

**THE IMPACT OF HYBRIDIZATION ON MORPHOLOGICAL
VARIATION IN *Xiphophorus* FISHES**

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

The Impact of Hybridization on Morphological Variation in *Xiphophorus* Fishes. (May 2013)

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Hybridization can produce novel phenotypic variation which may allow hybrid populations to evolve in novel directions relative to parentals. Hybridization can produce intermediate as well as extreme phenotypes with respect to the parent species. Furthermore, separate hybridization events may create phenotypically divergent populations compared to the parentals but also to other hybrid zones. The hybrid system of *Xiphophorus birchmanni* and *X. malinche* has been shown to be replicated independently in seven streams along an elevation gradient. The sword sexual ornament is an extension of the ventral portion of the caudal fin that has been secondarily lost in *X. birchmanni* but persists in *X. malinche* and is polymorphic in hybrids. Female *X. birchmanni* have lost a preference for the sword and female *X. malinche* and hybrids are indifferent to males with swords. To determine if these replicated hybrid zones have unique introgression of the sword, morphometric data of the parental species and hybrids were collected across the hybrid zones. Though there was a general trend for the increase of the sword as one moved towards a more *X. malinche*-like population, the hybrid zones displayed variation for the introgression of the sword.

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CHAPTER I

INTRODUCTION

Hybridization is an important mechanism for the generation of novel phenotypic variation in populations (1). Its evolutionary importance has been recognized in plants for several years, though it is now becoming clear that it is equally important in animals (2). The novel variation produced by hybridization can be acted upon by natural and sexual selection and can yield hybrid populations which are more fit than their parental species (1, 3). Typically, the hybrid population exhibits more variation than each parental species and this variation can sometimes exceed the combined variation in both parental species (1). As a result, hybrids may be able to use niches that are inaccessible to the parent species (1, 4). Thus, hybridization can provide fodder for the evolution of adaptations (4).

Transgressive segregation, a phenomenon whereby hybrids exhibit extreme phenotypes relative to parents has been suggested as a means to increase evolvability of hybrid populations. But less explored is the fact that repeated hybridization events should produce unique phenotypic variation relative to not only parentals but also other hybridization events. Hybridization produces a genetic architecture which has reduced covariance between traits and increased variance among traits (5). This conformation of the genetic architecture is unstable and will change considerably in only a few generations (6). As a result, hybrid populations may diverge from one another in very short time spans. Furthermore, the phenotypic outcome of hybridization is dependent of the degree of divergence between parentals and will produce hybrids which differ phenotypically relative to hybrids from other parental pairings (7).

Studies examining novel phenotypic variance in hybrid populations have largely focused on non-sexual traits (8). Strong directional female choice can deplete genetic and phenotypic variance in male traits (9). This brings forth the ‘lek paradox’, where females mate based on additive genetic benefits for their offspring, despite the reduction of additive male genetic variation in male traits due to directional female preference, therefore inhibiting additive genetic benefits for their offspring (9). Because hybridization produces novel variation, variation in male sexual ornaments may be reintroduced into a population where directional female preference has previously depleted it.

The swordtails *Xiphophorus birchmanni* and *Xiphophorus malinche* are found in rocky streams of Hidalgo, Mexico (10). They are part of the monophyletic northern group of swordtails and produce natural hybrids. Male swordtails are characterized by an extension of their caudal fin, known as the sword. The sword is a low-cost sexual ornament that develops after sexual maturity (11). Male *X. malinche* display a sword whereas male *X. birchmanni* have secondarily lost the sword (8). Hybrids are polymorphic with respect to the sword ornament (8). Although female preference for the sword is an ancestral trait in *Xiphophorus*, it is variable across the phylogeny. Female pygmy swordtails, *X. nigrensis*, and female *X. malinche* are indifferent to the sword (12, GGR, unpublished data). Female *X. birchmanni* have secondarily lost the preference for the sword while female *X. hellerii* have a preexisting bias for swords that may have arisen from a perceived larger body size in males (11, 13). In addition, parentals and hybrids differ with respect to habitat, with *X. malinche* being found in highland streams and *X. birchmanni* in low elevations and hybrids at mid-elevation (14). Prior work has shown that those hybridization events are replicated in seven separate streams (8, 14). Gene flow between these hybrid zones is

prevented by a number of geographic factors, including mountain ridges and long downstream distances (14). Given that female *X. malinche* are indifferent to the sword and female *X. birchmanni* have lost their preference for the sword, hybrids that are genetically *X. malinche* should exhibit *X. birchmanni* morphology with respect to the sword – that is, reduced or absent swords. The aim of this project is to determine whether these replicated hybrid zones display unique patterns of introgression of the sword.

CHAPTER II

METHODS

Male *X. malinche*, *X. birchmanni*, and hybrids were collected between 2003 and 2012. Nine hundred and thirty-seven males were collected from 17 populations across 8 drainages and preserved in 95% ethanol. In order to measure the sword sexual ornament, lateral photos of mature males were taken. A ruler was placed on the background of each photo for scale standardization. Sword extension length (hereafter sword length) was defined per Rosenthal et al. 2003 as “pronounced, pigmented elongation of the lower rays of the caudal fin”. Using tpsDig, the images were digitized and a landmark was placed at the end of the caudal fin and the tip of the sword (15). The landmark coordinates were extracted and Euclidean distances between the coordinates were estimated using R (16).

To determine the degree of hybridization in each population I used previously published genotype data (14). Seven hundred and seventy-six individuals were genotyped using single nucleotide polymorphisms (SNP) at one mitochondrial and three nuclear intron loci. A hybrid index was constructed by calculating the proportion of *X. malinche* alleles in each population. Thus, populations with a hybrid index of 0 were considered pure *X. birchmanni*, populations with an index of 1 were considered pure *X. malinche*. An analysis of variance (ANOVA) was performed with sword length as the dependent variable and hybrid index and drainage as independent variables. I also included the interaction of hybrid index and drainage into the regression model which evaluated the divergence in sword length between drainages and hybrid composition.

CHAPTER III

RESULTS

There was a significant relationship between sword length and hybrid index (Table 1) and between sword length and drainage (Table 1). Sword length increases as one moves from *X. birchmanni* sites to *X. malinche* sites (Figure 1; Table 1). Sword introgression differed by drainage, as indicated by a significant interaction term of hybrid index and drainage (Table 1; Figure 1). The patterns of sword introgression varied across hybrid zones as well as within hybrid zones, as populations with the same hybrid index differed in mean sword length. The large standard deviation present in Figure 1 indicates variation within populations, though this could be a result of differences in sample sizes for each population.

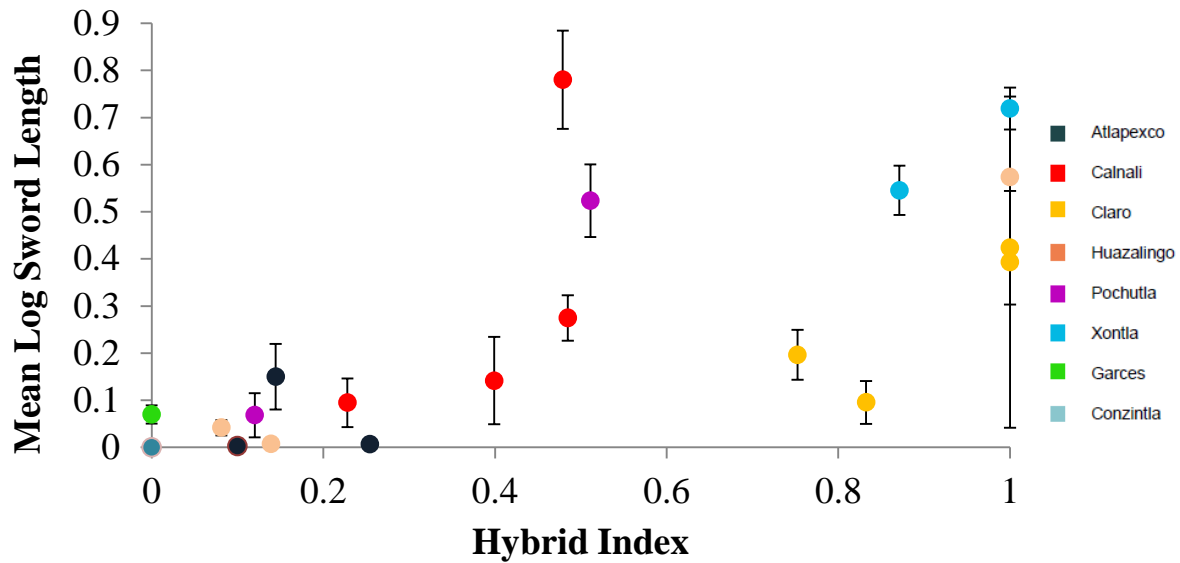


Figure 1: The relationship between hybrid index and sword length by drainage.

Table 1: Analysis of variance of hybrid index and drainage on sword length.

Effect	DF	Sum of Squares	F	P
Hybrid Index	1	192.77	25.52	<0.0001
Drainage	5	207.86	5.50	<0.0001
Hybrid Index x Drainage	5	117.01	3.10	0.0093

CHAPTER IV

CONCLUSIONS

We found that separate hybridization events have produced unique patterns of sword introgression (Figure 1). The patterns of introgression varied by drainage as well as among populations within the same hybrid zones. The hybrid zones are independent and gene flow is prevented due to geographic barriers. They also differ in population structure, with some populations having minimal introgressive hybridization and others consisting of only hybrids. These differences in population structure could be due to differential selection regimes and patterns of hybridization and may explain the variation in sword introgression within drainages, as populations with similar hybrid indices still varied in mean sword length (14).

These results are relevant to the “paradox of the lek” which relates the depletion of genetic and phenotypic variance of male sexual ornaments by strong directional female choice (9). Female directional choice on additive genetic male traits results in the eventual loss of additive genetic male traits in a population. If choice confers any additive genetic benefits upon offspring, females are ultimately depleting any additive genetic benefit by exercising directional choice. Several ideas have been proposed as potential resolutions for the paradox of the lek (9, 17). The genic capture hypothesis is a popular contender for the resolution of the lek paradox and is dependent on the idea that secondary-sexual traits are condition dependent and this condition has large additive genetic variance (9). Though this has been supported by condition-dependent secondary sexual traits, this is an unlikely resolution for the swordtail system. The sword is a low-cost ornament and while the genic capture hypothesis suggests female select ornamented males in order to pass on ‘good genes’ to their sons, there seems to be higher selection against

the introgression of the sword in swordtails. Indirect genetic effects conceded to daughters may allow for the persistence in directional female selection. These indirect genetic benefits are the result of gene expression in one individual conferring phenotypic effects to another. For the lek to be resolved by indirect genetic benefits, females, by way of their maternal behavior, provide their daughters with ‘good genes’ when choosing ornate males. Again, while this is a viable hypothesis in other systems, the sexual ornament is being selected against by female swordtails (17). Change in mutation rates due to female choice has also been proposed as a solution for the paradox of the lek. Mathematical models have been developed to test this hypothesis and have found an increase in the number of mutations as female choice increases. However, though the models pose a promising solution, the current models require several assumptions, such as linkage between mutation modifiers and the locus of interest, which seems unlikely in swordtails given the hybridization events (9). My results indicate that the lek paradox could be resolved by introgression from divergent populations or closely related species.

The ANOVA revealed a significant relationship between sword length and hybrid index (Table 1). Populations that are more *X. birchmanni*-like have shorter swords while populations that are more *X. malinche*-like had longer swords (Figure 1). This general pattern may be maintained via mate choice. Female *X. malinche* and hybrids are indifferent to the sword and *X. birchmanni* show disdain for the sword (12,13, GGR, unpublished data). Thus, introgression of the sword by *X. malinche*-like hybrids is likely buffered by the preferences of female *X. birchmanni* and *X. birchmanni*-like hybrids.

Further work can be done to explore the degree of variation in the hybrid zones. The number of individuals sampled throughout the populations was not consistent and additional sampling may help reduce inconsistencies in Figure 1. Performing a similar analysis on other sexual traits and non-sexual traits may further elucidate the relationship between hybridization and morphological variation.

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