THE ARCHITECTURE OF PHENOTYPES IN A NATURALLY HYBRIDIZING

COMPLEX OF *XIPHOPHORUS* FISHES

A Dissertation

by

JAMES BRADLEY JOHNSON

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Approved by:

Chair of Committee,	Gil G. Rosenthal
Committee Members,	Adam G. Jones
	Kirk O. Winemiller
	Lee A. Fitzgerald
Head of Department,	Thomas McKnight

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ABSTRACT

The origin and maintenance of phenotypic variation has generated considerable interest among students of functional morphology, sexual selection and behavioral ecology. In particular, hybridization has been suggested as a phenomenon which may generate novel phenotypic variation. In this dissertation I focus on the *Xiphophorus birchmanni - X. malinche* hybrid system to assess the role of hybridization in altering behavioral, morphological, sexual and non-sexual traits. I determine the relationship between the sword sexual ornament and body condition to support previous work which suggests that the sword is an inexpensive means to increase apparent size. My findings support the prediction that, while body size is condition-dependent, the sword is not. I show a trend toward hybrid populations displaying increased phenotypic variance and reduced phenotypic integration in sexual ornaments and body size. These findings provide evidence for a potential answer to a central question in the study of sexual selection, that of reduced genetic and phenotypic variance in sexual ornaments as the result of persistent direction selection generated by female choice. I take advantage of reduced phenotypic integration in hybrids allowing the evaluation of locomotor performance across a broad range of multivariate trait values. Sexual ornaments did not impair swimming performance per se. Rather, the sword negatively affected performance only when paired with a sub-optimal body shape. I evaluated how natural hybridization changes the relationship between boldness and anti-predator response. In poeciliid fishes, bold individuals have increased survival in the presence of predators.

This non-intuitive observation may result from bold individuals being more likely to engage in anti-predator behaviors. Counter to my prediction, bold individuals were less likely to perform a fast-start response to a predator threat. This correlation was consistent among populations and species but was only significant in hybrids. My findings suggest that hybridization could influence correlations between behavioral traits in a manner similar to that documented for morphological traits.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

An individual's success in finding mates, evading predators, acquiring resources and locating suitable habitat depends on its phenotype. This concept was succinctly described by Arnold (1983; Figure 1). In this view of trait function, the phenotype is composed of morphological, physiological and behavioral traits which in turn, influence performance (i.e. how well the individual performs an ecologically relevant task) and it is performance which directly influences fitness.
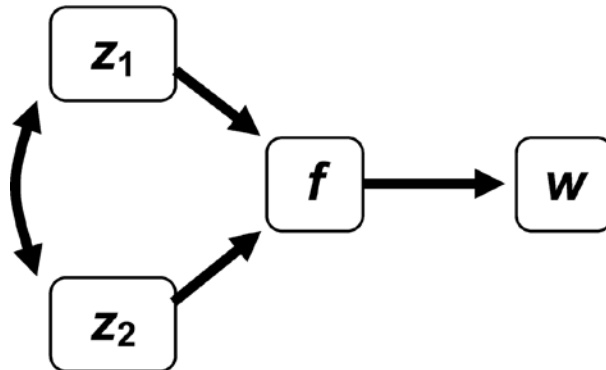
Figure 1 The morphology, performance and fitness paradigm. Variance in traits (z1 & z2) as well as covariance between traits (double-headed arrows) influence performance (f) which directly affects fitness (w). Modified from Arnold (1983).

In effect, the direction and intensity of selection on the phenotype can be decomposed into two components, selection on performance (e.g. greater running speed, more matings, etc.) and the mechanistic relationship between performance and traits (Arnold

1

1983). But the capacity for evolutionary change in both performance and traits depends, of course, on the extent to which phenotypic variation is associated with heritable genetic variation (Lande 1979; Lande and Arnold 1983). A more challenging problem arises from the fact that traits can be strongly inter-correlated, i.e. integrated phenotypes or as Pigliucci (2003) describes "complex patterns of covariation among functionally related traits in a given organism". Phenotypic integration can influence performance, fitness and ultimately evolution (Pigliucci 2003; Badyaev 2004). For example, suites of traits such as those that compose feeding structures are often shown to be highly integrated as their reduced integration could degrade the functionality of that aspect of the phenotype (Cheverud 1982; Klingenberg 2008). In addition, covariation between traits may also result in phenotypic trade-offs if the direction of selection conflicts with multidimensional variation in traits (Lande and Arnold 1983; Ashman 2003; DeWitt and Langerhans 2003). Covariation may also compensate for suboptimal or costly traits (Rundle and Bronmark 2001; Oufiero and Garland 2007). Conversely, covariation between traits may also exaggerate the apparent cost of other traits, which I explore in Chapter 4.

While phenotypic integration has been addressed largely in the context of morphology and physiology, covariation among behavioral traits will also influence performance and fitness. The behavioral integration literature has largely developed independently from that of phenotypic integration, and several terms have been developed to describe integrated suites of behaviors: "behavioral syndromes" (Sih et al. 2003; Sih et al. 2004a), "personality" (Gosling 2001; Dingemanse and Reale 2005),

"temperament" (Réale et al. 2007) and "coping styles" (Koolhaas et al. 1999) which I argue are simply special cases of phenotypic integration. I explore behavioral trait integration in Chapter 5.

How well an individual gains mating events, a performance trait is, just as with non-sexual performance traits, influenced by the phenotype (Kodric-Brown 1993). For example, a male's sexual ornaments may influence a female's willingness to mate with him (Andersson and Simmons 2006). Sexual aspects of the phenotype as subject to the same requirements for phenotypic evolution such as adequate phenotypic and genetic variance (e.g. the paradox of the lek; Kirkpatrick and Ryan 1991) as non-sexually selected traits (Mead and Arnold 2004). While the sexual traits may gain mating opportunities may also interfere with other performance traits (i.e. costs), such as avoiding predation (Basolo and Wagner 2004; Hernandez-Jimenez and Rios-Cardenas 2012). Not surprisingly, the structure of covariation between traits may alleviate costs (e.g. compensation of costs of sexually selected traits; Oufiero and Garland 2007) or exaggerate the apparent costs of sexual ornaments thus making them appear more costly than they are in reality (Chapter 4).

Most studies of phenotypic integration have focused on comparisons between populations within a species or comparisons between non-introgressing taxa (but see Grant and Grant 1997). Yet an emerging paradigm in evolutionary biology is that of hybridization as a creative force in evolution (reviewed by Arnold and Hodges 1995; Barton 2001; Seehausen 2004). Arguably few phenomena can alter the genetic and phenotypic architecture as greatly as hybridization It has long been debated as to

3

whether hybridization was a positive force or an evolutionary dead end (reviewed by Arnold and Hodges 1995; Dowling and Secor 1997). Yet over the last decade the opinion has emerged that hybridization can be a source of evolutionary novelty providing new variation among and covariation between traits. These alterations to the genetic and phenotypic architecture will unsurprisingly influence performance (Hawkins and Quinn 1996; Rosenfield et al. 2004; Fitzpatrick 2008; Beecham et al. 2009; Johnson et al. 2010) and fitness (Arnold and Hodges 1995; Arnold et al. 2008). Guillaume and Whitlock (2007) modeled the genetic effects of hybridization and found that introgression increased genetic variance and decreased the genetic covariances. These prediction are evident in empirical work, in particular that with transgressive segregation, or the production of extreme phenotypes (relative to parentals) in hybrids which can result in hybrid populations with increased phenotypic variance relative to parental lines (reviewed by Rieseberg et al. 1999). In addition, hybrids may also display altered covariation between traits which is commonly found to result in reduced phenotypic integration, although some empirical studies have found that hybridization may in fact increase trait covariation (Grant and Grant 1994; Seehausen 2004). Not surprisingly, hybridization has been suggested as a means for populations to utilize new resources which may ultimately lead to adaptation. For example, Rieseberg et al (2003) showed that novel trait variation produced via hybridization reproduced in synthetic hybrids sunflowers (*Helianthus* spp.) was genetically and phenotypically similar to that of established sunflower population occupying extreme habitats and that trait variation generated by hybridization facilitated the utilization of those habitats. Not surprisingly

4

the novel phenotypic variation generated via hybridization and the utility of this novel

variance has led some to suggest that hybridization has played a role in adaptive

radiations, in particular that of east African cichlid communities (Seehausen 2004;

Albertson et al. 2005; Joyce et al. 2011) and Galapagos finches (Grant et al. 2004; Grant

et al. 2005). Hybridization has also played a significant role in the success of several

invasive plant species (Ellstrand and Schierenbeck 2000) as well as providing

agricultural cultivars with increased disease resistance by back-crossing with resistant

relatives (Goodman et al. 1987).

Swordtail fishes of the genus *Xiphophorus* range from northern Mexico to

Honduras in habitat ranging from headwater mountain streams to coastal lowlands along

the eastern slope of the Sierra Madre Oriental (Kallman and Kazianis 2006). Swordtails

display extensive phenotypic variation, particularly in sexual traits and in preferences for

those traits. For example, males vary greatly among individuals, populations, and species

in body size. Large body size is a preferred trait by females in the genus (Rosenthal and

Evans 1998); even some populations of female pygmy swordtails (*X. pygmaeus*) show

preference for larger heterospecific males (Ryan and Wagner 1987; Hankison and

Morris 2002). Males also exhibit considerable variation in dorsal fin size, yet female

preference for these traits is variable across the genus. For example, in *X. variatus*

females prefer larger dorsal fins which may mimic large male size (MacLaren et al.

2011) while in *X. hellerii* female show no preference for enlarged dorsal fins (MacLaren

and Daniska 2008) and in *X. birchmanni* females prefer small dorsal fins (Fisher et al.

2009).

Not surprisingly, a preponderance of studies of sexual communication in swordtails concern the eponymous ventral extension of the caudal fin, noted by Darwin (1871) as an example of extreme sexual ornamentation. The sword is thought to have evolved as an inexpensive means to elicit a preexisting female bias for large apparent size (Basolo 1998b; Rosenthal and Evans 1998; Chapter 2). Female preference for the sword has been lost several times in the genus (Reviewed in chapter 2). In addition, males of several species lack or have greatly reduced swords (Reviewed in chapter 2).

I explore the role of hybridization in altering the both sexual and non-sexual traits, both morphological and behavioral, in a natural system of hybrid swordtails. The swordtails *X. birchmanni* and *X. malinche* hybridize in the Rio Calnali and surrounding drainages in Hidalgo, Mexico. Previous work has suggested that hybrids show extended variation in morphological traits relative to parentals (Rosenthal et al. 2003). I evaluate the relationship between the sword, body size and male condition in *X. malinche* in chapter 2. In chapter 3 I evaluate the role of hybridization in affecting variation in and covariance between three sexual traits (sword length, dorsal fin size and body size). In chapter 4 I explore the relationship between body shape and the sword ornament in affecting swimming performance by taking advantage of reduced phenotypic integration between both traits as a result of hybridization. In chapter 5 I evaluate how hybridization between these species has altered behavioral integration.

CHAPTER II

NEGATIVE CONDITION-DEPENDENCE OF A SEXUAL ORNAMENT IN

XIPHOPHORUS MALINCHE

Introduction

It is generally advantageous for individuals to choose mates that are in good

physical condition. Good-condition mates are less likely to transmit parasites to sexual

partners or offspring (Martinez-Padilla et al. 2012); further, physical condition may be

positively correlated with a male's genetic contribution with respect to viability, or

"good genes" (Kotiaho 2001). Accordingly, condition-dependence has become a

cornerstone for contemporary reformulations of handicap or indicator models of sexual

ornament evolution (Zahavi 1975; Grafen 1990; Cotton et al. 2004). Condition-

dependence postulates that the honesty of sexual signals is maintained by natural

selection, which imposes greater costs of sexual ornaments for males bearing "bad

genes" relative to males bearing "good genes" (Kotiaho 2001). Since overall physical

condition is a proxy for "good genes", females should evolve preferences for condition-

dependent sexual traits (Cotton et al. 2004; Bondhuriansky and Rowe 2005).

While the direct benefits of mating with good-condition males are

straightforward, the connection between condition-dependence and "good genes" in the

context of mate-preference evolution remains controversial (Kirkpatrick 1986; Prum

2010). Meta-analyses have found that a preferred partner's genetic contribution accounts

for a negligible fraction of variance in offspring viability (Møller and Alatalo 1999;

Prokop et al. 2012). Several workers have suggested that "good genes" hypotheses are too often invoked as a default explanation for the evolution of sexual ornaments, often with minimal evidence and without excluding alternative models (Prum 2010; Számadó 2011; Prum 2012). Notably, sexual traits can also evolve as the result of preexisting biases in female sensation or perception (Ryan 1990; Ryan and Rand 1993). Accordingly, sexual selection can favor male traits that are uncorrelated or negatively correlated with "good genes" and condition as long as they elicit preferences from females (Brooks 2000; Dale 2000).

The sexually-dimorphic "sword" extension of the caudal fin of swordtails (Teleostei: *Xiphophorus*) is an example of an ornament that has evolved in response to a preexisting preference (Basolo 1990, 1995; Rosenthal and Evans 1998). Female preference originated prior to the evolution of the ornament itself (Basolo 1990, 1995). However, the preference has weakened since the origin of the ornament; outgroup taxa have stronger preferences for swords than do swordtails (Basolo 1998a), and the preference for swords has been lost (Rosenthal et al. 2002) and reversed (Wong and Rosenthal 2006) in some swordtail lineages. It has been suggested that the sword may constitute an inexpensive way for males to project large apparent size (Basolo 1998b; Rosenthal and Evans 1998). The sword increases the risk of attacks by visual predators (Rosenthal et al. 2001; Basolo and Wagner 2004), and the sword is metabolically costly during courtship displays for at least one species, *X. montezumae* that possess particularly long swords (Basolo and Alcaraz 2003). Studies investigating locomotor performance cost for the sword ornament infrequently find evidence of the sword

8

impairing locomotion (Ryan 1988; Royle et al. 2006b; Kruesi and Alcaraz 2007; Baumgartner et al. 2011). Furthermore, when negative correlations between locomotor performance and the sword have been found, it is likely driven by covariation between the sword and non-sexual traits which directly influence performance thus giving the appearance that the sword is costly (Chapter IV). Furthermore, Basolo (1998b) showed that food-restricted males continued to grow swords even as body growth ceased, while males fed ad libitum invested in both traits. Thus, the sword ornament should either be independent of condition or negatively related with condition. I evaluated the relationship between the sword, body size, and condition in a natural population of the highland swordtail, *X. malinche*.

Materials and Methods

Collection – Fish were collected from *X. malinche*'s type locality on the Río Claro at Tlatzintla, Hidalgo, Mexico (GPS: 20 52' 51" N 98 47' 56" W) during May of 2011. Males were immediately euthanized using MS-222. Specimens were then photographed on their right side with a digital camera (Nikon D90; Nikon, Tokyo, Japan) fitted with a 60mm macro lens (AF Nikkor; Nikon, Tokyo, Japan) and mounted to a copy-stand. Specimens were preserved in 10% formalin.

Morphometrics – For each digital image I measured sword length from the edge of the caudal fin to the distal end of the sword in mm. Body size was estimated as centroid size, a common metric of body size used in landmark-based morphometrics that provides a two-dimensional metric of size similar to lateral projection area (MacLaren and Rowland 2006). To calculate centroid size, I digitized 9 landmarks on each image

(Figure 2). Landmarks included (1) upper lip, (2) eye, (3) anterior insertion of the dorsal

fin, (4) posterior insertion of the dorsal fin, (5) dorsal insertion of the caudal fin ray, (6)

ventral insertion of the caudal fin ray, (7) posterior insertion of the gonopodium (8)

anterior insertion of the gonopodium and (9) the ventral occlusion of the operculum

cover (Figure 2). The nuchal hump (10) and belly (11) were interpolated from right

angles from half the distance (shown as dotted lines) between landmarks 1 and 3 for

landmark 10 and 8 and 9 for landmark 11, respectively. Centroid size was calculated as

the summed squared distances for each landmark to the centroid of the landmark

configuration for each specimen. Centroid body size was log transformed for further

analysis.
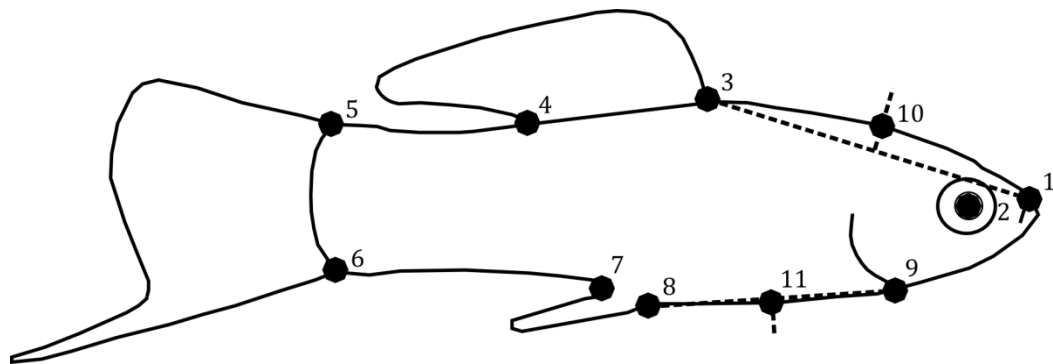
Figure 2 An illustration of a *X. malinche* male with digitized landmarks used to
calculate centroid size.

Upon sexual maturity, the anal fin of male swordtails differentiates into an

intromittent organ, the gonopodium. Gonopodium development typically occurs before

the onset of male secondary sexual characteristics, such as the sword. To account for

variation in male development, I scored gonopodium development on a 4 point scale: 1 represented no obvious gonopodium but the individual had testes, 2 represented a non-rigid but narrowed gonopodium, 3 a semi-rigid gonopodium, 4 fully rigid and developed gonopodium.

Body condition was estimated using lipid extraction (Meffe and Snelson Jr 1993; Heulett et al. 1995; Tobler 2008). Visceral organs were removed and the body of the specimen was placed in a drying oven (65° C) for 5 days. Specimens were removed and weighed to the nearest 0.001 g on a Mettler Toledo PG203-S balance and placed individually in vials. Each specimen was completely covered in lipid solvent (petroleum ether). Lipid solvent was changed daily for 5 days. Specimens were then removed and placed back into the drying oven for 24hrs and reweighed. Condition was estimated as the proportion of dry mass which was fat (mass before extraction – mass after extraction / mass before extraction; Tobler 2008). Proportion of mass which was fat was arc-sine square root transformed for further analysis.

I used model selection (Johnson and Omland 2004) to evaluate the relationship between morphology and fat content using the glmulti package (Calcagno and de Mazancourt 2010; R-Project 2011). My initial model included log centroid body size, log sword length and gonopodium index as independent variables and fat content as a dependent variable. Body size and sword length included as independent variables because I was interested in these morphological variables as predictors of condition. Alternatively, a MANOVA approach where morphology was dependent on condition could be employed but would not provide parameter estimates of

11
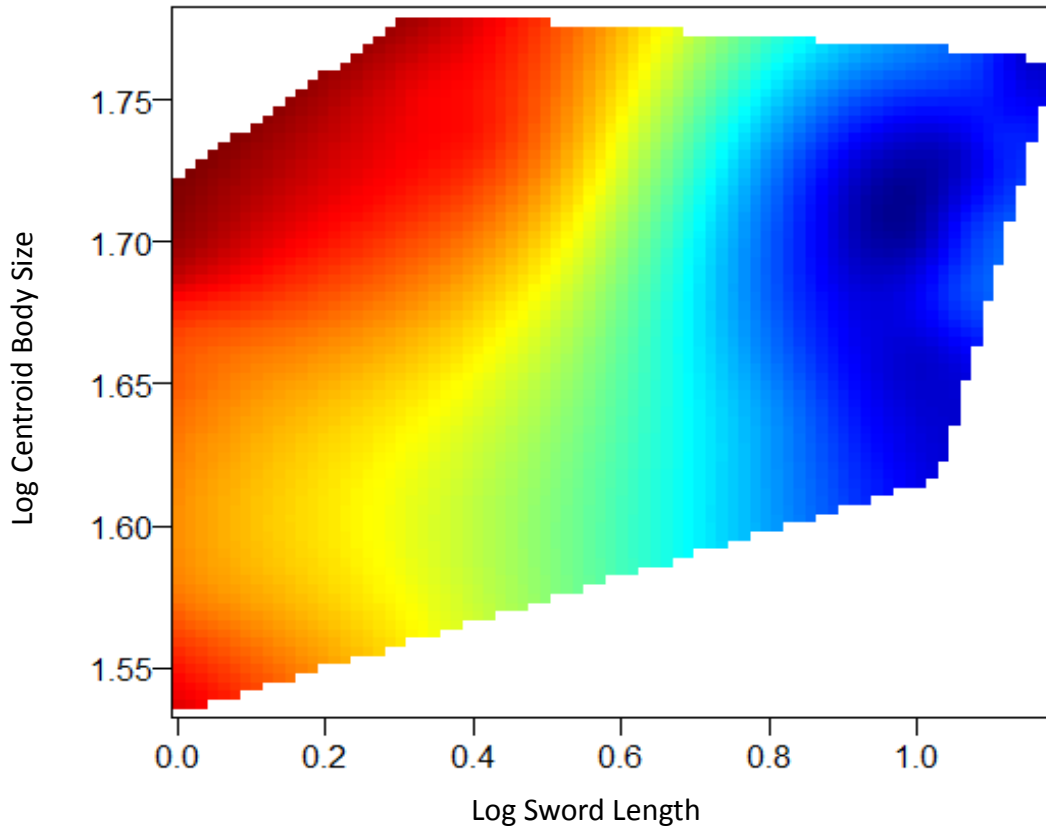
Figure 3 Surface plot describing the relationship between log sword length, log centroid body size and condition. Red indicates morphology which is associated with high condition (greater fat content) and blue indicates poor condition. Estimation of surfaces was performed in R (R-Project 2011) using the fields package.

body size or sword length nor allow the evaluation of their interaction. All possible

models including the full (all main effects and pairwise interaction terms) and null

(intercept only) were evaluated using Akaike Information Criteria (finite sample

correction, AICc, Johnson and Omland 2004). Main effects were retained if interaction

terms were found to improve model fit. Models which differ within two AICc units from

the model with the lowest AICc are considered equally supported (Burnham and

Anderson 2002). The relative importance of main effects and interaction terms were

evaluated using the sum of the relative evidence weights for each model in which a

given term appears (importance value > 0.8 are considered important; Buckland et al. 1997; Calcagno and de Mazancourt 2010). To visualize the relationship between morphological variables and condition non-parametric thin-plate spline regression was used to create a surface plot using the Fields package in R (R-Project 2011).

Results

The best-supported model explaining variation in condition (fat content) included both log sword length and log centroid size (Table 1). While both morphological traits were significantly related to condition (Table 2), the relationship was in opposite directions, with log centroid body size being positively related to condition ($\beta = 0.29$; Figure 3) and log sword length negatively related to condition ($\beta = -0.19$; Figure 3). Relative weights suggest that log sword length was of greater importance to model fit relative to log centroid body size or gonopodium development (Figure 4).

Discussion

Handicap models of sexual trait evolution suggest that fitness is maximized by mating with good-condition males and that condition dependent sexual ornaments provide females an honest signal to a male's condition (Zahavi 1975; Grafen 1990; Cotton et al. 2004). We should therefore expect ornaments that are favored by mate choice to positively correlate with condition (Andersson 1986; Iwasa et al. 1991; Bonduriansky 2007). This is indeed the pattern I found for body size in *X. malinche* males which supports the findings by Basolo (1998b) that male *X. hellerii* only invest in body growth when reared on high food diets. While a positive relationship between body size and condition is consistent with the notion of body size as an indicator of "good

13

genes", I cannot exclude the hypothesis that latent sensory biases for body size (Ryan 1990) exist independently any underlying discernment of male condition.
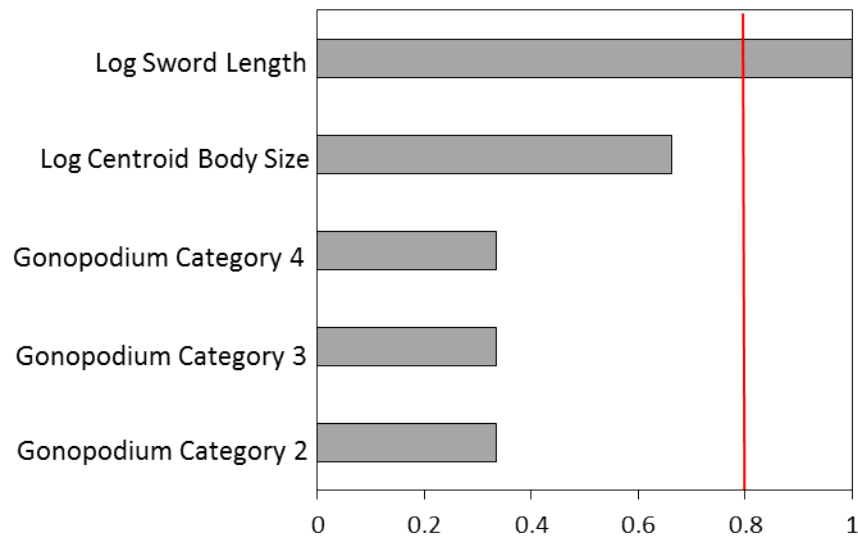
Figure 4 The summed relative weights (importance) for the five best-supported model effects.

By contrast, my findings suggest that the sword cannot represent an honest signal of male quality as it is negatively condition-dependent. Our findings are consistent with previous work on the origin and evolution of preferences for swords. Comparative studies by Basolo (1990, 1995) showed that the sword evolved in response to a preexisting bias on the part of females. Basolo (1998b) further found that male *X. hellerii* fed ad libitum invested in both sword length and body size, but males reared in a food-restricted treatment only invested in sword growth. Rosenthal and Evans (1998) argued that the sword constitutes a metabolically inexpensive way to elicit a female preference for large apparent size: female *X. hellerii* preferred animations of males with

longer swords over animations with no sword, but preference for the sword vanished

when females were presented animations of larger-bodied males. Thus, the sword

exploits a preexisting bias for large male size by providing a relatively inexpensive

means to increase male apparent size without the added expense of increasing body size

(Basolo 1998b; Rosenthal and Evans 1998). Thus the sword is effectively a dishonest

representation of male body size.

Since the sword's origin, the preference for swords has been weakened (*X.*

hellerii, Basolo 1998a), lost (*X. nigrensis*, Rosenthal et al. 2002; *X. malinche*, GGR

unpublished data), or reversed (*X. birchmanni*, Wong and Rosenthal 2006). By contrast,

females retain the ancestral preference for larger-bodied males (*X. nigrensis*, Ryan et al.

1992; *X. birchmanni*, Fisher et al. 2009). There are several scenarios that could account

for the loss of preference for swords in *Xiphophorus*. Fish predators attend more to

males with swords (Rosenthal et al. 2001) and there could be association costs to

females in close proximity to males with swords. The negative condition dependence of

the sword may mean that males with swords are more likely to transmit parasites or

pathogens during social interactions with females (Martinez-Padilla et al. 2012).

Negative condition-dependence may indeed be a sign that swords are correlated with

"bad genes", assuming that condition is indeed correlated with heritable variation in

fitness (Zahavi 1975; Grafen 1990; Cotton et al. 2004).

In addition, signal honesty may vary in time and space (Schluter and Price 1993;

Rands et al. 2011; Zollman et al. 2013). For example, Schluter and Price (1993) modeled

Table 1 Result from model selection. For all models condition is the dependent variable.

Model (Independent Variables)	AIC	Weights	ΔAIC
Body Size + Sword Length	-132.74	0.44	0.00
Gono Devl + Sword Length + Gono Devl*Sword Length	-131.41	0.22	1.33
Body Size + Sword Length + Sword Length*Body Size	-130.22	0.12	2.52
Sword Length	-129.88	0.10	2.85
Gono Devl + Body Size + Sword Length + Gono Devl*Sword Length	-129.07	0.07	3.67
Gono Devl + Body Size + Sword Length + Sword Length*Body Size + Gono Devl*Sword Length	-126.02	0.02	6.72
Gono Devl + Body Size + Sword Length	-125.52	0.01	7.22
Gono Devl + Sword Length	-124.49	0.01	8.25
Gono Devl + Body Size + Sword Length + Sword Length*Body Size	-122.75	0.00	9.98
Gono Devl + Body Size + Sword Length + Gono Devl*Body Size	-122.59	0.00	10.14
Gono Devl + Body Size + Sword Length + Gono Devl*Body Size + Gono Devl*Sword Length	-121.24	0.00	11.50
Gono Devl + Body Size + Sword Length + Sword Length*Body Size + Gono Devl*Body Size	-119.51	0.00	13.23
Gono Devl + Body Size + Sword Length + Sword Length*Body Size + Gono Devl*Body Size + Gono Devl*Sword Length	-117.66	0.00	15.08
Gono Devl	-86.52	0.00	46.22
Gono Devl + Body Size	-84.13	0.00	48.60
Gono Devl + Body Size + Gono Devl*Body Size	-75.89	0.00	56.85
Body Size	-71.21	0.00	61.53
Intercept Only (Null Model)	-70.20	0.00	62.54

preference for two male traits of varying honesty and found that female preference should evolve for the trait that maximizes both honesty and detectability. Thus, a less than honest signal which is highly detectable may be preferred over a more honest but less detectable sexual signal (Schluter and Price 1993). If the relationship between honesty and detectability changes, female preference may likewise evolve and even result in loss of both the preference and the male trait (Wiens 2001). It may be that initial evolution of the elaborately colored sword which is often accented by males during courtship displays was more detectable to ancestral female swordtails relative to male body size (a more honest signal of male condition). If females evolved greater detectability to differences in male body size but honesty of the sword and body size remained in equilibrium it could explain the loss of female preference for the sword and occurrence of the sword in the males of several *Xiphophorus* species. I present these as potential hypothesis for further investigation.

Table 2 Results from the best supported model

Effect	SS	DF	F	*P*
Log Centroid Body Size	0.011	1	5.25	0.027
Log Sword Length	0.301	1	139.91	<0.001
Residuals	0.084	39		

An alternative hypothesis to handicap models of sexual preference-ornament evolution is that of preexisting bias as a result of female perception (Ryan 1990; Kirkpatrick and Ryan 1991). The preexisting bias hypothesis is particularly attractive as it does not require natural selection to maintain signal honesty (Prum 2010, 2012). Yet

mechanisms of sexual trait evolution (e.g. good genes or preexisting bias) are not mutually exclusive (reviewed by Muñoz et al. 2011). This holistic view of sexual ornament evolution is congruent with both the findings of this study in addition to previous work regarding male investment in body size and sword growth in *Xiphophorus* fishes. I suggest that dishonest sexual signals that mimic honest signals and female preferences for those dishonest signals should be more variable among species than honest signals and preferences. The ability of males to mimic expensive structures with cheap ones may provide a partial explanation for the diversity of male traits and female preferences.

CHAPTER III

HYBRIDIZATION AND THE STRUCTURE OF PHENOTYPIC VARIATION IN

SWORDTAILS (TELEOSTEI: *XIPHOPHORUS*)

Introduction

Understanding the origin and maintenance of genetic and phenotypic variation is

a significant problem in evolutionary biology (Bull 1987; Wagner and Altenberg 1996).

Ultimately, most novel variation is the result of mutation (Carroll 2008), but other

effects such as environmental (Casanueva et al. 2012), epigenetic effects (Richards

2006), genetic drift (reviewed by Mitchell-Olds et al. 2007) and introgression

(Guillaume and Whitlock 2007) also play a major role in genetic and phenotypic

diversity. In particular, introgression between closely related species (i.e. hybridization

Seehausen 2004) has can produce dramatic changes in the genetic and phenotypic

architecture of populations. There are two general ways in which hybridization can alter

phenotypes. Phenomena such as transgressive segregation can produce individuals that

exceed the phenotypic range of either parental species, therefore contributing to

increased phenotypic variance in hybrid populations (Figure 5A; Rieseberg et al. 1999).

In addition, hybridization can also reduce phenotypic integration (i.e. reduced covariance

between traits; Figure 5A; Albertson et al. 2005). Both increased variation and reduced

integration may affect the capacity of a hybrid population to evolve (assuming the

phenotype is representative of the genotype) by increasing the amount of variation for

19
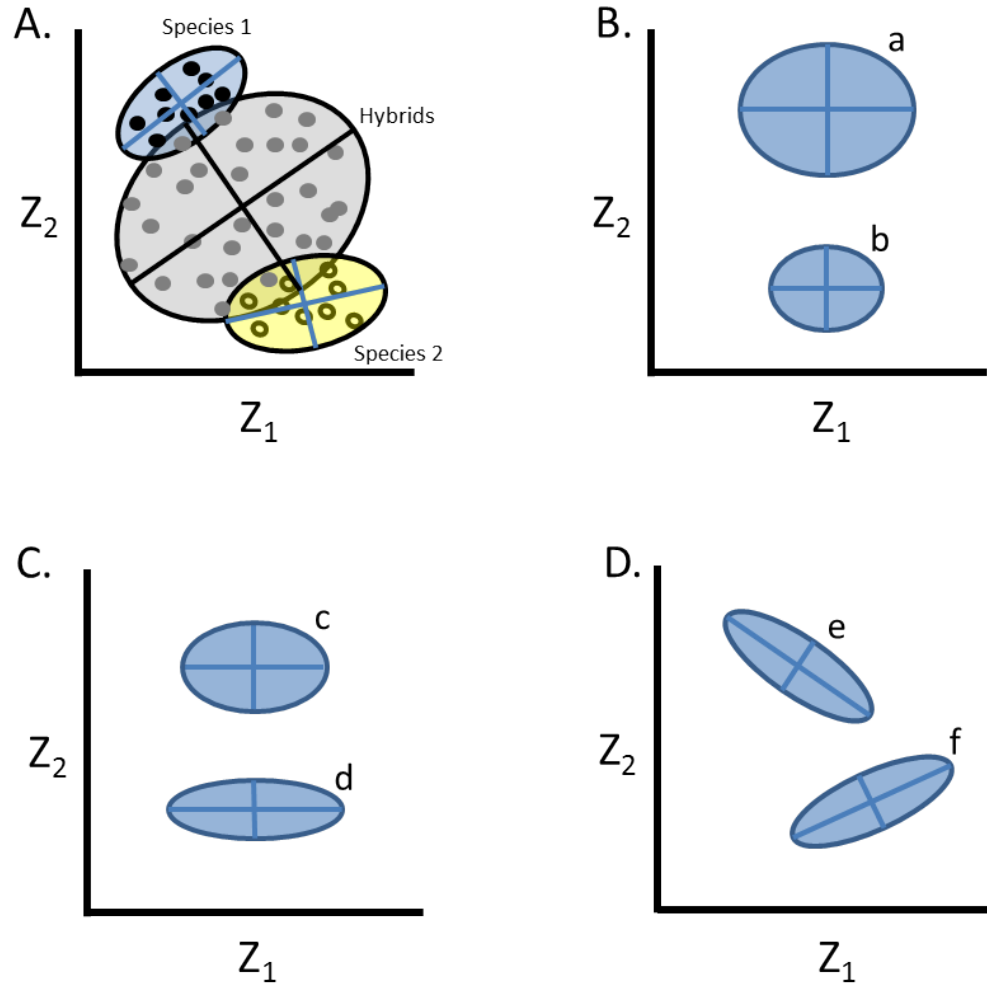
Figure 5 A, hybridization between two species for two traits (Z_1 and Z_2). Hybridization between two closely related species can result in hybrids which are intermediate to or even occupy parental phenotypic space which can translate into increased phenotypic variance of hybrid populations when compared to parental populations. Hybridization may also reduce phenotypic integration as a result of hybrid individuals with a greater diversity of trait combinations relative to parental species (i.e. the larger ellipse for hybrids in A). B, increased phenotypic variance will produce a **P** matrix which occupies more space (is larger; a) than a comparatively smaller **P** (b). C, decreased phenotypic integration will result in a **P** matrix with a reduced primary axis of variation ($\mathbf{P}_{max}$). Compare the long axis of the ellipse c to that of a more integrated **P** matrix, d. D, the orientation between **P** matrices may also differ (e and f).

selection to act upon and reducing the possibility of phenotypic trade-offs (Guillaume and Whitlock 2007).

In quantitative genetics, a multivariate phenotype can be summarized by the $\mathbf{P}$ matrix, which is composed of trait variances as diagonal elements and trait covariances as off-diagonal elements (Lande 1979). $\mathbf{P}$ can be decomposed into orthogonal vectors and corresponding vector lengths (Jones et al. 2003). These components can shed light on the standing phenotypic variation on which selection may act and assuming the phenotypic architecture is representative of the genetic architecture are insightful in understanding the evolutionary potential of the population in question (Schluter 1996; Arnold et al. 2001). For example, increased phenotypic variation translates into increased size of $\mathbf{P}$ (Figure 5Ba vs. 5Bb; Jones et al. 2003). Decreased covariances between traits (i.e. reduced phenotypic integration) results in a $\mathbf{P}$ matrix with low eccentricity (i.e. more basketball shaped; Figure 5Cc; Jones et al. 2003) with less difference between its major axis of variation ($\mathbf{P}_{max}$) and other subsequent orthogonal axes of variation. By contrast, increased phenotypic integration produces high eccentricity (i.e. cigar shaped; Figure 5Cd; Jones et al. 2003) and greater directionality due to a larger major axis. Furthermore, $\mathbf{P}$ matrices may differ in their orientation (Figure 5De and 1Df). Comparatively, a larger $\mathbf{P}$ matrix with a reduced major axis (variance more spread among other axes of variation; a rotund $\mathbf{P}$ matrix) will more easily move across the adaptive landscape relative to a small, oblong $\mathbf{P}$ (Jones et al. 2003; Guillaume and Whitlock 2007). Hybridization should increase phenotypic variance and

21

decrease covariances, producing a larger and more rotund **P** (Guillaume and Whitlock 2007).

In this study, I explore phenotypic variation, integration in sexually selected male traits a natural hybrid system of *Xiphophorus* fishes. The swordtail fishes, *X. malinche* and *X. birchmanni* form a hybrid systems in the mid-elevational reaches were both species meet (Rosenthal et al. 2003). In addition, hybrids are at least as fit as parentals where hybrids are found (i.e. bounded hybrid superiority; Culumber et al. 2012). Males of the two species differ dramatically with regards to morphology (Rauchenberger et al. 1990; Rosenthal et al. 2003). *Xiphophorus malinche* males are similar to other swordtails and express the sword. *Xiphophorus birchmanni*, by contrast, completely lack or only express very small swords (~ 1mm); males bear an enlarged dorsal fin and have larger bodies (a male trait which is universally preferred by females in this system). Previous work suggests that hybrids between the two species occupy a broad region of morphospace.

Materials and Methods

Sexually mature males (N = 736) were collected in the *X. malinche- X. birchmanni* hybrid system in northeastern Hidalgo state, Mexico, between 2003 and 2010 using baited funnel traps, baited with dog food. The localities included in this study (Table 3) were the subject of previous work by Culumber et al. (2010) using 3 autosomal and one mitochondrial SNP markers to characterize population structure and the extent of hybridization. I defined a population as *X. malinche* if the mean number of *X. malinche* alleles was greater than 80%, *X. birchmanni* less than 20% and hybrids

22

between 20 and 80%. Digital images were collected from the left and right side of specimens that had either been preserved in 95% EtOH or lightly anesthetized with tricaine methanesulfate (MS-222). I digitized 10 landmarks on each image and calculated the inter-landmark distances (mm) for 15 separate measures of the body and fins (Table 4; Figure 6). All distances were log transformed for analysis.

Table 3 Taxa, elevation and sample sizes by locality.

Locality	Code	Taxon	Hybrid Index	Elevation	N
Aguazarca	AGUA	Hybrid	0.48	981	55
Ahuamole	AHUA	*X. birchmanni*	0.12	869	23
Apantla	APTL	*X. malinche*	0.83	352	57
Calnali Low	CALL	Hybrid	0.23	920	36
Calnali Mid	CALM	Hybrid	0.48	1007	122
Chicayotla	CHIC	*X. malinche*	1	1003	72
Cocalaco	COCA	*X. birchmanni*	0.14	450	33
Garces	GARC	*X. birchmanni*	0	299	40
Malila	MALI	*X. malinche*	0.95	1364	26
San Pedro	SPED	*X. birchmanni*	0.08	384	48
Spider	SPDR	*X. malinche*	0.87	921	64
Tlatzintla	TLTZ	*X. malinche*	1	658	50
Tlatemaco	TLMC	Hybrid	0.71	480	110

To provide a two-dimensional measure of body size, I performed principal components analysis (PCA) on log transformed body measures (measurements 1-12 and 14, Table 4, Figure 6). Because the variables were all in the same units (mm), an eigen decomposition of the covariance matrix was used in the PCA. The first principal component loaded positively on all morphometric measures and explained 86% of the variation in body morphology and was correlated with a 1 dimensional measure of size,

standard length ($F_{1,822} = 75204.73$, $R^2 = 0.99$, $P = <0.001$). Body-size PC1, log dorsal fin width and log sword extension length were retained for further analysis.
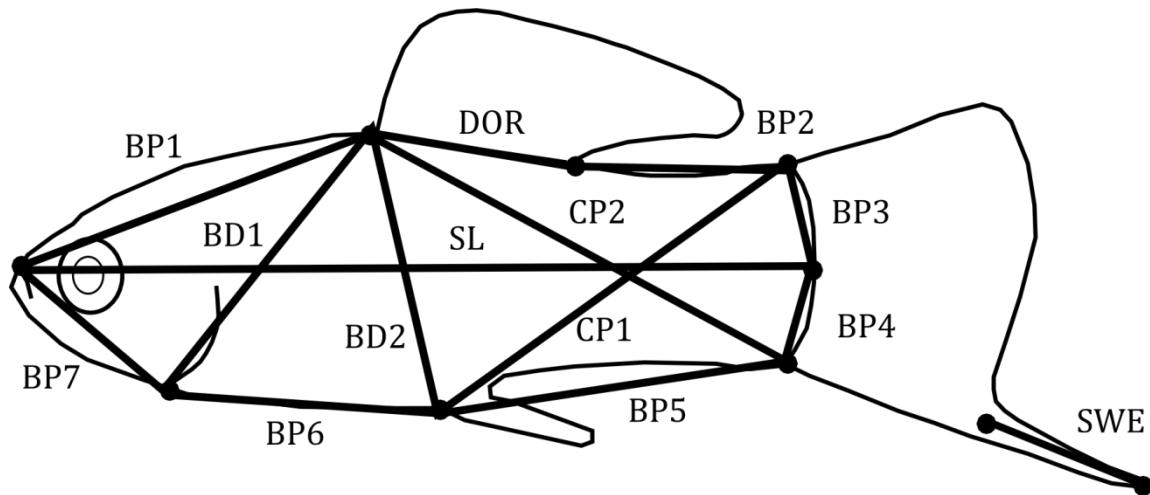
Figure 6 Inter-landmark distances used in the morphometric analysis (N = 14). Measurement details are given in Table 4.

To test the hypothesis that hybrid populations occupy greater morphospace (increased phenotypic variance) relative to parental populations, I used the summed eigenvalues derived from an eigendecomposition on the **P** matrix for body size PC1, log dorsal fin width and log sword extension length for each locality (Jones et al. 2003; Guillaume and Whitlock 2007). The eigenvalues describe the length of a given eigenvector and their total represents the total phenotypic space occupied by a given population (Jones et al. 2003; Guillaume and Whitlock 2007). Summed eigenvalues for parentals and hybrid populations were compared using one-way ANOVA.

Numerous indices of integration exist (reviewed by Haber 2011) but the relative standard deviation of the eigenvalues ($SD_{rel}(\lambda)$; Pavlicev et al. 2009) has been

determined to perform better in comparison with other indices thus it was used to estimate integration between body size, log dorsal fin width and log sword extension length for each locality. This index represents an estimation of the variance in distribution of eigenvalues which describes the degree to which traits are correlated (Pavlicev et al. 2009). Specifically, an eigen-decomposition of a correlation matrix for a highly integrated set of traits will yield a larger dominant eigenvalue relative to a less integrated suite of traits (i.e. it will explain a larger portion of the total variance). For a suite of traits which is less integrated the distribution of eigenvalues will be more even. Thus, for a highly integrated phenotype the eigenvalue standard deviation should be larger, indicating that the distribution of eigenvalues is less even. I adjusted $SD_{rel}(\lambda)$ for differences in sample size by population (Wagner 1984; Cheverud et al. 1989) by subtracting the random expected level of integration (T-1/N) according to a model of random pleiotropy (Wagner 1984). Integration across taxa was compared using one-way ANOVA on $SD_{rel}(\lambda)$. To evaluate phenotypic orientation (i.e. direction of $\mathbf{P}_{max}$; Fig. 1D) of hybrid and parental populations, $\mathbf{P}$ matrices (phenotypic variance-covariance matrices) were calculated for each of the 13 sample locations using body size PC1, log dorsal fin width and log sword extension length. Several methods have been developed to compare covariance matrices in general and $\mathbf{P}$ and $\mathbf{G}$ matrices specifically (reviewed by Roff et al. 2012).

I applied the random skewers method which is commonly used to compare $\mathbf{P}$

Table 4 Distance measurements (mm) used in the truss analysis.

Measurement	Distances Between Features
1. Standard Length (SL)	Premaxilla to the posterior midpoint of the caudal peduncle.
2. Body Perimeter 1 (BP1)	Premaxilla to the anterior insertion of the dorsal fin.
3. Body Perimeter 2 (BP2)	Posterior insertion of the dorsal fin to the dorsal insertion of the caudal fin.
4. Body Perimeter 3 (BP3)	Ventral insertion of the caudal fin to the posterior midpoint of the caudal peduncle.
5. Body Perimeter 4 (BP4)	Posterior midpoint of the caudal peduncle to the ventral insertion of the caudal fin.
6. Body Perimeter 5 (BP5)	Posterior insertion of the gonopodium to the ventral insertion of the caudal fin.
7. Body Perimeter 6 (BP6)	Anterior insertion of the gonopodium to the ventral edge of the operculum cover.
8. Body Perimeter 7 (BP7)	Ventral edge of the operculum cover to the upper lip.
9. Body Depth 1 (BD1)	Ventral edge of the operculum cover to the anterior insertion of the dorsal fin.
10. Body Depth 2 (BD2)	Anterior insertion of the dorsal fin to anterior insertion of the gonopodium.
11. Caudal Peduncle 1 (CP1)	Anterior insertion of the gonopodium to the dorsal insertion of the caudal fin.
12. Caudal Peduncle 2 (CP2)	Anterior insertion of the dorsal fin to the ventral insertion of the caudal fin.
13. Dorsal Fin Width (DOR)	Anterior to the posterior insertion of the dorsal fin.
14. Sword Extension Length (SWE)	Edge of the caudal to the tip of the sword.

matrices and performs well in this application (Cheverud and Marroig 2007). The random skewers tests for association between two or more covariance matrices by estimating a mean vector correlation of the response to random vectors (i.e. random skewers) and comparing this randomized vector correlation to the observed mean vector correlation. If hybrid **P** matrices differ from parentals, then one would a priori expect hybrid **P** matrices to show reduced matrix correlations with parental **P** matrices.

Results

Populations differed in phenotypic variance differed among populations (i.e. size of their **P** matrices; eigenvalue sum ANOVA: $F_{2,10} = 9.56$, P = 0.005; Figure 7A). Tukey's HSD comparisons indicated that *X. birchmanni* occupied significantly less phenotypic space than hybrids (mean difference = 0.1, lower CI = 0.036, upper CI = 0.165) and *X. malinche* (mean difference = 0.07, lower CI = 0.008, upper CI = 0.13). Whereas the mean phenotypic space occupied by hybrids was the largest, it was not significantly greater than the phenotypic space occupied by *X. malinche* (mean difference = 0.03, upper CI = -0.03, 0.093). Phenotypic integration, measured as the relative standard deviation of the eigenvalues ($SD_{rel}(\lambda)$), did not significantly differ among taxa (ANOVA: $F_{2,10} = 1.87$, P = 0.20; Figure 7B). Random skewers matrix correlations indicated similar P matrix orientation both among and between hybrid and *X. malinche* localities, whereas *X. birchmanni* localities frequently differed from other *X. birchmanni* sites as well as hybrid and *X. malinche* sites (Table 5). Inspection of the dominant eigenvectors indicates that among hybrids (Table 6) and *X. malinche* (Table 7)
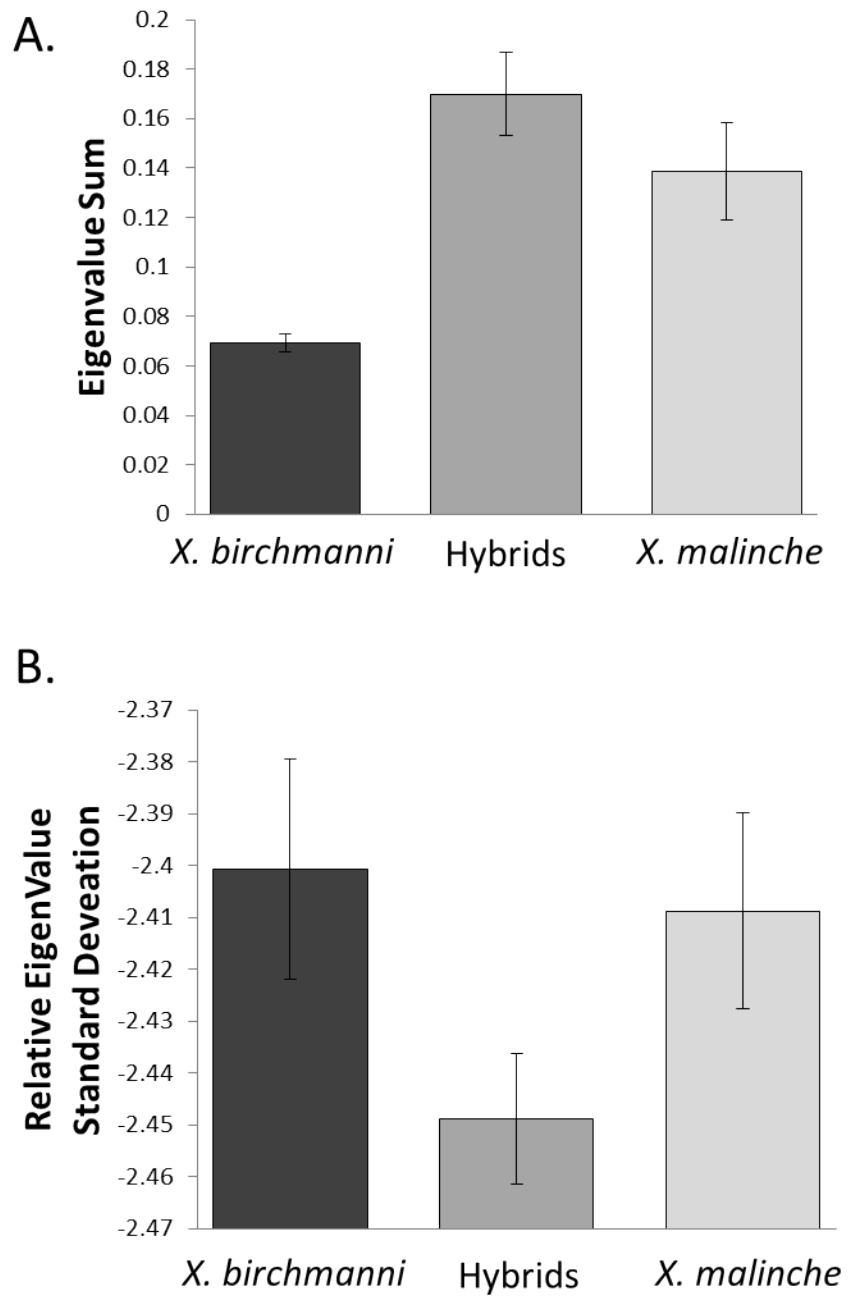
Figure 7 Means and standard errors for taxon differences in A. eigenvalue sum and B. relative eigenvalue standard deviation.

phenotypic variation was oriented toward sword length, whereas in *X. birchmanni* it was oriented towards body size (Table 8).

Discussion

Hybridization can increase phenotypic variance in male traits in hybrid populations relative to parentals (Figure 7A). All else being equal, strong, persistent directional selection should erode genetic variation and therefore phenotypic variation. This concept often has been asserted for male sexual ornaments, whereby female preferences should remove all but the more preferred male phenotypes, yet variation in male ornaments paradoxically persists. Resolutions to this "paradox of the lek" (Kirkpatrick and Ryan 1991) have been proposed such as the capture of genetic variance by condition dependent sexual traits (Rowe and Houle 1996), evolving mutation rates and indirect genetic benefits (reviewed by Kotiaho et al. 2008). But less explored is the prospect that introgression between closely related groups or species could introduce novel variation in female preferences and male traits into a population (reviewed by Kotiaho et al. 2008). Introgression, whether among species or among genetically divergent subpopulations may resupply genetic and phenotypic variance in male traits previously depleted by strong, direct female choice.

The prediction that hybridization reduces phenotypic integration was not supported at the alpha level of 0.05 (Figure 7B). The lack of distinction between taxa may reflect relatively low power in my analysis. While this data set consists of 736 males, the analysis is conducted at the population level with 4 hybrid, 5 *X. malinche* and

Table 5 Results from **P** matrix comparisons (random skewers) between 13 populations of *X. birchmanni*, *X. malinche* and hybrids. Significant correlations are shown in bold.

Taxon		*X. birchmanni*				Hybrid				*X. malinche*				
Taxon	Population	AHUA	COCA	GARC	SPED	CALL	AGUA	TLMC	CALM	MALI	TLTZ	APTL	SPDR	CHIC
X. birchmanni	AHUA	1.00												
	COCA	0.69	1.00											
	GARC	**0.99**	0.75	1.00										
	SPED	0.79	**0.97**	0.83	1.00									
Hybrid	CALL	**0.99**	0.67	**0.97**	0.77	1.00								
	AGUA	**0.92**	0.61	**0.90**	0.71	**0.96**	1.00							
	TLMC	0.86	0.44	0.83	0.55	**0.92**	**0.95**	1.00						
	CALM	**0.93**	0.55	**0.91**	0.65	**0.97**	**0.98**	**0.98**	1.00					
X. malinche	MALI	**0.93**	0.56	**0.91**	0.67	**0.97**	**0.98**	**0.98**	**0.99**	1.00				
	TLTZ	0.82	0.38	0.79	0.49	0.89	**0.93**	**1.00**	**0.97**	**0.96**	1.00			
	APTL	**0.93**	0.50	**0.91**	0.60	**0.95**	**0.91**	**0.94**	**0.96**	**0.95**	**0.92**	1.00		
	SPDR	**0.97**	0.58	**0.95**	0.68	**0.98**	**0.95**	**0.95**	**0.98**	**0.98**	**0.92**	**0.99**	1.00	
	CHIC	0.86	0.48	0.84	0.59	**0.92**	**0.98**	**0.99**	**0.98**	**0.98**	**0.98**	**0.91**	**0.94**	1.00

4 *X. birchmanni* sampling localities. These sites were chosen because molecular data were available to establish their taxonomic status (hybrids or parental) and adequate sample size could be obtained. That being said, these results do suggest a trend toward reduced phenotypic integration in hybrid populations (Figure 7B) which may be biologically meaningful and at least a partial confirmation of my prediction that hybridization will reduce phenotypic integration. Generally speaking, reduced phenotypic integration should make a population less subject to phenotypic trade-offs (Pigliucci and Marlow 2001; Pigliucci 2003). Female *X. birchmanni* express disdain for

Table 6 Primary eigenvectors (largest shown in bold) for hybrid localities.

Locality	Trait	Vector
AGUA	Dorsal Fin Width	-0.1267
	Sword Extension Length	**0.9603**
	Body Size PC1	-0.2487
CALL	Dorsal Fin Width	-0.1620
	Sword Extension Length	**-0.7944**
	Body Size PC1	-0.5855
CALM	Dorsal Fin Width	-0.0256
	Sword Extension Length	**-0.9938**
	Body Size PC1	-0.1080
TLMC	Dorsal Fin Width	-0.0383
	Sword Extension Length	**-0.9991**
	Body Size PC1	-0.0182

the sword ornament (Wong and Rosenthal 2006) but preference for large male body size (Fisher et al. 2009). If the pooled populations are considered, for male *X. malinche* the sword and body size are more integrated than that of hybrids (r hybrids = 0.04, P = 0.37;

r *X. malinche* = 0.29, P = <0.01). Thus it may be more likely on average for female *X.*

birchmanni to outcross with a male from a hybrid population vs. that of *X. malinche* as

Table 7 Primary eigenvectors (largest shown in bold) for *X. malinche* localities.

Locality	Trait	Vector
APTL	Dorsal Fin Width	-0.1545
	Sword Extension Length	**-0.9231**
	Body Size PC1	-0.3523
CHIC	Dorsal Fin Width	-0.0430
	Sword Extension Length	**0.9914**
	Body Size PC1	-0.1239
MALI	Dorsal Fin Width	-0.0357
	Sword Extension Length	**-0.9990**
	Body Size PC1	-0.0275
SPDR	Dorsal Fin Width	-0.1447
	Sword Extension Length	**-0.9090**
	Body Size PC1	-0.3910
TLTZ	Dorsal Fin Width	-0.0161
	Sword Extension Length	**-0.9999**
	Body Size PC1	-0.0035

she would be more likely to encounter a male hybrid that is both large bodied and lacks

or has a small sword. In addition, female *X. birchmanni* prefer males with large bodies

but small dorsal fins which result in a preference for a conflicting trait combination as

body size is strongly correlated with dorsal fin size (Fisher et al. 2009). Yet, my data

suggest that male hybrids also express similar strong correlations between dorsal fin size

and body size (r hybrids = 0.92, p = <0.01; r *X. birchmanni* = 0.96, p = <0.01). Thus

hybridization may not eliminate all potential conflicts between suites of traits.

Table 8 Primary eigenvectors (largest shown in bold) for *X. birchmanni* localities.

Locality	Trait	Vector
AHUA	Dorsal Fin Width	-0.2129
	Sword Extension Length	-0.6594
	Body Size PC1	**-0.7210**
COCA	Dorsal Fin Width	-0.3826
	Sword Extension Length	-0.0399
	Body Size PC1	**-0.9230**
GARC	Dorsal Fin Width	-0.3036
	Sword Extension Length	-0.5322
	Body Size PC1	**-0.7903**
SPED	Dorsal Fin Width	-0.3462
	Sword Extension Length	-0.0547
	Body Size PC1	**-0.9366**

The hypothesis that hybrid phenotypes should be oriented differently than those parentals was not supported. Matrix correlations (Table 5) suggest that the orientation of hybrids is largely in line with that of *X. malinche* and both are primarily oriented along the phenotypic axis of sword length (Table 6 and 7) while *X. birchmanni* were phenotypically oriented with respect to body size (Table 8). For *X. birchmanni* this result is not surprising given that males typically lack swords or at best have extremely short ones. Thus, while hybrids show increased variance and marginal evidence of reduced trait integration, the orientation of that variation does not diverge from one of the parentals (*X. malinche*). In addition, female *X. birchmanni* show disdain for the sword (Wong and Rosenthal 2006), while *X. malinche* and hybrids are ambivalent (GGR unpublished data). As mentioned above, while it may be the case that, with respect to mating with *X. birchmanni* females, hybrid populations could benefit from reduced

33

correlations between sword length and body size over *X. malinche*, they could be constrained by the fact that direction of the increased variance in hybrid sexual traits is in the direction that female *X. birchmanni* dislike (Van Homrigh et al. 2007).

Female choice is complex and inherently multivariate (Brooks and Endler 2001; Candolin 2003; Van Homrigh et al. 2007), and thus understanding variation in and between male traits is imperative to a clear understanding of sexual selection. My results suggest that hybridization can alter the phenotypic architecture of male traits. This alteration of male trait variance may provide novel phenotypes for female choice to act but is dependent on the orientation of that variance relative to female preference. Yet for increased phenotypic variance to result in differences in evolvability of hybrid populations relative to parentals the novel hybrid variance must be heritable. Furthermore, for sword extension length and body size previous work suggests that the social environment, such as the presence of adult males during early life can delay maturity in young males causing them to mature at a larger size with longer swords (Sohn 1977; Walling et al. 2007). Environmental factors that influence phenotypic expression could make it impossible to interpret phenotypic data in light of possible evolutionary change (Grant and Grant 1995).

CHAPTER IV

SEXUAL ORNAMENTS, BODY MORPHOLOGY, AND SWIMMING

PERFORMANCE IN NATURALLY HYBRIDIZING SWORDTAILS (TELEOSTEI:

XIPHOPHORUS)

Introduction

A pervasive view in the study of sexual selection is that sexual ornaments are costly to the bearer, and that these costs are integral in conveying information to choosers as to the "quality" of signaler (See Hunt et al. 2004 for a discussion on the nebulous term "quality"; reviewed by Mead and Arnold 2004). Yet, sexually-selected ornaments occur as part of a suite of traits that interact to determine fitness (Arnold 1983; Ballentine 2006; Lailvaux and Irschick 2006; Oufiero and Garland 2007). Just as the benefits of sexual ornaments depend on their interaction with other traits, so do their costs (reviewed by Oufiero and Garland 2007; Husak and Swallow 2011). In particular, exaggerated morphological structures like elongated feathers, fins, or horns are often assumed to impair locomotor performance; the evidence for such performance costs, however, is far from conclusive (reviewed by Oufiero and Garland 2007). The lack of consensus likely emerges from the fact that it is ultimately the multivariate phenotype, including both sexually-selected and non-sexual traits, that affects locomotor performance (Arnold 1983; Oufiero and Garland 2007). As a result, the interactions between sexual and non-sexual traits and locomotor performance can be complex (Oufiero and Garland 2007; Wilson et al. 2010). This complexity could, in theory, result

in sexual ornaments appearing more costly if paired with other traits which negatively influence locomotor performance. As a result, information stemming from elaborate sexual traits may provide little reliable information to individuals choosing mates.

Ideally, to evaluate the fitness effects of sexually-selected traits in a multivariate context, we would like to be able to decouple correlated traits to study an array of multivariate phenotypes in which traits vary independently. Arguably few natural phenomena can alter multivariate phenotypes as dramatically as hybridization (Rieseberg et al. 1999; Seehausen 2004). Hybridization can alter trait variances and covariances, thereby producing hybrid phenotypes that occupy a greater volume of phenotypic space relative to parental phenotypes (Grant and Grant 1994; Rieseberg et al. 1999; Seehausen 2004). This novel phenotypic variation can in turn affect ecologically relevant tasks such as locomotor performance (Hawkins and Quinn 1996; Semlitsch et al. 1999; Fitzpatrick 2008; Johnson et al. 2010). I demonstrate that novel hybrid trait variation and its influence on locomotor performance can be used as a 'natural laboratory' (Hewitt 1988) for evaluating functional relationships between traits and performance. For example, any assessment of the influence of a given trait on locomotor performance will be confounded by its co-expression with other trait values (i.e. trait correlations; Arnold 1983; Ghalambor et al. 2003; Ghalambor et al. 2004). Correlations among traits are often reduced in hybrids (Parsons et al. 2011), thus expanding the range of trait combinations in which to evaluate locomotor performance.

Here I take advantage of novel trait variation in natural hybrids of the swordtail fishes *Xiphophorus birchmanni* and *X. malinche* (Rosenthal et al. 2003; Culumber et al.

2011) to evaluate the relationship between sexual ornaments, non-sexual traits and locomotor performance. *Xiphophorus malinche* males are similar to other swordtails and express an extension of the ventral rays of the caudal fin known as a sword (Rauchenberger et al. 1990). *Xiphophorus birchmanni*, by contrast, have secondarily lost the sword; however, males bear an enlarged dorsal fin (Rauchenberger et al. 1990). *Xiphophorus birchmanni* are also deeper-bodied, particularly in the mid-section, anterior region of the body, relative to *X. malinche*. Hybrids between the two species occupy a broad region of morphospace encompassing both parental species (Rosenthal et al. 2003). Thus, these fish differ radically with respect to not only sexual ornaments but also body morphology, which itself can have dramatic effects on swimming performance (reviewed by Blake 2004; Langerhans and Reznick 2010).

I evaluated the interaction between sexual ornaments (the sword and dorsal fin) and non-sexual traits (body shape) in locomotor performance in both unsteady (fast-start) and steady (endurance) swimming modes. First, I determined if hybrids suffered reduced swimming performance relative to parentals. Second, I used novel phenotypic variation in a hybrid population to determine if body morphology is compensating for or exaggerating locomotor costs of sexual ornaments. Finally, I explored to what extent variation in hybrid performance is attributable to morphological and genetic similarities to parentals.

Materials and Methods

Animals were collected between May-June 2010 from sites previously identified as *X. birchmanni*, *X. malinche* or hybrid (Rosenthal et al. 2003; Culumber et al. 2011).

Parental males were collected from two *X. birchmanni* populations (Río Garces: n = 15

and Río Coacuilco: n = 7) and two *X. malinche* populations (Chicayotla n = 13 and

Tlatzintla: n = 6). The hybrid population Tlatemaco (n = 35) was chosen because it is

highly admixed, with sampled alleles in Hardy-Weinberg equilibrium and with no

significant linkage disequilibrium among marker loci (Culumber et al. 2011). In

addition, the hybrid population is morphologically intermediate to both parentals (JBJ

and GGR, unpublished data; see Results). Furthermore, I evaluated phenotypic

integration between body shape, body size, sword length and dorsal fin size (see

"Morphometrics" below) for each species within this sample of individuals using the

relative standard deviation of the eigenvalues estimated from the correlation matrix

(Pavlicev et al. 2009). Integration between these traits was lowest in hybrids ($SD_{rel}(\lambda)$ =

0.45) followed by *X. malinche* ($SD_{rel}(\lambda)$ = 0.58) and *X. birchmanni* ($SD_{rel}(\lambda)$ = 0.79).

Animals were transported to the laboratory facilities at Texas A&M University in

College Station, Texas. Individuals were housed by population in 76-l aquaria and

maintained in the lab for three weeks on a 12:12 light cycle and on a diet of algae flake,

de-capsulated Artemia eggs, and bloodworms (*Glycera* sp.) prior to the start of

swimming performance trials. Males were housed individually in 13l aquaria between

trials to track individual identity.

I performed endurance swimming performance trials using a Brett type swim

tunnel (Kruesi and Alcaraz 2007; Langerhans 2009b). The apparatus consisted of a flow-

through tunnel with a test section (length = 45.7cm, depth = 7.6cm, width = 7.6cm) with

a matrix of plastic drinking straws upstream in order to minimize turbulent flow and a

downstream grate to prevent the fish from leaving the test chamber. The apparatus was submerged in a 284-l aquarium and powered by a Leader Provort 540a propeller pump (Ladson SC, USA). Fish were tested using a modification of the protocol used by Royle et al. (2006a). Water temperature was maintained at 21 ± 1°C. Fish were placed individually in the test section and given 5 min to acclimatize. At the conclusion of the 5 min, flow was slowly increased to 20 cm s-1 for 5 min then increased to 30 cm s-1 for 1 min. Once the 30 cm s-1 interval was completed the flow was slowly increased to the test flow of 45 cm s-1 (~10.8 L s-1). This flow rate is the upper limit of critical swimming speed (U_{crit}) in a comparative study of *Xiphophorus* swimming performance (C.E. Oufiero, personal communication). Exhaustion was defined following the criterion used in other studies of steady swimming performance (e.g. Ryan 1988; Ojanguren and Braña 2000; Royle et al. 2006a). If a fish stopped swimming during the 45 cm s-1 test period, the fish would end up being impinged against the back grating that covers the water outflow channel. If the fish remained pinned for 5 sec the test chamber was tapped once, and if the fish did not resume swimming, the trial was ended and the time recorded. If the fish failed to exhaust by 25 min, the trial was terminated and the exhaustion time was recorded as 1500 sec. In a preliminary data set (N = 61), 92% of the fish that swam for 25 min (21%) continued to swim for >60 min.

Fast-start swimming performance trials were conducted by placing an individual fish in a 16.21 cm X 65 cm arena filled with filtered tap water to a depth of 4 cm. The test arena was illuminated using compact fluorescent lamps to minimize heating of the test arena. Water temperature was maintained at 21 ± 0.2°C. Each fish was allowed 5

min to acclimate to the tank before the trial began. Fast-start behavior (i.e. c-starts,

Domenici and Blake 1997) were elicited by startling the fish by striking the bottom of

the test arena with one end of a wooden dowel (6.4 mm diameter, Langerhans 2009a). A

high speed video camera (Casio Exilim Pro EX-F1, Casio Computer Co., Tokyo, Japan)

recorded each fast-start event at 300 frames per second. I waited 5 min between

successive trials for recovery. A total of three trials per fish were retained for analysis.

Trials where fish jumped out of the water or ran into the walls of the test arena were

excluded from analysis. For each video, the tip of the snout and center of mass were

digitized for every frame starting one frame before the fishes' movement began to the

15th frame following movement (i.e. 53 ms of video was analyzed). To estimate center

of mass, a line was fitted along the dorsal midsection of the fish in each frame starting at

the tip of the snout to the end of the caudal peduncle. The point on the line which

corresponded to half the standard length of the fish (as measured from the tip of the

snout) provided an estimate of the fish's center of mass. Digitizing error was minimized

using a mean-square quintic spline (Walker 1998). Four fast-start swimming

performance measures which have been shown to influence survival with predators

(Walker et al. 2005; Langerhans 2009a) were calculated for each trial: net distance

travelled maximum velocity, maximum acceleration and average rotational velocity.

Maximum values for each individual were retained for analysis (Langerhans 2009a). To

reduce dimensionality, I subjected the data to principal components analysis (PCA). I

performed two PCAs. The first PCA assessed differences between parentals and hybrids.

The first PC score summarizing variation in fast-start swimming performance in

parentals and hybrids explained 56% of the variation in swimming performance. The second PCA was used in the analysis of morphology and locomotor performance in hybrids and included only hybrids. The first PC from this analysis explained 62% of the variance in swimming performance in hybrid males. For both PCAs, evaluation of loading scores indicated that larger PC1 scores corresponded to increasing values for all four fast-start parameters.

The order in which individuals were subjected to performance trials (endurance or fast start) was randomized. I also evaluated possible order effects using two generalized linear models (GLMs) with either fast-start swimming performance (fast-start PC1) or endurance swimming performance (time to fatigue) as dependent on which trial (fast-start or endurance trial) was first. For these and all further GLMs (see below) which evaluate fast-start swimming performance the error distribution was specified as Gaussian whereas models evaluating endurance swimming performance was specified as a gamma error distribution. Both models (fast-start: $F_{1,74} = 0.67$, P = 0.41; endurance: $F_{1,74} = 0.06$, P = 0.80) indicated that order of trial had no effect on swimming performance.

At the conclusion of both swimming performance trials, fish were anesthetized using tricaine methanesulfate (MS-222). I took a lateral image of the right side of the body using a Nikon D90 digital camera with a 50mm lens (Nikon, Tokyo, Japan) mounted to a copy stand, and removed a small portion of the upper caudal fin for genetic analysis (see below). From each image, 13 landmarks were digitized using tpsDig (Rohlf 2010a; Figure 8). Landmarks included (1) upper lip, (2) eye, (3) anterior insertion of the

dorsal fin, (4) posterior insertion of the dorsal fin, (5) dorsal insertion of the caudal fin

ray, (6) ventral insertion of the caudal fin ray, (7) posterior insertion of the gonopodium,

or intromittent organ (8) anterior insertion of the gonopodium and (9) the ventral

occlusion of the operculum cover (Figure 8).
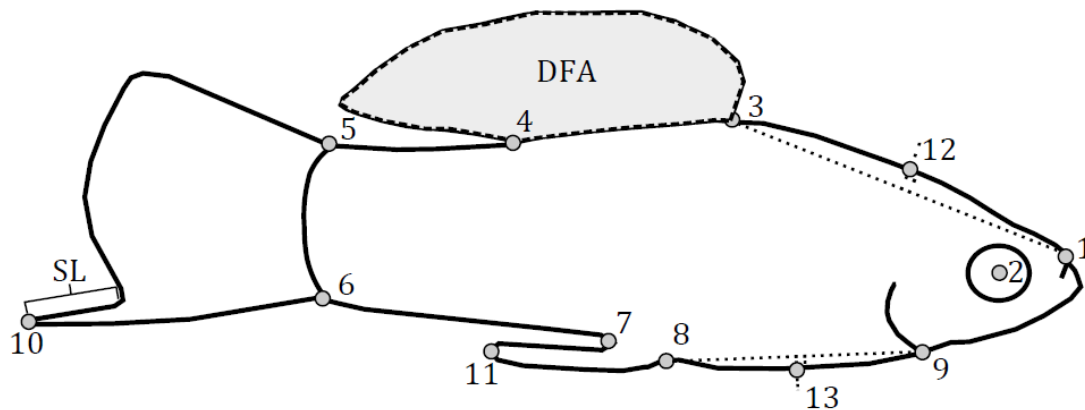
Figure 8 Illustration of landmark locations, sword length (SL) and dorsal fin area (DFA, shaded area) measurements.

The sword (10) and gonopodium tip (11) landmarks were subject to idiosyncratic

differences in orientation in the images, i.e. position of the sword or gonopodium in a

given image. These differences were removed by rotating both the sword and

gonopodium tip (landmarks 10 and 11, respectively) to 45^0 relative to the centerline of

the body (Adams 1999). Semi-landmarks for the nuchal hump (12) and belly (13) were

interpolated from right angles from half the distance (shown as dotted lines) between

landmarks 1 and 3 for landmark 12 and 8 and 9 for landmark 13, respectively. These

semi-landmarks were taken into account during landmark alignment (Bookstein 1991;

Zelditch et al. 2004). Landmark coordinates were then subjected to generalized

Procrustes superimposition where coordinates were translated, scaled and rotated, i.e.

aligned (Bookstein 1991; Zelditch et al. 2004). From the aligned coordinates, I

calculated partial warps and uniform components (i.e. the weight matrix) which describe

localized shape variation and uniform shearing in the X and Y dimensions, respectively

(Rohlf et al. 1996; Zelditch et al. 2004). A body size statistic, centroid size, was also

calculated as the square root of the sum squared distances for each individual's landmark

configuration to its centroid (Zelditch et al. 2004). Alignment, calculation of the weight

matrix and centroid size were performed using tpsRelw (Rohlf 2010b). I also measured

standard length (mm), sword extension length (mm) and dorsal fin surface area (mm2,

Figure 8). Centroid size, sword length and dorsal fin area were log transformed.

To determine the genomic composition (*X. malinche* vs. *X. birchmanni*) of each

hybrid individual I genotyped hybrid males after Culumber et al. (2011) using one

mitochondrial marker and three unlinked intron SNPs. Each hybrid male was assigned a

hybrid score based on the number of *X. malinche* alleles it bore at the four marker loci,

ranging from zero to seven (one allele at the mitochondrial marker and two at each of the

nuclear markers). Based on allele frequencies sampled in 2007, and given that these are

physically unlinked markers, the probabilities that a fish assigned a hybrid index of 7

(i.e. pure *X. malinche*) or 0 (pure *X. birchmanni*) is in fact a hybrid are 0.12 and 6 X 10-8

respectively. Thus, I believe that a marker-based hybrid index is a reasonable proxy for

similarity at a whole-genome level.

43

I compared swimming performance among hybrids and parentals using two GLMs where swimming performance (either fast-start PC1 or log fatigue time) was dependent on species-type (*X. malinche*, *X. birchmanni* or hybrid). Differences between species-types were evaluated using Tukey's multiple contrast. To account for population effects, I also included population nested in species-type.

Because I was interested in body shape per se, I excluded landmarks 10 (sword tip) and 11 (gonopodium; Figure 8); otherwise, alignment and calculation of partial warps and uniform components were as presented above (see Morphometrics). Partial warps and uniform components were subjected to PCA to reduce dimensionality (Rohlf 1993; Zelditch et al. 2004). The first two principal components accounted for 23% and 19% of the variance in hybrid body shape, respectively, and were retained for further analysis. I evaluated the effect of morphological traits (both ornaments, body shape PC 1 and 2 and log centroid size) on swimming performance (either fast-start PC 1 or time to fatigue) with model selection (Johnson and Omland 2004) using the glmulti package (Calcagno and de Mazancourt 2010) in R (R-Project 2011). All possible models including the full (all main effects and pairwise interaction terms) and null (intercept only) were evaluated using Akaike Information Criteria (finite sample correction, AICc, Johnson and Omland 2004). If interaction terms were retained their respective main effects were likewise retained, i.e. the principle of marginality was observed (Calcagno and de Mazancourt 2010). Models which differ within two AICc units from the model with the lowest AICc are considered equally supported (Burnham and Anderson 2002). I also evaluated relative importance of main effects and interaction terms individually

using the sum of the relative evidence weights for each model in which a given term appears, terms which exceed an importance value of 0.8 were considered important (Buckland et al. 1997; Calcagno and de Mazancourt 2010). If sexual ornaments are costly per se, one would expect a negative relationship between ornament and locomotor performance to be retained in well-supported models (models with low AICc scores). Furthermore, significant interaction terms between body shape and ornaments would suggest that ornaments are being compensated for or exaggerated by body shape and are not costly per se. Significant interaction terms were visualized using non-parametric thin-plate spline regression to create a performance surface (Arnold 2003). These methods have been used to evaluate fitness surfaces, which are conceptually identical to performance surfaces (Lee et al. 2008). Estimation of performance surfaces was performed in R (ver. 2.1.3.1, 2011 2011) using the fields package.

To determine if observed differences in morphology and swimming performance between parentals were mirrored by variation among hybrids, I evaluated vectors describing morphological variation between both parental species, and between hybrids that differed in performance. Specifically, I tested whether morphological differences between hybrids that did and did not exhaust in the endurance swimming trials were consistent with morphological differences between *X. malinche* and *X. birchmanni*. I performed this only for the endurance data, since species did not significantly differ in fast-start response (see Results). If the relationship between morphology and endurance in hybrids mirrors species differences (e.g. hybrids that did not exhaust are more morphologically similar to their better performing parental) one might a priori expect the

orientation between both vectors to be parallel. I included both the body and the sword

ornament (landmark 10) and the gonopodium (landmark 11), which has been shown to

influence swimming performance in other poeciliids (Langerhans et al. 2005). Partial

warps and uniform components were size-adjusted by taking residuals in a MANOVA

model where the partial warp and uniform components were dependent on log centroid

size ($F_{22, 54} = 6.09$, P = <0.001). Means of each size-adjusted partial warp and uniform

component were calculated for *X. birchmanni*, *X. malinche*, hybrids that exhausted and

hybrids that did not exhaust, and evaluated using MANOVA (Collyer and Adams 2007).

Using these means, two vectors were created: the first described variation between the

parental species, *X. birchmanni* and *X. malinche* and the second described variation

between hybrids that exhausted and hybrids that did not exhaust following the methods

described in Collyer and Adams (2007). I determined if the two vectors were oriented

similarly by calculating the angle between them (Collyer and Adams 2007). Permutation

tests (1000 iterations) were used to evaluate the significance of the observed angle

between vectors using a residual randomization approach (Collyer and Adams 2007). P-

values were calculated to evaluate the null hypotheses that the two vectors are parallel

(Collyer and Adams 2007). Analysis of phenotypic vectors was performed in R (R-

Project 2011) using a modification of the script provided in Collyer and Adams (2007).

To visualize the differences between vectors (Collyer and Adams 2007), I subjected the

partial warps and uniform components to PCA (Rohlf 1993; Zelditch et al. 2004; Collyer

and Adams 2007). The first two PC scores explained 75% and 7% of the variance

respectively. Both PC1 and PC2 were size-adjusted using MANOVA ($F_{2,74} = 27.43$, P =

<0.001). I then plotted the mean and standard error of residual PC1 and PC2 scores for

X. birchmanni and *X. malinche*, hybrids that exhausted, and hybrids that did not exhaust.

These means were used to create vectors describing morphological change between

parentals and hybrids in residual PC space. In addition, I visualized a performance

surface describing variation in performance in morphological space using non-

parametric thin-plate spline regression (see above). The performance surface is provided

for heuristic purposes and is independent of the calculation of the means, standard errors,

vectors and contrasts.

To evaluate the relationship between fast-start swimming performance and

genetic similarity of hybrids to parental species, I performed two GLMs where

swimming performance (either fast-start PC1 or log fatigue time) was dependent on

hybrid index.

Results

ANOVA revealed significant variation in fast-start (fast-start PC 1) among

species-types (*X. birchmanni*, *X. malinche*, or hybrids) and populations (whole model

test: $F_{4,71} = 2.812$, $P = 0.032$). Fast-start means differed among species types ($F_{2,71} =$

3.144, $P = 0.049$). Tukey's HSD post-hoc analysis indicated that mean fast-start for

hybrids was significantly greater than mean fast-start for *X. birchmanni* (difference

between means = 0.930, SE = 0.397, $z = 2.343$, $P = 0.05$). *Xiphophorus malinche* was

intermediate in fast-start performance (Figure 9A) and was not significantly different

from hybrids (difference between means = -0.647, SE = 0.416, $z = -1.556$, $P = 0.264$) or

X. birchmanni (difference between means = 0.283, SE = 0.457, z = 0.619, P = 0.809).

Population nested in species-type was not significant ($F_{2,71}$ = 2.479, P = 0.091).

Variation in species-type and population effects with respect to endurance swimming performance was also significant (whole model test: $F_{4,71}$ = 5.706, P = <0.001). Effect tests for species-type indicated significant variation between parentals and hybrids with respect to endurance swimming performance ($F_{2,71}$ = 5.776, P = 0.005). Hybrids and *X. birchmanni* showed the greatest endurance (Figure 9B). Tukey's HSD post-hoc analysis suggests that fatigue time was not significantly different between hybrids and *X. birchmanni* (difference between means = -4.537e-05, SE = 1.213e-04, z = -0.374, P = 0.924). *Xiphophorus malinche* had lower performance in endurance trials
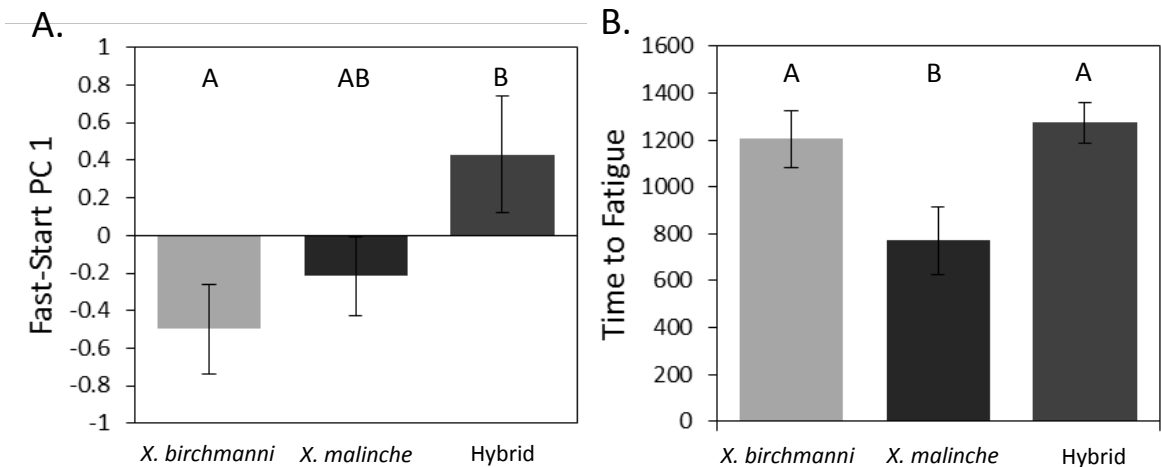
Figure 9 Means and standard errors for A. fast-start and B. endurance swimming performance by species-type. Letters indicate similarities between means determined with Tukey's HSD.

(Figure 9B). Tukey's HSD indicated that both hybrids (difference between means = -5.116e-04, SE = 1.785e-04, z = 2.866, P = 0.011) and *X. birchmanni* (difference between means = 4.662e-04, SE = 1.897e-04, z = 2.458, P = 0.036) were significantly less likely to fatigue than *X. malinche*. Population nested in species-type was also significant ($F_{2,71}$ = 19.677, P = <0.001).

Table 9 Results from an exhaustive model selection search (top 15 models shown) where hybrid fast-start swimming performance is dependent on sexual ornament (sword length and dorsal fin area), body shape (body shape PC 1 and PC2), body size (centroid size) and all pairwise interactions.

Model Terms- (Dependent Variable: Fast-Start PC 1)	AICc	ΔAICc
Null (intercept only)	135.300	0.000
Body Shape PC 2	136.905	1.604
Sword Length	137.008	1.708
Centroid Size	137.454	2.154
Body Shape PC 1	137.629	2.329
Dorsal Fin Area	137.642	2.342
Body Shape PC 2 + Sword Length	138.603	3.303
Body Shape PC 2 + Centroid Size	139.112	3.812
Sword Length + Dorsal Fin Area	139.221	3.921
Centroid Size + Sword Length	139.329	4.028
Body Shape PC 2 + Dorsal Fin Area	139.350	4.050
Body Shape PC 1 + Body Shape PC 2	139.392	4.091
Body Shape PC 2 + Sword Length + Sword Length*Body Shape PC 2	139.491	4.190
Body Shape PC 1 + Sword Length	139.566	4.266
Body Shape PC 1 + Centroid Size	139.759	4.459

Model selection indicated that morphology had little association with fast-start swimming performance in hybrids, as the null model was found to have the lowest AICc score (Table 9). Furthermore, no terms exceeded the 0.8 threshold of relative importance (Figure 10).
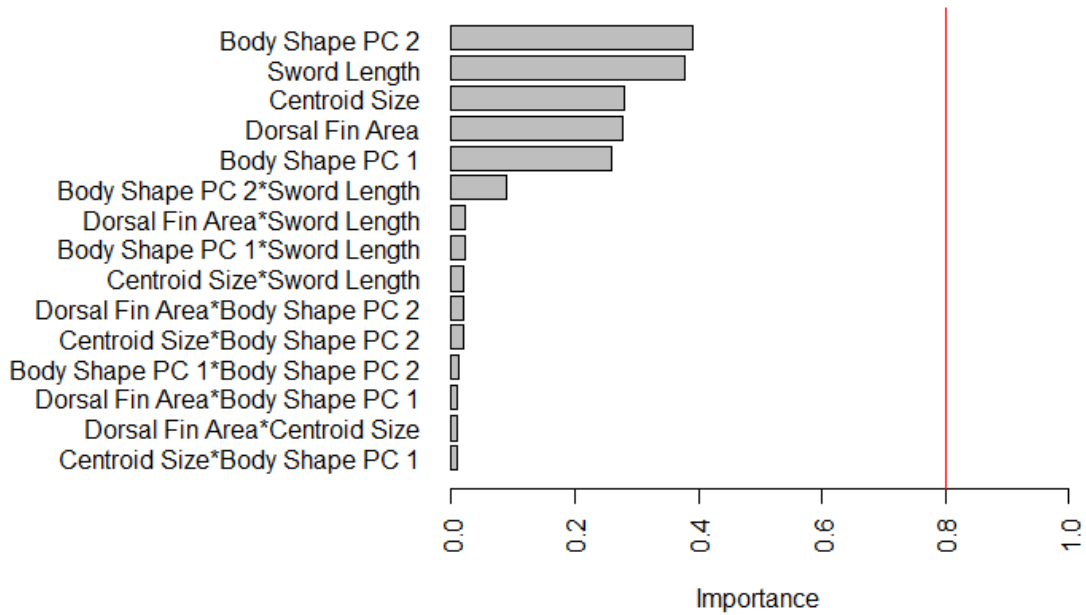
Figure 10 Importance scores for each morphological variable (and pairwise interaction terms) predicting fast-start swimming performance.

By contrast, morphological variation did influence endurance swimming performance. A model with body shape PC 1, centroid size, sword length, dorsal fin area and an interaction term of sword length and body shape PC 1 had the lowest AICc score (Table 10). P-values for this model indicate that the interaction term of body shape PC 1 and sword length was significantly related to time to fatigue (Table 11). Among the 10 equally supported models body shape PC 1, sword length and their interaction appeared in all (Table 10). In addition, importance values for body shape PC 1, sword length and

Table 10 Results from an exhaustive model selection search (top 15 models shown) where hybrid endurance swimming performance is dependent on sexual ornament, body shape, body size and all pairwise interactions.

| Model Terms – (Dependent Variable: Time to Fatigue)	AICc	ΔAICc
Body Shape PC 1 + Centroid Size + Sword Length + Dorsal Fin Area + Sword Length*Body Shape PC 1	517.969	0.000
Body Shape PC 1 + Sword Length + Sword Length*Body Shape PC 1	518.338	0.369
Body Shape PC 1 + Centroid Size + Sword Length + Sword Length*Body Shape PC 1	518.660	0.691
Body Shape PC 1 + Sword Length + Dorsal Fin Area + Sword Length*Body Shape PC 1	518.855	0.887
Body Shape PC 1 + Body Shape PC 2 + Centroid Size + Sword Length + Sword Length*Body Shape PC 1 + Sword Length*Body Shape PC 2	519.289	1.320
Body Shape PC 1 + Centroid Size + Sword Length + Dorsal Fin Area + Sword Length*Body Shape PC 1 + Dorsal Fin Area*Sword Length	519.296	1.327
Body Shape PC 1 + Centroid Size + Sword Length + Dorsal Fin Area + Sword Length*Body Shape PC 1 + Dorsal Fin Area*Centroid Size	519.395	1.426
Body Shape PC 1 + Body Shape PC 2 + Centroid Size + Sword Length + Dorsal Fin Area + Sword Length*Body Shape PC 1 + Sword Length*Body Shape PC 2	519.425	1.456
Body Shape PC 1 + Body Shape PC 2 + Sword Length + Body Shape PC 2*Body Shape PC 1 + Sword Length*Body Shape PC 1	519.455	1.486
Body Shape PC 1 + Centroid Size + Sword Length + Dorsal Fin Area + Sword Length*Body Shape PC 1 + Sword Length*Centroid Size	519.935	1.967
Body Shape PC 1 + Body Shape PC 2 + Sword Length + Sword Length*Body Shape PC 1 + Sword Length*Body Shape PC 2	520.266	2.297
Body Shape PC 1 + Body Shape PC 2 + Centroid Size + Sword Length + Body Shape PC 2*Body Shape PC 1 + Sword Length*Body Shape PC 1	520.314	2.345
Body Shape PC 1 + Body Shape PC 2 + Sword Length + Sword Length*Body Shape PC 1	520.339	2.370
Body Shape PC 1 + Centroid Size + Sword Length + Sword Length*Body Shape PC 1 + Sword length*Centroid Size	520.407	2.439
Body Shape PC 1 + Body Shape PC 2 + Centroid Size + Sword Length + Body Shape PC 2*Body Shape PC1 + Sword Length*Body Shape PC 1 + Sword Length*Body Shape PC 2	520.533	2.564
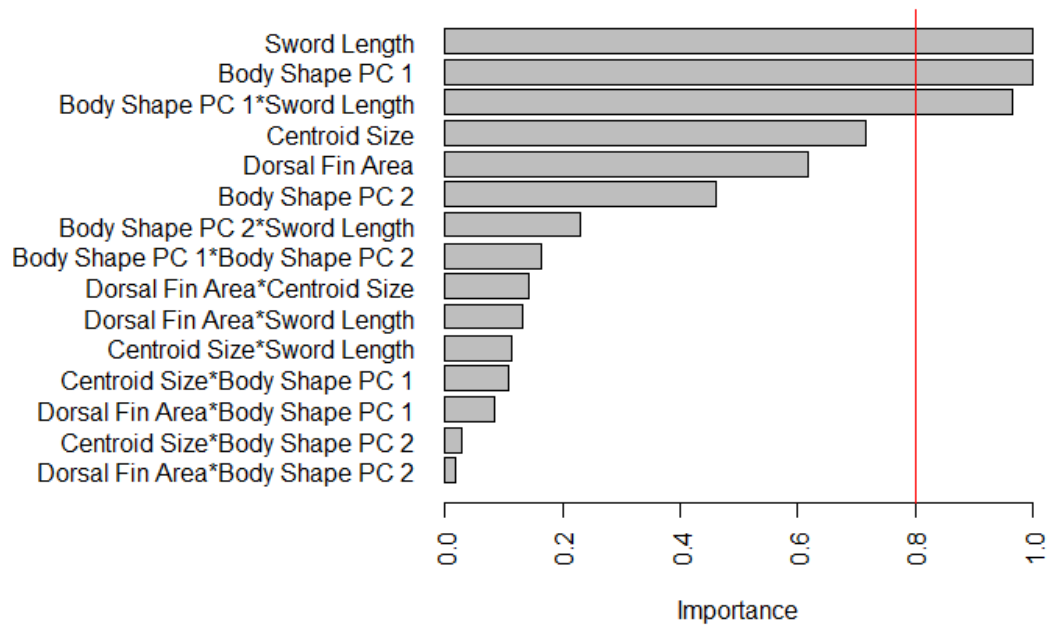
Figure 11 Importance scores for each morphological variable (and pairwise interaction terms) predicting endurance swimming performance.

their interaction term were the only terms which exceeded the 0.8 importance threshold

(Figure 11). Visualization of the interaction term of body shape PC 1 and sword length

shows that sword length had a negative effect on endurance swimming only when paired

with a small anterior body shape (high PC1 scores; Figure 12).

Table 11 Results for the best supported model predicting time to fatigue (i.e. endurance swimming performance).

Term	SS	$F_{1,29}$	P
Body Shape PC 1	0.043	0.166	0.686
Centroid Size	0.429	1.648	0.209
Sword Length	2.547	9.794	0.004
Dorsal Fin Area	0.220	0.845	0.365
Body Shape PC 1*Sword Length	2.270	8.728	0.006
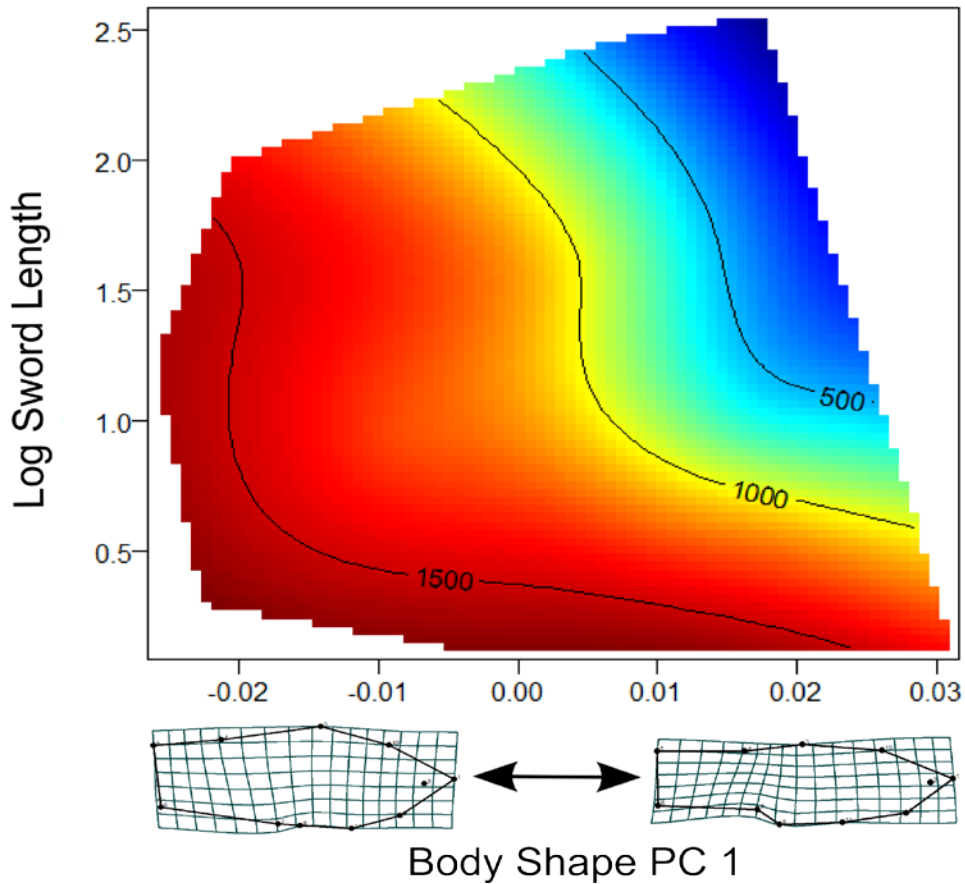
Figure 12 The interaction of log sword length and residual body shape PC1 on time to fatigue (in seconds) illustrated using thin-plate spline regression (morphology predicts performance; lambda = 0.01). Red represents longer endurance time. Contour lines and numbers indicate exhaustion time. End points for body shape PC 1 were visualized using thin-plate-spline visualization (Zelditch et al. 2004) and were magnified by a factor of 3 (Langerhans et al. 2004; Ward and McLennan 2009; Haas et al. 2010).

MANOVA indicated significant differences in morphology between parental species and between exhausting and non-exhausting hybrids ($F_{66, 156.14} = 4.94$, P = <0.001). Vectors describing morphological variation between parental species and

between exhausting and non-exhausting hybrids were parallel in orientation ($\theta = 20.9^0$,

$P_{rand} = 0.99$) and differed in length ($D_{parental} = 0.13$, $D_{hybrid} = 0.05$, $P_{rand} = 0.002$). Hybrid

fish that did not exhaust were predominantly *X. birchmanni*-like morphologically. These

fish had, on average, deeper, more anterior-allocated bodies, shorter, deeper caudal

peduncles, larger dorsal fins and very short or absent swords (Figure 13).

By contrast, genetic similarity to the two parent species did not predict

performance. There was no association between hybrid index for fast-start ($F_{4,30} = 1.98$,

$P = 0.12$) or endurance swimming performance ($F_{4,30} = 0.15$, $P = 0.96$).
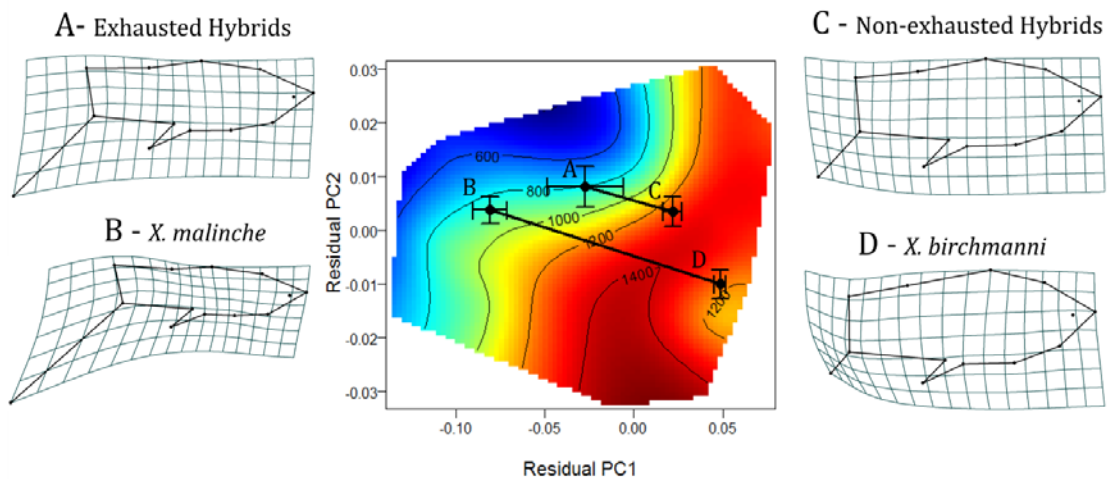
Figure 13 Variation in morphology and swimming performance between parental species (*X. malinche* and *X. birchmanni*) and between hybrids that exhausted and hybrids that did not. Red represents longer endurance time. Contour lines and numbers indicate exhaustion time. Means and standard errors of residual PC 1 and residual PC 2 for parentals and hybrids are shown. Mean values for each group were visualized using thin-plate-spline visualization and were magnified by a factor of 3.

Discussion

Despite ample evidence to the contrary (Kokko et al. 2006; Rendall et al. 2009; Prum 2010, 2012), it remains a common assumption that sexual signals encode information about "quality". A central pillar of this argument is that sexual ornaments are often costly to express (Zahavi 1975; Andersson 1994). The honesty of sexual signals is then enforced by condition-dependence, whereby individuals in good "condition", which is itself a nebulous term (see Hill 2011) are better equipped to bear these costs (reviewed by Cotton et al. 2004; Hill 2011). We have known for some time, however, that correlated traits can obscure the detection of the true targets of selection (Arnold 1983; Lande and Arnold 1983). Thus, to accurately evaluate the true costs of sexual ornaments, we need to first, not assume a priori that they are costly and, second, consider how sexual and non-sexual traits interact to influence performance. My data suggest that the apparent locomotor costs of the sword are a by-product of the sword being paired with a sub-optimal body shape. Fish that performed well in the endurance swimming had a narrow, tapered caudal peduncle and increased anterior body depth (Figure 12, Figure 13). These results are congruent with theoretical and empirical findings describing the relationship between body morphology and steady swimming performance (reviewed by Langerhans and Reznick 2010). Specifically, a narrow, tapered caudal peduncle and increased anterior body depth, as found in *X. birchmanni* and *X. birchmanni*-like hybrids, should benefit endurance swimming performance by minimizing drag and maximizing thrust (reviewed by Langerhans and Reznick 2010). Visualization of the significant interaction effect of PC1 and sword length suggests that

55

sword length is only costly when paired with a relatively poorly performing body shape (i.e. a small anterior body and a shallow caudal peduncle; Figure 13). In line with my findings, Kruesi and Alcaraz (2007) found that *X. montezumae* males with more rotund bodies had greater swimming performance, and incurred relatively little change in swimming performance after sword removal. Furthermore, I found no association between any aspect of morphology (sexually-dimorphic or otherwise) and fast-start swimming performance (Table 9; Figure 10). Thus in this system, sexual ornaments by themselves incur little, if any, cost to locomotor performance.

There have been mixed results on the relationship between swimming performance and the sword ornament in *Xiphophorus*: some studies have found a negative relationship between swimming performance and presence of the sword (Kruesi and Alcaraz 2007), others have found no relationship (Ryan 1988; Baumgartner et al. 2011), and one has even found positive effects of the sword on swimming performance (Royle et al. 2006b). This lack of consensus may in part be due to variation in the presence of compensatory traits (Oufiero and Garland 2007; Husak and Swallow 2011). In addition, my findings suggest that non-sexual traits can produce apparent "costs" of correlated ornaments, and highlight the importance of evaluating both the costs and the benefits of sexually selected traits from a multivariate perspective. Thus, the sword as an indicator, at least with respect to locomotor ability, is at best an unreliable signal.

Just as the locomotor costs of the sword are equivocal, so are other potential costs of the sword. For example, Basolo (1998b) found that *X. hellerii* invest in both sword growth and body size when resources are unrestricted but allocate growth to only

56

sword length when resources are limited. This supports the hypothesis that the sword

evolved as a means for males to increase apparent size while not paying the cost of

investing in increased body size (Basolo 1998b; Rosenthal and Evans 1998). On the

other hand, the conspicuousness of the sword increases predation risk (Rosenthal et al.

2001; Basolo and Wagner 2004; Hernandez-Jimenez and Rios-Cardenas 2012). In *X.*

montezumae, where the sword is exaggerated to the point that it exceeds body length, it

increases metabolic demands (Basolo and Alcaraz 2003). These costs of the sword could

be interpreted as handicaps (Zahavi 1975). Natural selection acting in opposition to

sexual selection is, however, by no means exclusive to indicator models. Fisherian

models (Fisher 1915) include such constraints on sexual ornaments (Lande 1981;

Pomiankowski and Iwasa 1998), as do models of signal evolution in response to

preexisting receiver biases (Ryan 1990). The fact that there are some costs to the sword

in some contexts is therefore consistent with multiple scenarios of trait and preference

evolution.

Morphological differences between hybrids that became exhausted and hybrids

that did not paralleled the differences in morphology and performance observed between

the parental species (Figure 13). Specifically, hybrids that did not exhaust were more *X.*

birchmanni-like morphologically (large anterior body region and reduced or absent

swords) and the body shape of exhausted hybrids was similar to *X. malinche*

morphologically (small anterior body region and longer swords; 13). The robust body

shapes of *X. birchmanni* and *X. birchmanni*-like hybrid males are those predicted if

selection favors increased steady swimming performance (see above; reviewed by

Langerhans and Reznick 2010). Furthermore, based on the small number of marker loci genotyped, performance was independent of whole-genome similarity to one parental species or the other. The lack of a whole-genome signature of performance coupled with parallel effects of morphology both among species and within hybrids that are consistent with biomechanical predictions makes it unlikely that variation in latent traits, such as physiology (Jayne and Lauder 1993) has obscured my interpretation.

Locomotor performance has a profound impact on the fitness of animals. In fishes, fast-start swimming performance is associated with predator evasion (Webb 1986; Katzir and Camhi 1993; Walker et al. 2005; Langerhans 2009a), whereas endurance swimming could influence competitive interactions, locating suitable micro-habitat, and dispersal (Plaut 2001; Domenici 2003; Blake 2004). Furthermore, improved or compromised locomotor performance may indicate differential vulnerability to predators or dispersal ability of hybrid populations over parentals (Semlitsch et al. 1999; Rosenfield et al. 2004; Fitzpatrick 2008). I observed that hybrids performed as well if not slightly better than the best-performing parental species in both fast-start and endurance swimming performance (Figure 9). These findings are consistent with previous observations in the *birchmanni-malinche* hybrid system. For example, I observed that fish with a *X. birchmanni*-like body shape performed better in endurance swimming trials (Figure 12, Figure 13). Previous work indicates that the *X. birchmanni*-like morphology is overrepresented in the hybrid zone (Rosenthal et al. 2003). Thus the prevalence of *X. birchmanni*-like morphology among hybrid populations may result from improved endurance swimming performance and thus dispersal ability of *X.*

birchmanni like hybrids. Furthermore, hybrid male phenotypes are not costly with respect to sexual selection; indeed, females of both parental species fail to prefer conspecifics over hybrids in mate-choice trials, and express preferences for some hybrid phenotypes (ZW Culumber and GGR, unpublished data). Thus, hybrids in this system do not appear to suffer a fitness cost of hybridization, and may in fact have greater fitness at the intermediate elevations where they are found (Culumber et al. in press).

There has been a tendency in the literature to interpret correlations between sexual ornaments and aspect of viability and condition in light of the assumption that natural selection has shaped sexual signals to confer information to the female about male "quality" (Prum 2010; Hill 2011; Prum 2012). Yet, simple correlations can be deceptive, as the potential costs of an ornament are functionally tied to non-sexual aspects of the phenotype. Thus, the interpretation that sexual ornaments are linked to viability is inherently complex and context-dependent, and may be invalid even if correlations are found between ornament elaboration and performance costs.

A growing body of work has highlighted the importance of natural hybridization in generating phenotypic novelty (Grant and Grant 1996; Seehausen 2004; Parsons et al. 2011). This phenotypic novelty encompasses both increased variation in traits, and altered patterns of integration among traits. Studies of trait function can benefit from the opportunity to perform natural experiments on complex suites of traits.

CHAPTER V

BOLDNESS AND PREDATOR EVASION IN NATURALLY HYBRIDIZING

SWORDTAILS (TELEOSTEI: *XIPHOPHORUS*)

Introduction

Correlations among morphological (Relyea and Auld 2004), life-history (Stearns 1989) and behavioral traits (Sih et al. 2004a; Dingemanse and Reale 2005; Réale et al. 2007) may affect fitness by generating phenotypic trade-offs, which have the potential to affect the evolvability of populations (Arnold et al. 2001; Ashman 2003). For morphological traits, hybridization can rapidly and dramatically alter trait correlations resulting in hybrid populations that differ in their evolvability relative to parentals (Grant and Grant 1996; Seehausen 2004; Albertson and Kocher 2005). While considerable attention has been given to factors which influence behavioral integration, such as social context (Sih et al. 2004a; Mainwaring et al. 2011), predation (Huntingford 1976; Bell 2005; Dingemanse et al. 2007) and life-history (Wolf et al. 2007; Biro and Stamps 2008) surprisingly little attention has been paid to the role that natural hybridization may play in changing correlations among behavioral traits. Here I explore hybridization's influence on the relationship between two behavioral traits, boldness and predator evasion.

Boldness, or the propensity of individuals to engage in risky behavior (Wilson et al. 1994; Wilson 1998; Brown et al. 2005), can be beneficial in the acquisition of mates (Godin and Dugatkin 1996; Reaney and Backwell 2007) and resources (Ward et al.

60

2004; Stamps 2007) but is often presumed to expose individuals to greater predation risk (Sih et al. 2004a; Sih et al. 2004b; Stamps 2007). However, while there are several examples of lower survivorship of bold individuals (Dugatkin 1992; Wilson et al. 1993; Bremner-Harrison et al. 2004) the pattern is not ubiquitous. Indeed, a number of studies have demonstrated higher survivorship of bold individuals (Godin and Davis 1995; Réale and Festa-Bianchet 2003; Fuiman et al. 2010; Smith and Blumstein 2010). Costly or suboptimal trait values can be compensated for by other traits (Mikolajewski and Johansson 2004; Oufiero and Garland 2007). Thus, the counterintuitive finding that boldness increases survival with predators may arise from bold behavior being compensated for by anti-predator behaviors, such as evasive responses to predator threats. If boldness is compensated for by anti-predator behaviors, we should expect to observe positive correlation between both traits. I evaluated the relationship between boldness and predator evasive responses to predators in a naturally hybridizing complex of the northern swordtails *Xiphophorus malinche* and *X. birchmanni* (Rosenthal et al. 2003; Culumber et al. 2011). In other poeciliids, individuals sympatric with predators are bolder (*Brachyraphis episcopi*, Brown et al. 2005; *Poecilia* spp., Riesch et al. 2009; Harris et al. 2010), and bolder fish have higher survivorship in the presence of fish predators (Godin and Davis 1995; Smith and Blumstein 2010). My goals were twofold. First, I sought to determine whether boldness covaries with fast-start behavior, which has been demonstrated to improve success in surviving attacks by fish (Walker et al. 2005; Langerhans 2009a) and bird (Katzir and Camhi 1993) predators. Thus, boldness should be positively correlated with the tendency to respond to a predation threat.

61

Second, I evaluated the impact of hybridization on the individual-level relationship between these two behavioral traits.

Materials and Methods

Sexually mature male and female *Xiphophorus* were collected during May and June 2007 using baited funnel traps, from six sites as described in (Culumber et al. 2011): two *X. birchmanni*: Garces and Huitznopala; two *X. malinche*: Chicayotla and Malila; and two hybrid: Tlatemaco and Calnali-mid. Fish were temporarily housed in aerated coolers and transported to the Centro de Investigaciones Científicas de las Huastecas "Aguazarca"(CICHAZ) in Calnali, Hidalgo, Mexico) where they were housed, by population, in 76-liter aquaria on a natural light/dark cycle, for at least 48 hours before trials began.

Fish were placed individually into a circular plastic tank (57 cm top diameter X 47cm bottom diameter X 25cm depth) filled with water to a depth of 15 cm. At the center of the tank, four small rocks provided cover. Natural behavior of *Xiphophorus* from my sample sites is to seek cover in the substrate, and all fish took cover in the rocks immediately upon release. In preliminary trials, fish remained in shelter for the duration of the trial if placed in isolation in the tank. In the wild and in captivity, *Xiphophorus* shoal with other swordtails (Wong & Rosenthal 2005; Buckingham et al. 2005). I therefore placed five juvenile *Xiphophorus* (standard length 10-15 mm) in an 8-liter plastic aquarium along the side of the tank to provide visual shoaling cues. I measured two indices of boldness, latency to emerge from shelter (Brown et al. 2005; Wilson and Godin 2009; Cote et al. 2010; Harris et al. 2010) and proportion of time spent out of

cover (Harcourt et al. 2010). If a fish did not emerge within 10 minutes the trial was ended and the fish was assigned a latency to emerge of 600 seconds. Latency to emerge was log transformed (Brown et al. 2005) and proportion of time out was arcsine square root transformed (Harcourt et al. 2009). The two measures of boldness were correlated (r = -0.64, P = <0.0001). Therefore, I used principal components analysis (PCA) on both measures to create a combined index of boldness. The first principal component explained 82% of the variance in boldness. Loadings indicated that high PC1 scores were associated with greater proportion of time out (i.e. boldness) and low PC scores with greater latency to emerge (i.e. shyness).

Immediately after boldness trials, I used a simulated predator attack to measure the propensity to perform a fast-start. Each subject was placed in a plastic tank adjacent and identical to that used for the boldness trials, but entirely bare, and given 5 minutes to acclimatize. A cardboard model of an Amazon kingfisher (*Chloroceryle amazona*) was then released down a monofilament line stretched taut over the test tank (bird drop length = 325cm, bird model height = 284cm, bird model drop angle = 30°). Fish responses were recorded with a Panasonic PVGS59digital camcorder at 30 frames per second. I recorded whether or not the fish responded to the stimulus by initiating a fast-start. I operationally scored a fish as performing a fast-start if, during the period the bird model passed over the test pool, the fish brought its snout toward its caudal peduncle (forming a C shape; Domenici and Blake 1997). All other behaviors, including swimming backward slowly (N = 4), cessation of swimming (N = 29) and no apparent change in swimming behavior (N = 71), were operationally scored as "no response".

I used logistic regression to estimate the effect of taxon (hybrid, *X. birchmanni* or *X. malinche*), population nested in taxon, and sex on whether or not an individual performed a fast-start. Analysis of variance (ANOVA) was used to investigate the effects of taxon, population nested in taxon, sex, and fast-start (yes or no) on boldness (PC 1). To evaluate if the relationship between fast-start and boldness differed among taxa, I calculated correlation coefficients for hybrids and parentals, respectively. All analyses were performed using JMP software (ver. 9.0.0, SAS Institute, Inc., Cary, NC).

Results

Logistic regression indicated that taxa (*X. birchmanni*, *X. malinche*, and hybrids) and populations within taxa both varied in their likelihood to perform a fast-start (χ^2taxon = 16.8, DF = 2, P = 0.0002, population nested in taxon, $\chi^2_{pop[taxon]}$ = 25.09, DF = 3, P = <0.0001). *Xiphophorus birchmanni* performed fast-starts more often (48.5% of trials, N = 66; fig. 1), hybrids fast-started slightly less often (38.3% of trials, N = 47) and *X. malinche* were least likely to fast-start (16.2% of trials, N = 74). The odds of a fast-start in *X. birchmanni* were equivalent to that of hybrids (odds ratio = 1.39, P = 0.46). By contrast, the odds of a fast-start in *X. malinche* were significantly different than in *X. birchmanni* (odds ratio = 7.08, P = <0.0001) and hybrids (odds ratio = 5.11, P = 0.005). There was no sex difference in propensity to fast-start (χ^2_{Sex} = 2.67, DF = 1, P = 0.1024). ANOVA indicated that taxa ($F_{2,179}$ = 8.34, P = 0.0003), and population nested in taxa ($F_{3,179}$ = 3.63, DF = 3, P = 0.014) were variable in boldness. *Xiphophorus birchmanni* were boldest (least-square mean = 0.44, SE = 0.16) and significantly bolder than both *X. malinche* (Tukey's HSD: P = 0.001) and hybrids (Tukey's HSD: P = 0.002).

Xiphophorus malinche were shyest (least-square mean = -0.34, SE = 0.15) and did not

differ from hybrids (least-square mean = -0.40, SE = 0.18) with respect to boldness

(Tukey's HSD: P = 0.96).
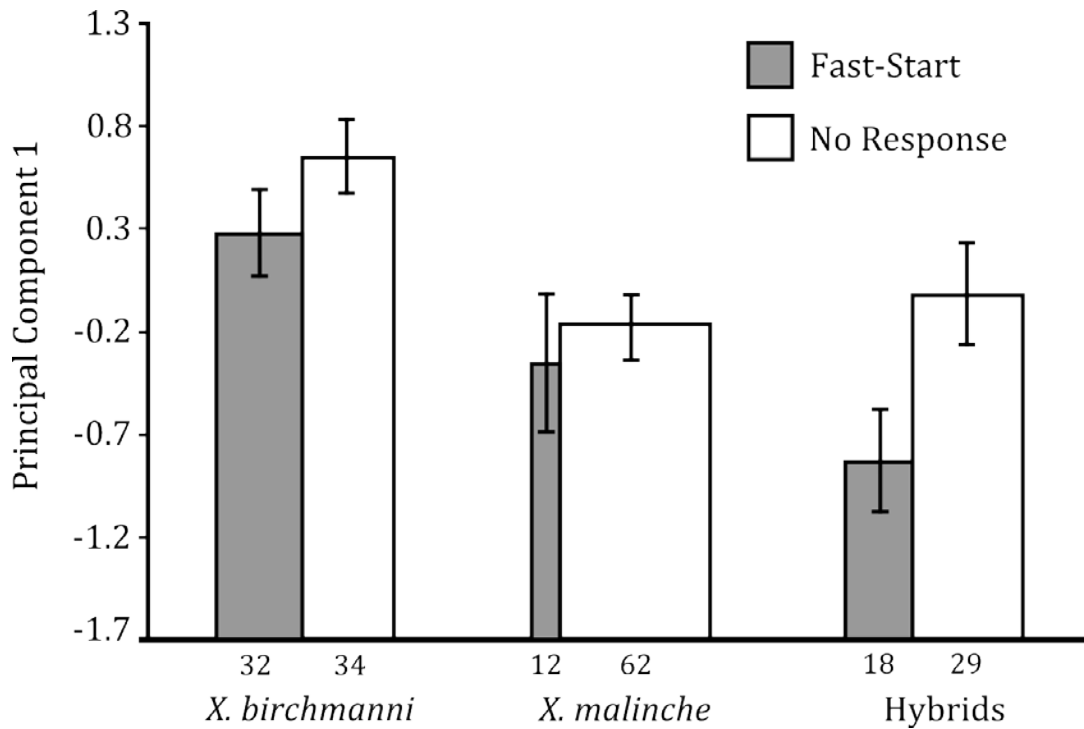
Figure 14 Mean boldness for *X. birchmanni, X. malinche,* and hybrids that performed
fast-starts in simulated predator strikes. Width of bars indicates proportion of individuals
who performed a fast-start. Sample sizes per group are indicated below each bar.
Boldness is represented as the first principal component explaining latency to emerge
and proportion of time out of shelter.

The relationship between boldness and fast-start was weak ($F_{1,179}$ = 3.76, DF = 1,

P = 0.054) but consistent among taxa with bolder fish being less likely to fast-start

(Figure 14). Correlation coefficients indicated that the relationship between fast-start and

boldness was only significant in hybrids (r = -0.31, N = 47, p = 0.036) but not in *X.*

birchmanni (r = -0.16, N = 66, p = 0.19) nor *X. malinche* (r = -0.05, N = 74, p = 0.67).

Males and females did not differ in boldness ($F_{1,179}$ = 0.1, DF = 1, P = 0.3194).

Discussion

Hybridization has been proposed as a powerful means of relaxing evolutionary constraints via altered trait correlations (Seehausen 2004; Parsons et al. 2011). However, the suggested benefits of hybridization are dependent on hybrids displaying reduced phenotypic integration and thus being less constrained with respect to selection (Seehausen 2004; Parsons et al. 2011). I expected that selection should favor individuals who reap the benefits of boldness (growth, Ward et al. 2004; increased mating opportunities, reviewed by Smith and Blumstein 2008) while minimizing predation costs via increased likelihood to display anti-predator behaviors (Fuiman et al. 2010). Thus, I hypothesized that boldness would covary positively with the tendency to perform a common anti-predator behavior, a fast-start response (Katzir and Camhi 1993; Walker et al. 2005; Langerhans 2009a). Surprisingly, I found a weak effect in the opposite direction where bolder fish were less likely to fast-start. This was only significant in hybrids (Figure 14).Thus, in hybrid populations, it is likely that swordtails that emerge from shelter sooner and that spend more time in the open (i.e. bold individuals) increase their likelihood of being attacked by an aerial predator due to their reduced propensity to fast-start.

In some circumstances, hybridization may actually increase genetic constraints in hybrid populations. Grant and Grant (1994) showed that hybrid Galápagos finches (*Geospiza* ssp.) displayed increased genetic correlations between morphological traits if

66

both parental species had similar allometric trajectories. Thus, if genetic constraints are operating, the observed phenotypic correlation seen in hybrids must arise from a genetic correlation between shyness and likelihood to fast-start (Dochtermann and Roff 2010; Dochtermann 2011). There are at least three factors that could account for a genetic correlation between shyness and likelihood to fast-start, i.e. linkage disequilibrium (LD) between sets of loci associated with both behavioral traits.

First, the Calnali-mid population that I tested is a mix of *X. malinche*, *X. birchmanni*, backcross, and later-generation hybrids (Culumber et al. 2011), so there should be high LD between *X. malinche* and *X. birchmanni* genomic regions. However, at Tlatemaco swordtails form an unstructured, panmictic population and the correlation between boldness and fast-start is markedly stronger at Tlatemaco ($r = -0.23$) versus Calnali-mid ($r = -0.07$). Second, higher LD (higher genetic covariance) could arise from correlational selection favoring shy fish that are more sensitive to risk. This is contrary to studies of geographic variation and predator response indicating that bold fish should be more risk-sensitive (Fuiman et al. 2010; Smith and Blumstein 2010). It is therefore unlikely that correlational selection would have produced a correlation in the opposite direction.

A final possibility is that the correlation arises from pleiotropy, i.e. that is the same set of genetic loci underlying much of the variation in both traits. Pleiotropic effects are consistent with the hypothesis that individuals in many species vary along a 'proactive-reactive' axis. In mammals, a common neural mechanism underlies variation ranging from reactive coping styles, where individuals are highly sensitive to

67

environmental stimuli, to proactive coping styles where individual behavior is less dependent on environmental cues (Coppens et al. 2010). A similar mechanism might also underlie both predator response and exploratory behavior in swordtails. Future genetic mapping studies of *Xiphophorus* hybrids could pinpoint genomic regions associated with the proactive-reactive axis.

Natural hybrids have provided numerous insights into the nature and consequences of phenotypic integration among multiple morphological traits. Only a handful of studies have examined hybridization in the context of behavioral traits such as mate choice (Hatfield and Schluter 1996; Grant and Grant 1997; Fisher et al. 2006), vocalizations (Grant and Grant 1997; Qvarnstrom et al. 2006) anti-predator behaviors (Fitzpatrick 2008), and there is little if any work on how hybridization affects correlated suites of behaviors. Furthermore, despite a growing body of literature suggesting that common underlying mechanisms should affect multiple behaviors in a range of contexts (O'Connell and Hofmann 2012), we know relatively little about the genetic architecture and functional trade-offs associated with suites of behavioral traits (Dochtermann and Roff 2010). Given the potential for hybridization to alter genetic architecture (Guillaume and Whitlock 2007), and given the promise of genetic mapping studies in non-model organisms in light of next-generation sequencing techniques, hybrids may prove useful in studying the evolutionary genetics of multivariate behavioral phenotypes.

CHAPTER VI

CONCLUSIONS

Trait variation is a prerequisite for phenotypic evolution to occur, thus its

maintenance is a central problem in evolutionary biology (Mitchell-Olds et al. 2007;

Carroll 2008). I evaluated hybridization as a mechanism that increases phenotypic

variation in behavioral, morphological, sexual and non-sexual traits. The hybrid zone of

X. birchmanni and *X. malinche* provided a unique opportunity to evaluate a diverse suite

of traits. Previous work has suggested that the sword is an inexpensive means to increase

apparent size (Basolo 1998; Rosenthal and Evans 1998), and larger body size is a trait

that *Xiphophorus* females prefer (Reviewed in Chapter 2). The results presented in

Chapter 2 support the interpretation of the sword as a dishonest sexual signal. My

findings also support the prediction that hybridization alters phenotypic architecture, but

not to the degree that hybrids have diverged from parentals. I argue that hybridization

could provide a potential answer to "the paradox of the lek" (Borgia 1979; Kirkpatrick

and Ryan 1991). Yet this potential resolution would hinge on how much genetic and

phenotypic variation produced by hybridization was oriented toward male trait optima as

defined by female preference (Arnold 1992; Blows and Hoffmann 2005; Van Homrigh

et al. 2007). Furthermore, reduced phenotypic integration in hybrids can allow

hypotheses of trait function to be evaluated by providing a wide range of multivariate

trait values for assessment (Chapter 4). Chapter 4 illustrates the complexity of sexual

ornaments with respect to their interaction with other aspects of the phenotype.

Quantification of the costs of ornaments should involve estimation of the effects of, not just controlling for, covariance with non-sexual traits. Finally, hybridization affects all aspects of the phenotype, including behavioral traits (Chapter 5). With respect to trait correlations, hybridization may also strengthen statistical relationship between traits, which have been infrequently documented (but see Grant and Grant 1994). The role of hybridization in behavioral studies could be of considerable interest particularly in regards to behavioral syndromes.

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72

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