved in sword expression may be linked to genes affecting physiological tolerance of environmental variables (cf. Borowsky, 1990). Although predators show a bias for males with swords (Rosenthal et al., 2001) and females appear to reduce their preference for swords in areas of high predation risk (Rosenthal et al., 2002), it is unlikely that natural selection by predation is affecting sword distributions within the hybrid zone. The major visual predators of northern swordtails, characid fishes in the genus *Astyanax* and the eleotrid *Gobiomorus dormitor* (Rosenthal, 2000), have not been observed in the Río Calnali or Arroyo Pochutla.

The trait distributions within the hybrid zone suggest that females may be permissive with respect to the specific form taken by visual traits. Female *X. helleri* show a strong preference for

males with swords over males without (Basolo, 1990), but this preference is abolished when a swordless male is made to appear large (Rosenthal and Evans, 1998). Females may prefer males with more exaggerated traits (Ryan, 1998) but may be relatively agnostic as to what form these traits take. This account is supported by the fact that, in our study, no one male trait appears to have introgressed throughout the hybrid zone (cf. Parsons et al., 1993). Vertical bar patterns, sword length, and dorsal fin height are highly variable across the entire study area, suggesting that none of these traits has enjoyed a huge advantage with respect to sexual selection. This may be the case especially if some sexually dimorphic traits, such as aspect ratio, exhibit transgressive values exceeding parental phenotypes. Two males may differ drastically in a suite of male traits and yet be equally attractive to females.

The striking phenotypic differences between male *X. malinche* and *X. birchmanni* constitute extreme endpoints from the standpoint of visual signal morphology, and yet recombinant phenotypes thrive in natural populations, producing "transgressive" individuals outside the natural range of variation in either parental species. Recombinant traits are the rule, and most individuals typed exhibit novel trait combinations. These traits appear to interact in nonintuitive ways, with a presumably preferred trait, the sword, failing to introgress into swordless populations. The biogeography of *X. malinche* and *X. birchmanni* suggests that this system can provide valuable insights about sexual selection and species recognition. *Xiphophorus birchmanni* are sympatric with the closely related *X. cortezi*, which share characteristics like regular vertical bars and  $S_C$  at several localities (Rauchenberger et al., 1990); no hybrids have been detected over multiple samplings since 1987.

Multiple-trait complexes and their concomitant preferences have traditionally proven intractable to study. The *X. birchmanni*/*X. malinche* system permits both an assessment of the fitness consequences of recombinant phenotypes in the wild, and measurement of female preferences for recombinant traits (e.g., Rosenthal and Evans, 1998, Rosenthal et al., 2002). The species group should prove a powerful system for testing hypotheses about the evolution of male traits and female preferences.

#### ACKNOWLEDGMENTS

We thank M. Morris, M. Kirkpatrick, M. Servedio, I. Schlupp, and three anonymous reviewers for comments on drafts of this manuscript

and E. Atarod, J. Bosch, A. Cadavid, C. Elmore, T. Flores Martinez, J. Kingston, M. Kirkpatrick, R. Long, T. Prude, B. Wagstaff, P. Sturm, and M. Woods for assistance in the field and laboratory. We thank the government of Mexico for permission to collect and conduct fieldwork (permiso de pesca de fomento 020398-213-03). Animal research was conducted in compliance with Mexican state and federal laws and with Animal Research Protocol 01960301 of the Institutional Animal Care and Use Committee of the University of Texas, Austin. This work was funded by National Science Foundation grants IBN 96-30230 to MJR and 97-01008 to GGR and MJR. FJGL and XfDRR are indebted to the Instituto Tecnológico de Ciudad Victoria for support.

#### LITERATURE CITED

- BASOLO, A. L. 1990. Female preference for male sword length in the green swordtail (Pisces: Poeciliidae). *Anim. Behav.* 40:332-338.
- . 1995a. Phylogenetic evidence for the role of a preexisting bias in sexual selection. *Proc. R. Soc. Lond. B. Biol. Sci.* 259:307-311.
- . 1995b. A further examination of a preexisting bias favoring a sword in the genus *Xiphophorus*. *Anim. Behav.* 50:365-375.
- BOROWSKY, R. 1990. Habitat choice by allelic variants in *Xiphophorus variatus* (Pisces, Poeciliidae) and implications for the maintenance of genetic polymorphism. *Evolution* 44:1338-1345.
- , M. McCLELLAND, R. CHENG, AND J. WELSH. 1995. Arbitrarily primed DNA fingerprinting for phylogenetic reconstruction in vertebrates: the *Xiphophorus* model. *Mol. Biol. Evol.* 12:1022-1032.
- CARSON, H. L., K. Y. KANESHIRO, AND F. C. VAL. 1989. Natural hybridization between the sympatric Hawaiian species *Drosophila silvestris* and *Drosophila heteroneura*. *Evolution* 43:190-203.
- DOHERTY, J., AND R. HOY. 1985. Communication in insects III. The auditory behavior of crickets: some views of genetic coupling, song recognition, and predator detection. *Q. Rev. Biol.* 60:457-472.
- ENDLER, J. A., AND A. E. HOUDE. 1995. Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution* 49:456-468.
- KAZIANIS, S., D. C. MORIZOT, B. B. MCENTIRE, R. S. NAIRN, AND R. L. BOROWSKY. 1996. Genetic mapping in *Xiphophorus* hybrid fish: assignment of 43 AP-PCR/RAPD and isozyme markers to multipoint linkage groups. *Genome Res.* 6:280-289.
- MARCUS, J. M., AND A. R. McCUNE. 1999. Ontogeny and phylogeny in the northern swordtail clade of *Xiphophorus*. *Syst. Biol.* 48:491-522.
- MCDONALD, D. B., R. P. CLAY, R. T. BRUMFIELD, AND M. J. BRAUN. 2001. Sexual selection on plumage and behavior in an avian hybrid zone: experimental tests of male-male interactions. *Evolution* 55:1443-1451.
- MEYER, A., J. MORRISSEY, AND M. SCHARTL. 1994. Re-

- current origin of a sexually selected trait in *Xiphophorus* fishes inferred from a molecular phylogeny. *Nature* 368:539–542.
- MORIZOT, D. C., R. S. NAIRN, R. B. WALTER, AND S. KAZIANIS. 1998. The linkage map of *Xiphophorus* fishes. *ILAR Journal* 39:237–248.
- , AND M. E. SCHMIDT. 1990. Starch gel electrophoresis and histochemical visualization of proteins, p. 23–80. *In: Applications of electrophoresis and isoelectric focusing in fisheries management*. D. H. Whitmore (ed.). CRC Press, Boca Raton, FL.
- MORRIS, M. R., M. MUSSEL, AND M. J. RYAN. 1995. Vertical bars on male *Xiphophorus multilineatus*: a signal that deters rival males and attracts females. *Behav. Ecol.* 6:274–279.
- , W. E. WAGNER JR., AND M. J. RYAN. 1996. A negative correlation between trait and mate preference in *Xiphophorus pygmaeus*. *Anim. Behav.* 52: 1193–1203.
- , K. DE QUEIROZ, AND D. C. MORIZOT. 2001. Phylogenetic relationships among populations of northern swordtails (*Xiphophorus*) as inferred from allozyme data. *Copeia* 2001:65–81.
- NIJHOUT, H. F., AND D. J. EMLEN. 1998. Competition among body parts in the development and evolution of insect morphology. *Proc. Natl. Acad. Sci. USA* 95:3685–3689.
- PARSONS, T. J., S. L. OLSON, AND M. J. BRAUN. 1993. Unidirectional spread of secondary sexual plumage traits across an avian hybrid zone. *Science* 260: 1643–1646.
- PTACEK, M. 1998. Interspecific mate choice in sailfin and shortfin species of mollies. *Anim. Behav.* 56: 1145–1154.
- RAUCHENBERGER, M., K. D. KALLMAN, AND D. C. MORIZOT. 1990. Monophyly and geography of the Río Pánuco Basin swordtails (genus *Xiphophorus*) with descriptions of four new species. *Am. Mus. Novit.* 2975:1–41.
- ROSEN, D. E. 1979. Fishes from the uplands and intermontane basins of Guatemala: revisionary studies and comparative geography. *Bull. Am. Mus. Nat. Hist.* 162:269–375.
- ROSENTHAL, G. G. 2000. The behavioral ecology of visual signaling in swordtails. Unpubl. Ph.D. diss., Univ. of Texas, Austin.
- , AND C. S. EVANS. 1998. Female preference for swords in *Xiphophorus helleri* reflects a bias for large apparent size. *Proc. Natl. Acad. Sci. USA* 95:4431–4436.
- , T. Y. FLORES MARTINEZ, F. J. GARCÍA DE LEÓN, AND M. J. RYAN. 2001. Shared preferences by predators and females for male ornaments in swordtails. *Am. Nat.* 158:146–154.
- , M. J. RYAN, AND W. E. WAGNER JR. 2002. Secondary loss of preference for swords in the pygmy swordtail *Xiphophorus nigrensis* (Pisces: Poeciliidae). *Anim. Behav.* 63:37–45.
- RYAN, M. J. 1998. Sexual selection, receiver biases, and the evolution of sex differences. *Science* 281:1999–2003.
- , AND W. E. WAGNER JR. 1987. Asymmetries in mating preferences between species: female swordtails prefer heterospecific mates. *Ibid.* 236:595–597.
- , D. K. HEWS, AND W. E. WAGNER JR. 1990. Sexual selection on alleles that determine body size in the swordtail *Xiphophorus nigrensis*. *Behav. Ecol. Sociobiol.* 26:231–237.
- SANDERSON, N., J. M. SZYMURA, AND N. H. BARTON. 1992. Variation in mating call across the hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata*. *Evolution* 46:595–607.
- SCHARTL, M. 1995. Platyfish and swordtails: a genetic system for the analysis of molecular mechanisms in tumor formation. *Trends Genet.* 11:185–189.
- SINERVO, B., AND A. L. BASOLO. 1996. Testing adaptation with phenotypic manipulations, p. 149–185. *In: Evolutionary biology of adaptation*. M. R. Rose and G. V. Lauder (eds.). Academic Press, New York.
- VON HELVERSEN, D., AND O. VON HELVERSEN. 1975. Verhaltens genetische Untersuchungen am akustischen Kommunikationssystem der Feldheuschrecken (Orthoptera, Acrididae) II. Das Lautschema von Artbastarden zwischen *Chorthippus biguttulus* und *Ch. mollis*. *J. Comp. Physiol.* 104:301–323.
- WEIR, B. S., AND C. COCKERHAM. 1984. Estimating *F*-statistic for the analysis of population structure. *Evolution* 38:1358–1370.
- WIRTZ, P. 1999. Mother species—father species: unidirectional hybridization in animals with female choice. *Anim. Behav.* 58:1–12.
- WRIGHT, S. 1969. Evolution and the genetics of populations. Vol. 2. The theory of gene frequencies. Univ. of Chicago Press, Chicago.
- (GGR) STON UNIVERSITY MARINE PROGRAM, MARIN BIOLOGICAL LABORATORY, 7 MBL STREET, WOODS HOLE, MASSACHUSETTS 02543; (XFDRR, FJGL) LABORATORIO DE BIOLOGÍA INTEGRATIVA, INSTITUTO TECNOLÓGICO DE CIUDAD VICTORIA, CIUDAD VICTORIA, TAMAULIPAS 87010, MEXICO; (SK, DCM) UNIVERSITY OF TEXAS M. D. ANDERSON CANCER CENTER, SCIENCE PARK, RESEARCH DIVISION, SMITHVILLE, TEXAS 78957; (MJS) AQUATIC STATION, DEPARTMENT OF BIOLOGY, SOUTHWEST TEXAS STATE UNIVERSITY, SAN MARCOS, TEXAS 78666; (MJR) SECTION OF INTEGRATIVE BIOLOGY, SCHOOL OF BIOLOGICAL SCIENCES, UNIVERSITY OF TEXAS, AUSTIN, TEXAS 78712. E-mail: ggrman@biomail.ucsd.edu. Send reprint requests to GGR. Submitted: 26 Feb. 2002. Accepted: 6 Oct. 2002. Section editor: R. E. Wood.