

FEMALE PREFERENCE FOR COMPLEX MALE DISPLAYS IN HYBRIDIZING
SWORDTAILS

A Thesis

by

ZACHARY PIERCE CRESS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2008

Major Subject: Zoology

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Approved by:

Chair of Committee,	Gil G. Rosenthal
Committee Members,	Adam G. Jones
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Head of Department,	Thomas McKnight

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ABSTRACT

Female Preference for Complex Male Displays in Hybridizing Swordtails.

(December 2008)

Zachary Pierce Cress, B.A., Texas A&M University

Chair of Advisory Committee: Dr. Gil G. Rosenthal

Swordtail fishes of the genus *Xiphophorus* have been studied as a model of sexual selection for many years. Many single-trait manipulation studies have been performed, determining female preferences for individual male traits. I characterized how five traits (standard body length, body depth, dorsal fin width, sword length and vertical bar number) correlate to one another within natural variation of populations of *X. birchmanni*, *X. malinche* and three hybrid populations and created synthetic 3-dimensional animations exhibiting these traits within ranges of natural variation. I then performed choice tests on females of the above populations using a computer system that automatically played these stimulus videos and simultaneously tracked a female's position within a test tank to determine female preference for different male phenotypes.

Only *X. birchmanni* females showed significant preferences. Their preferences were in line with past research of univariate trait manipulation experiments. They showed significant preference for larger bodies and dorsal fins and smaller or no swords. They also showed a non-significant preference for vertical bar numbers.

My results also confirmed univariate studies in which *X. malinche* females showed reduced preference for conspecific males and being rather indifferent to the presence of swords. Hybrid females were also shown to have reduced preferences for any specific trait, suggesting that they express recombinant preferences, which can also be explained by reduced color vision at low levels of light.

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CHAPTER I

INTRODUCTION

Little is known about how sexual selection operates within natural hybrid populations. Hybrid zones can be a source of evolutionary novelty, creating both recombinant phenotypes and female preferences for male phenotypes, which in turn can alter the adaptive landscape of populations. I investigated female preferences for sexual traits within a hybrid zone of the fish genus *Xiphophorus*, as well as within parental species, and evaluated how these preferences co-vary with male phenotypic distributions in the wild.

Testing hypotheses about multiple preferences requires intensive sampling of female preference in multiple dimensions. Some studies (*e.g.* van Homrigh et al. 2007; Endler and Houde 1995) have conducted multivariate analyses of female preferences for naturally varying male phenotypes. A potentially more powerful approach is to use synthetic stimuli where the experimenter can precisely vary signal parameters, which has been used with considerable success for acoustic signals (*e.g.* Márquez et al. 2008; Orci 2007).

Testing hypotheses about multiple male traits in the visual domain has historically been difficult, but recent advances in video-playback technology have made it feasible to effect precise manipulations of singular male traits and present them to females (reviewed in Rosenthal 1999; Rowland 1999; Rosenthal Dissertation Chapter II). Synthetic animations (Künzler and Bakker 1999; Bakker et al. 1999; McKinnon and McPhail 1996;

This thesis follows the style of *Evolution*.

Rosenthal Dissertation Chapter V) permit the rapid manipulation of any combination of traits according to quantitative parameters (reviewed in Rosenthal in press; Rosenthal Dissertation Chapter III).

My thesis focuses on developing a novel, high-throughput system for performing controlled assays of female mating preferences. First, I designed novel, synthetic stimuli based on natural covariation among male visual traits. Second, I used an automated motion-tracking apparatus synchronized with a computer-based playback system to perform parallel mate-choice tests on multiple females, which allowed me to efficiently sample female preference space. This approach is detailed in Chapter II. Next, as discussed in Chapter III, I used this methodology to characterize multivariate preference functions in female *X. birchmanni*, *X. malinche*, and their hybrids in an effort to explain observed temporal and spatial patterns of trait distributions in natural populations.

CHAPTER II

A HIGH-THROUGHPUT ASSAY FOR MEASURING MULTIVARIATE MATING PREFERENCES

Introduction

Behavioral traits, by definition, involve responses to external stimuli in the environment (Barnard 2004). Many of the fundamental questions in animal behavior center around how animals respond to a large set of stimuli, varying in multiple dimensions both within and outside the range of existing phenotypic variation.

Modern technology allows us to precisely control stimulus inputs along multiple axes of variation, such as the use of robotic female satin bowerbirds to gauge changes in a male's courtship display (Patricelli et. al 2002) or the alteration of male túngara frog calls (Ryan 1980, Ryan 1983). More specifically, video playback has been used successfully in a variety of taxa and experiments such as species recognition and aggressive displays in anolis lizards (Macedonia et al. 1994), response to food-associated visual displays in fowl (Smith and Evans 2008), and even prey recognition in jumping spiders (Clark and Uetz 1990).

Computer animation technology, which has rapidly advanced in the last twenty years for the entertainment and visualization industries, allows precise control over many aspects of visual stimuli. Locating live animals with the exact visual traits needed for any test could yield a rather daunting, if not impossible task. Studies using synthetic stimuli created by computer animation to precisely alter visual cues have proven successful in swordtail fishes (Wong and Rosenthal 2006, Morris et al. 2006, Rosenthal 1999, Rosenthal 2000).

In order to study behavioral responses to large numbers of traits, we must be able to measure a large number of responses from many individuals. Automated motion tracking allows for reliable, high-throughput measurements of behavioral responses in large numbers of individuals (Jamain et al. 2007, Meshi and Bloch 2007). Working with a computer scientist from the Texas A&M Visualization Sciences department, Trisha Butkowski, I developed synthetic stimuli using state-of-the-art animation techniques employed by the entertainment industry. Working with engineers from Biobserve, a company that produces image-tracking systems, I then developed a system to integrate playback of animations with automated processing of subject animal behavior.

In this Chapter, I describe the steps involved in the construction of an array of synthetic stimuli representing variation in male visual signals in the northern swordtails *Xiphophorus birchmanni*, *X. malinche* and their hybrids. I then describe the approach used to play these stimuli back to multiple females simultaneously in concert with real-time recordings of behavioral responses.

Creating synthetic stimuli

Amongst swordtail fishes (family Poeciliidae) of the genus *Xiphophorus*, much is known about visual female preferences amongst species. Various male traits are taken into consideration by females, such as the presence and size of swords, an extension of the lower caudal fin rays, color, body size, and vertical barring patterns on the body (Rosenthal and Evans 1998, Basolo 1990, Kingston et al. 2003, Cummings et al. 2003, Morris et al. 1996, Morris et al. 1995).

As detailed in the next chapter, my goal was to characterize multivariate mating preferences in parental and hybrid populations of two naturally-hybridizing swordtail

fishes, *Xiphophorus birchmanni* and *X. malinche*. Accordingly, I focused on five morphological traits that are highly sexually dimorphic and show marked differences among parental species and hybrids (Rosenthal et al. 2003): body size (standard length), body depth, dorsal fin width, sword extension length, and the number of pigmented vertical bars on the side of the body. I did not attempt to vary color parameters; for a discussion of the limitations of using video playback to test hypotheses about color, see Fleishman et al. 1998, Fleishman and Endler 2000). *X. birchmanni*, *X. malinche* and hybrids perform a simple, conserved courtship display (Wong & Rosenthal 2006) which I held constant across animations.

Modeling

One base model was constructed around which all stimulus animations were made. Using Autodesk Maya 7.0, polygons were used to create a 3-dimensional shape around a reference photograph of *X. birchmanni*. A polygon face is a flat surface made when three or more points (vertices) are connected. The resulting mesh had 468 quad faces (a polygon with 4 vertices), the minimum amount of polygons that could be made without compromising visual quality of the model. Using the minimum number of polygons optimized the speed at which animations could be rendered (Derakhshani 2005). The dorsal, caudal and pectoral fins were made from 2-dimensional polygon meshes, meaning that, mathematically, they had no depth properties and no volume. Parameters such as body length and depth were directly editable in Maya 7.0.

Each mesh, or surface composed of adjoining polygons, (body, dorsal fin, caudal fin, pectoral fins) had its own texture files, which were networked with Adobe Photoshop CS2. With Photoshop networks, a texture image may be edited in Adobe Photoshop

CS2, with any changes automatically appearing in Maya. Images for each texture were taken from a high resolution photograph of *X. malinche*. Any number of vertical bars could be added to the body texture simply by darkening the image using the burn tool. As the meshes of the dorsal fin and sword were already larger than the maximum sizes needed for stimuli, their apparent sizes were edited by making invisible any portion of the texture image. The same base image for each model's body and fins were used to control for variation in color and shape of fins.

Fins, which are not completely opaque, were given some transparency by creating a transparency layer in those files' corresponding Photoshop files. Transparency layers were created by reducing a fin's colors to only grayscale values, inverting the black and white values, and then adjusting the contrast and brightness. Maya recognizes the color values from a transparency layer as a gradient from white (transparent) to black (opaque). These white and black values were edited until the fin, in Maya, appeared to show natural levels of transparency along with color. See Figures 1 through 3.

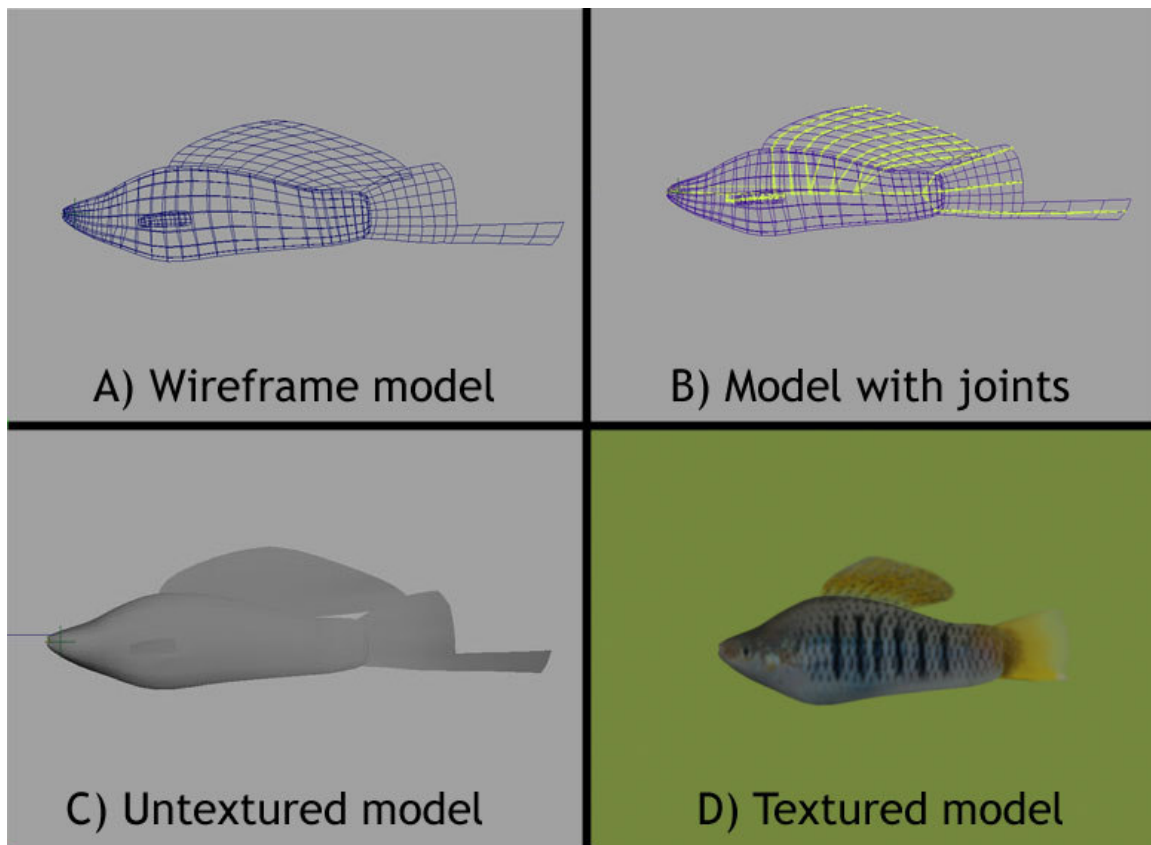


Figure 1. 3D Model Construction

All stimuli are constructed from the same model, whose dimensions and textures can be edited to create any stimulus. Fin transparency is visible against the green background.

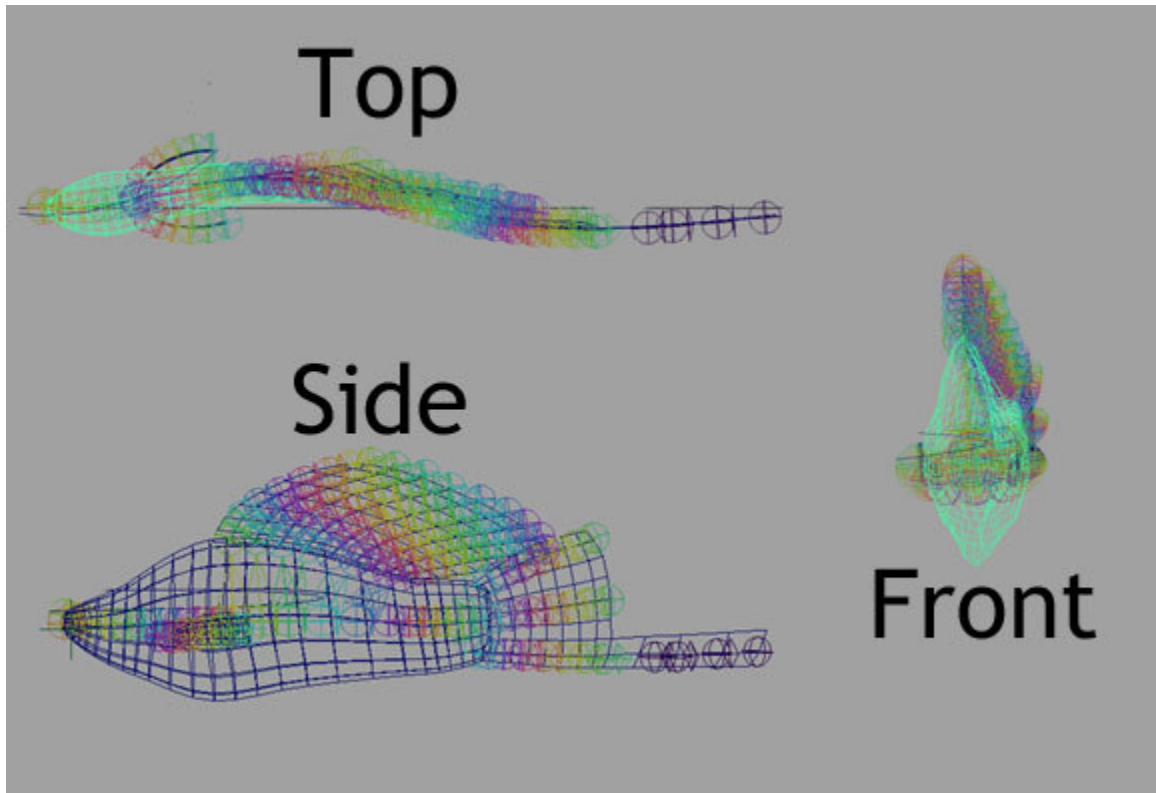


Figure 2. 3D Models

Models are fully three dimensional, giving the ability to move in three dimensions, recreating more accurate swimming motions.

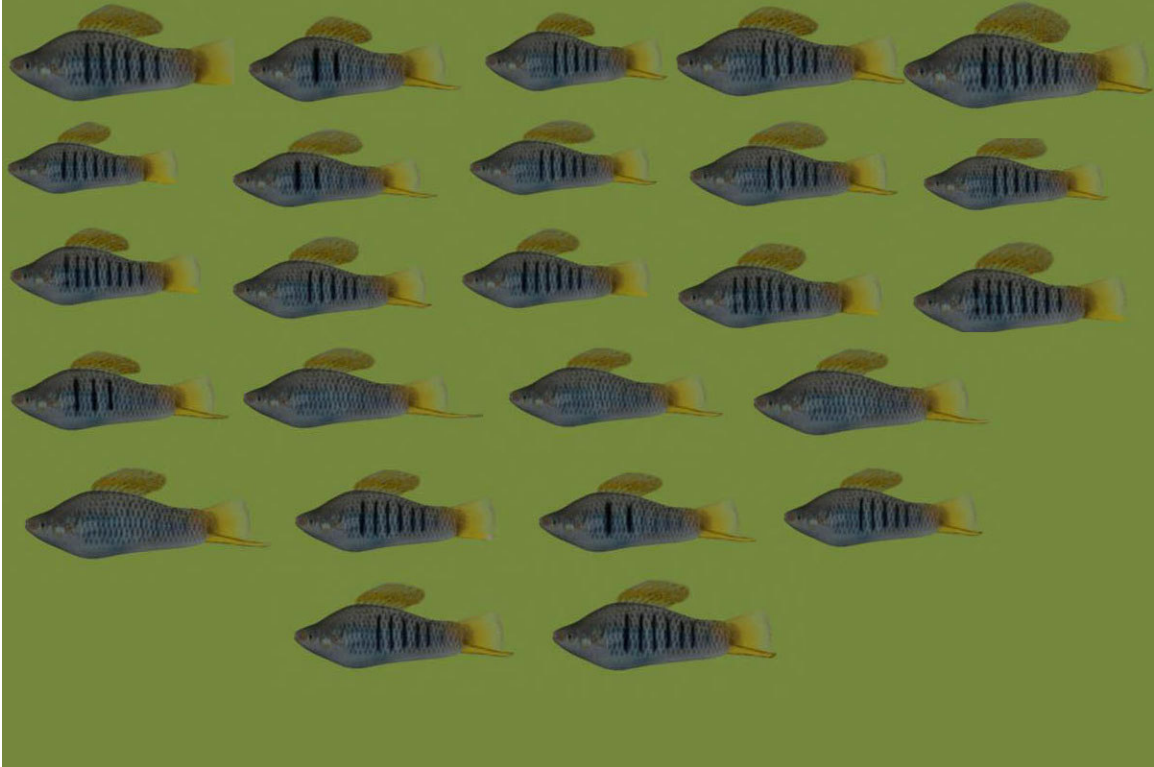


Figure 3 Stimulus Models

Twenty-five stimuli were created from the same base model, varying in length, depth, dorsal fin width, sword extension and vertical bar numbers.

Rigging

Rigging is the process of attaching a 3D mesh to rigid bones, which define the movement parameters of a model. Two identical models were created for the rigging process. One model was reactive to animation information (movement model) and was invisible when an animation was rendered. The other model (show model) was the model actually visible when an animation was rendered and whose size parameters (length, depth, fin size) were editable.

To allow the fish to contort and move in a side-to-side motion, the bones of the fish used spline inverse kinematics, in which IK handles are attached to the end of a joint system (the fish's snout) and the corresponding base of the IK system is attached farther up the skeleton hierarchy to a joint determined to be the root of that IK segment (Derakhshani 2005). The dorsal, caudal and pectoral fin joints were moved using forward kinematics, in which individual joints are rotated independently of one another. For the movement of the fin joints, set driven keys were used to place the fins in position. An attribute was added to a locator to control the size of the fins. Body movement locators were used to control the spline inverse kinematics, which move in X and Y space, to control swim movement.

Animation information could then be applied to the movement model. This information was then applied to the show model by parenting movement parameters of the movement model to the show model. For example, when a joint in the movement model is rotated, the corresponding joint of the show model rotates in exactly the same way.

The show model was then parented under a control object that allowed the model to be scaled to the appropriate length and depth. Two objects were created in Maya which could be edited to change the model's length and depth. An attribute was also created to show the maximum possible dorsal fin size for any given body size. This value was determined by the maximum scale of the dorsal fin times the scale that was currently applied to the length of the model.

Animation

A swimming cycle was then created for the model—a side to side movement that simulated a fish propelling forward through the water. An overhead video of a live male swimming in a straight line was recorded. This video was then divided into individual frames. The (X,Y) position of the fish's snout, dorsal fin insertion and caudal fin insertion were measured every third frame (or 0.1 s). These data were then used to determine the fish's side-to-side bending patterns (as they would be seen overhead) per body length moved through space.

Although the animations that were made were not overhead shots, we felt it important to recreate as many parameters of real swimming motion that we could allow for. Having accurate side-to-side body bending altered the way in which light reflects off of the model in the rendering program, creating more realistic shadowing, diffusion and specular properties.

Swimming speed was estimated by timing the average number of body lengths moved per second of several reference videos of courting males filmed in the wild. Combining this speed with swimming patterns created very life-like motions. All stimulus animated males swam at the same rate and with the same movement, but it is

possible to scale movement patterns with speed, creating life-like animations of fish swimming at any desired speed. The models were then scaled so as to appear life-sized on the computer monitors used for presentations (i.e. a model that represents a 25mm male is exactly 25mm on the monitor).

The background color of each animation was a neutral green (R: 117, G: 135, B: 61), which is an average color of the fish's ambient natural environment.

Videos were rendered at 640x480 (pixels) resolution in AVI format. Each video was then stitched to an identical video so that the two identical videos were side by side. These were then rendered so that each video file actually contained two identical stimulus males. This allowed for two focal females in adjacent tanks (not visible to each other) to be tested using the same monitors simultaneously.

Automated playback and motion tracking

The Biobserve Viewer system, along with the Monitortrainer plugin, allows for automated simultaneous playing of stimulus videos and real-time movement tracking of subjects. The Viewer system consists of three computers. One computer is employed by Monitortrainer and is dedicated to playing stimulus videos. One video camera is attached to each of the other two computers, each of which can track the position, movement and velocity of two subjects, allowing four subject fish to be tested at the same time. Monitortrainer synchronizes with the other two computers so that they can begin tracking a subject's movement the moment a stimulus video begins and stop tracking when the video is finished. Figure 4 shows a schematic diagram of how Biobserve works.

Each trial began with an acclimatization period 10 minutes of monochromatic green screen from both monitors, followed by simultaneous presentation of the 5 minute test stimuli. The female was then presented with 10 minutes of monochromatic green screens, followed by 5 minutes of the same stimulus sets, this time switched between monitors to correct for side bias. Viewer, synchronized with Monitortrainer, recorded subject females' positions only during the times when stimulus videos were being played. See Figure 4.

Monitortrainer can also control a software switch that allows four subject females to be removed, four new subject females to be put in and acclimatizing in an adjacent room while four other subject females are undergoing a trial and being tracked. This would permit a total of eight subjects to be tested within 40 minutes, vastly increasing the rate of behavioral data collection from past behavioral assays.

To ensure that all stimuli were identical except for the traits in question, the contrast, brightness and color values of each 16 inch Dell and Samsung CRT monitor were adjusted until they appeared to be identical by eye and independently confirmed by a second person. As two different brands of monitor were used, no subject female saw two different kinds of monitors. She either saw both stimuli on Dell monitors or both stimuli on Samsung monitors.

The test tanks were divided lengthwise into three equal sections (left and right "preference" zones and a central "neutral" zone) by lines drawn on the sides of the tank. Viewer allowed me to draw these zones into the system, which would then tell me if the subject was in an association zone or not at any given time.

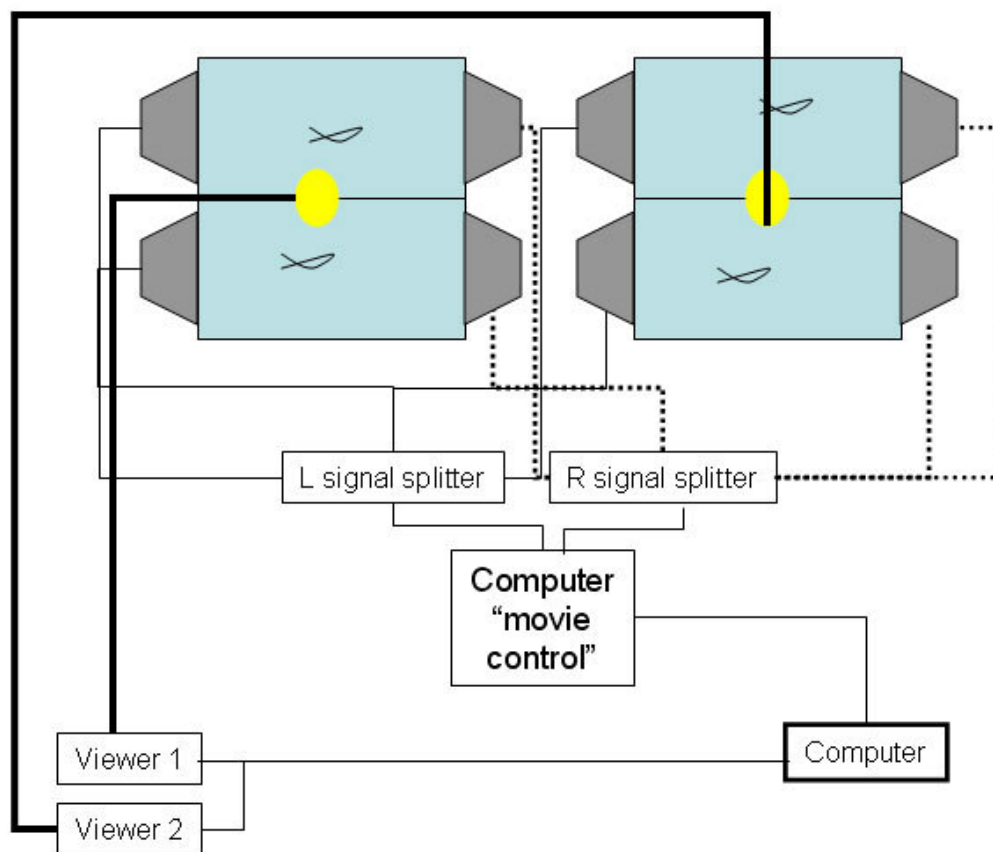


Figure 4 Experimental Design Schematic

Schematic diagram of how Biobserve Viewer works. Viewer 1 and Viewer 2 are computers that record a live fish's position through overhead cameras (yellow circles). A third computer plays stimulus videos.

Because the overhead cameras could not be placed directly above the centers of the tanks looking down at a perfectly perpendicular angle to the tanks, some small shadows at the edges of the tanks were visible to Viewer. This could have posed a problem as Viewer was set to detect a dark fish in a white tank. Viewer could then occasionally confuse a dark shadow with a dark fish (ghosting), giving unusable data. When ghosting occurs, the computer could mistakenly record the fish as being at one end of the tank at one moment and then at the other end 0.1s later. To correct for this, Viewer allowed any shadows to be painted out from the measurement area to ensure that the only object being detected were the subject fish.

Viewer outputs the data in a format that is easily exported for use in Microsoft Excel. For any given subject fish, it records her (X,Y) position (from an overhead camera) within her tank an average of 11 times per second, which zone she is in, her velocity at any given time and her maximum velocity during a trial, potentially opening the door for many different kinds of behavioral assays. See Figure 5. Association time is widely used as a measure of sexual response in *Xiphophorus* (Basolo 1990, Morris et al. 2003). Although it is a robust and repeatable measure that accurately predicts mating success (Cummings & Mollaghan 2006), association time has been criticized as being difficult to distinguish from a non-sexual shoaling response. Analysis of real-time female response data, for example using neural networks to parse complex response patterns in space and time (e.g. Piratla and Jayasumana 2002) might reveal a more specific metric of female mating preference.

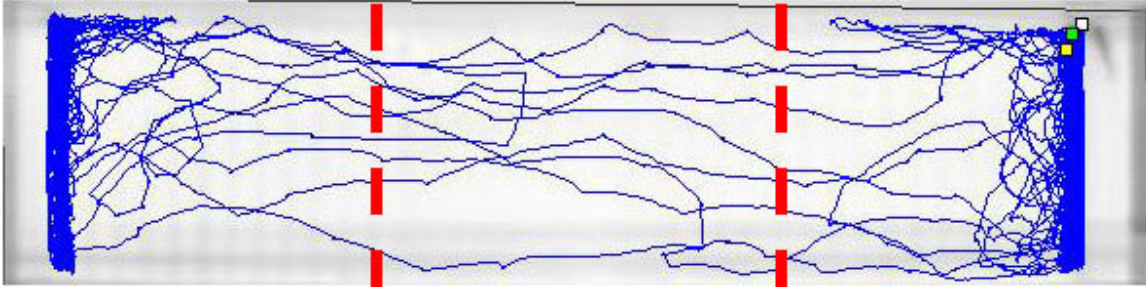


Figure 5 Viewer Data Output

Visual representation of Biobserve Viewer output. Looking down from an overhead camera, Viewer tracks the focal female's movement between association zones (at either end) and the neutral zone in the middle as stimuli are presented.

CHAPTER III
MULTIVARIATE MATING PREFERENCES AND THE EVOLUTION OF
XIPHOPHORUS HYBRID ZONES

Introduction

Countless studies of mate choice in animals have been conducted in which single traits were manipulated, often giving simple binary choices (Andersson 1994). In nature, however, animals typically display and perceive multiple cues simultaneously. These can be multimodal, in which more than one sensory modality is used by the receiver (Partan & Marler 2005, Rowe & Guilford 1999). Multiple traits can convey the same information (*e.g.* about direct or indirect benefits to females) redundantly, or one stimulus can be a noninformative accessory that enhances the perception of another, informative stimulus (Rowe 1999).

Candolin (2003) proposed that multiple signals in the context of mate selection can fall into three main categories: adaptive, non-adaptive and maladaptive, from the receiver's point of view. Of the adaptive signals, some are informative, such as multiple messages indicating different qualities, back-up cues indicating the same quality, species recognition and Fisherian signals. Some adaptive signals are uninformative, such as sensory exploitative signals or those which enhance detectability. Some signals may be either informative or uninformative, such as those signals which take advantage of multiple sensory environments. Non-adaptive cues, on the other hand, are uninformative

and not honest signals of male quality. Maladaptive signals are also uninformative to the receiver as they presumably increase intersexual conflict Candolin (2003).

If multiple signals convey the same information, they should tightly covary with signaler quality (Hebets and Papaj 2005). If multiple traits are all positively correlated with heritable components of fitness, they should covary accordingly. If for example, females preferred larger apparent body size and body length and depth are heritable traits, they should both scale with each other.

Natural hybrid zones present an intriguing opportunity to examine the role of trait correlations in sexual communication. Introgression of phenotypes from one population to another via hybrid zones relaxes genetic constraints and provides a sort of shortcut to phenotypic novelty. Within a hybrid zone, covariance among traits can potentially be broken and new combinations of traits could appear. Sexual selection can act in a range of ways on novel variation. At one extreme, recombination of parental male phenotypes in hybrid zones can be a source of novelty in species that exhibit a rare male mating advantage, in which males with novel, or rare, sexual phenotypes have a mating advantage over the majority of other males in the population. The argument with rare male effects is that novelty itself is attractive – with hybrids, they are not only novel but can exhibit specific preferences that happen to be outside the range of natural male variation; more generally, some hybrid phenotypes may be favored if female preferences extend outside the range of current male trait variation (Dal Molin 1979). Alternatively, recombinant phenotypes may be at a selective disadvantage with respect to males of either parental species.

Understanding how sexual selection acts on novel hybrid phenotypes is primarily complicated by two additional factors. First, females of the two parental species differ in their preferences. In a review, Wirtz (1999) showed that of 80 studies available at the time that analyzed the mitochondrial DNA of natural hybrids, 50 showed hybrids with mitochondrial DNA of only one of the two parental species. Second, asymmetric preferences between species can be frequency dependent, in which females of the rarer species mate with males of the more common species.

Unidirectional hybridization has been studied in *Bufo* toads, in which calling efforts of hybrid males were shown to be similar to those of one parental species (Malmos et al. 2001). Furthermore, those hybrids all contained mitochondrial DNA from the other parental species, indicating that all hybrids are the product of females of one species and males of the other. Another example of unidirectional hybridization and perhaps the most well-known study of unidirectional hybridization is of the white- and golden-collared manakins. In this system, secondary sexual traits of golden-collared manakins have spread into populations of white-collared manakins through a hybrid zone. Again, mtDNA haplotypes of only one species, the white-collared manakin, were found in hybrids, demonstrating that females of one species show preferences for males of the other species, but only in that one direction (Parsons et al. 1993).

Hybrid traits may be transgressive, intermediate between parental species, or characteristic of one of the parents (Bell & Travis 2006). Shaw (2000) showed that artificial backcross hybrids of Hawaiian *Laupala* crickets showed preferences intermediate between the two parent populations. Hybrid *Hyla* tree frog females were

shown preferences for hybrid male calls (Doherty and Gerhardt 1983). We addressed multivariate preferences of parental and hybrid females in a naturally-hybridizing complex of swordtail fish (Poeciliidae: *Xiphophorus birchmanni* and *X. malinche*; Rosenthal et al. 2003).

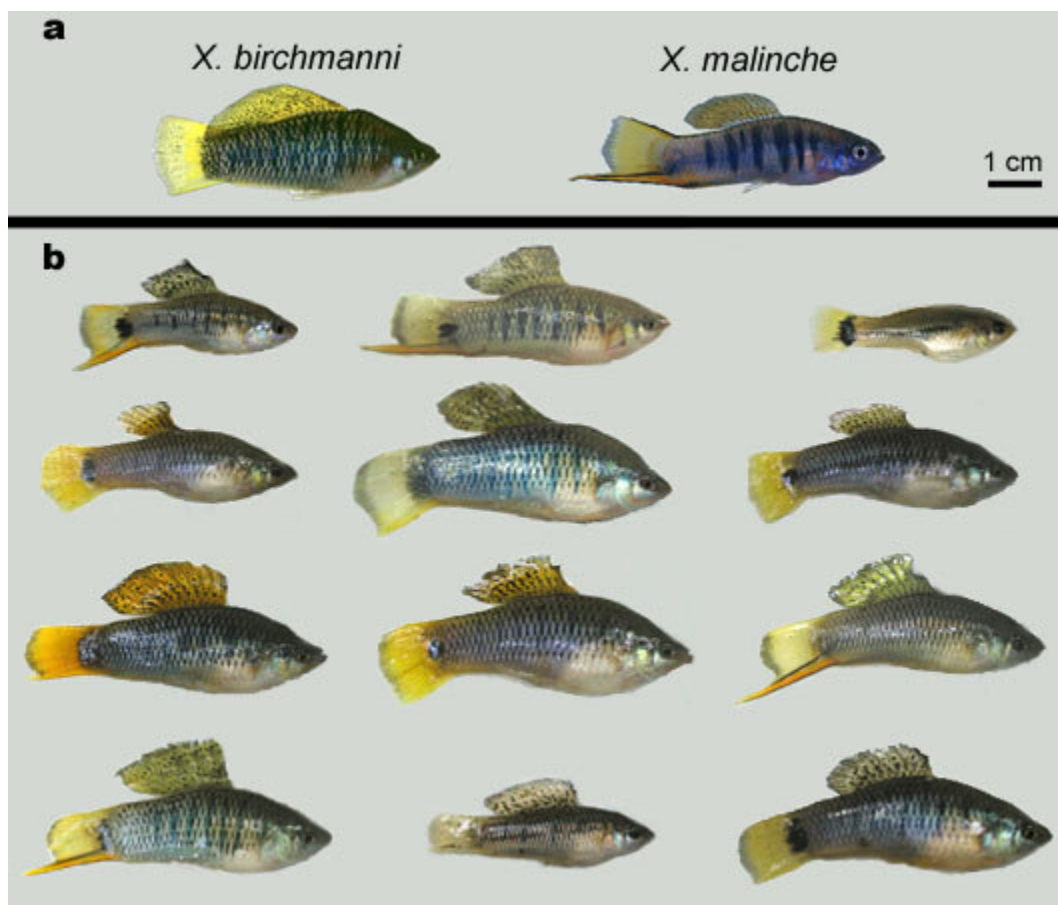


Figure 6 Parental and Hybrid Males

A) Male *X. birchmanni* and *X. malinche* and B) hybrid males with recombinant and novel phenotypes.

Although interspecific hybrids are generally less fit than parentals (for review, see Andersson 1994), *X. birchmanni/malinche* hybrids may be favored by natural selection at elevations intermediate between the montane *X. malinche* and the lowland *X. birchmanni* (Rosenthal et al, unpublished data). Further, from the point of view of female mate choice, hybrids have the possible advantage of expressing phenotypes not shown in parental species. These can include both novel combinations of traits, and transgressive values of individual traits (Mallet 2007). This is in fact the case in hybrid swordtail populations, where some males have extreme trait values and novel phenotype combinations (Rosenthal et al. 2003; Fig. 6).

Swordtails are a well-characterized model for visually-based female mating preferences. Females attend to multiple traits when choosing mates (reviewed in Rosenthal & García de León 2006). We focused on five focal traits likely to be under sexual selection: (a) the sexually-dimorphic *sword*, an extension of the lower caudal fin rays. Female preference for swords has been shown to predate the existence of swords (Basolo 1995) existence, but this preference has secondarily been lost among *X. birchmanni* females (Wong & Rosenthal 2006) and *X. malinche* females show no preference for swords. Sword length is shrinking over time in hybrid populations (GG Rosenthal et al., unpublished data). (b) *Body size* and (c) *dorsal fin width* are allometrically related in *X. birchmanni* (Fisher 2006), but the strength of the allometry is reduced in hybrids (Rosenthal et al. 2003). It is also known, at least in *X. birchmanni*, that females prefer large males, but show a preference for smaller dorsal fins, as large dorsal fins are an indicator of aggressiveness (Fisher & Rosenthal 2007). If body size

and dorsal fin size were genetically linked, which they likely are not, then hybrid males would not be able to match female preferences. Rosenthal *et al.* show, using principal components analysis, that hybrids do fall outside of the range of parental phenotypes (See figure 1, Rosenthal et al. 2003). Hybrid males thus can have large body size and smaller dorsal fins. A constraint on the evolution of the hypothetical perfect male with a large body and small dorsal fin is that males raise their dorsal fins in the presence of other males, signaling their level of aggressiveness. *(d) Body depth* may also increase apparent male size (Rosenthal & Evans 1998). Females in several swordtail species attend to *(e) Vertical bar number* (Morris et al. 1995; Hankison & Morris 2003).

Manipulating these traits in combination requires both precise control over stimuli, and the ability to efficiently perform numerous controlled behavioral trials. Construction and high-throughput playback of computer-animated test stimuli is detailed in here. I characterized female preference space in parental species and hybrids by presenting stimuli representing means and extremes of male covariation, allowing me to broadly assess female response to sexually dimorphic male traits. In any trial, females were given a choice between a mean male of her own population and either a mean male of another population or a male from one of four extremes from PCA space of her own population.

Methods

Study populations

Xiphophorus malinche were collected from the Arroyo Xontla, a tributary of the Río Calnali near the town of San Andrés Chicayotla, Hidalgo, México (20°55'26"N, 98°34'35"W) at an elevation of 1003 m. *X. birchmanni* were collected from the Río Garces, Hidalgo, México (20° 56' 24"N, 98° 16' 54"W) at an elevation of 229 m. Hybrids were collected at three different locations on the Río Calnali: Calnali-high (20°53'54"N, 98°37'39"W) at 1168 m, Calnali-mid (20°53'34"N, 98°36'36"W) at 1007 m, and Calnali-low (20°55'26"N, 98°34'35"W) at 920 m.

Stimulus array construction

Morphometric data were compiled from measurements taken using ImageTool (UTHSCSA) and ImageJ (Abramoff et al. 2004) from digital photographs taken of wild-caught individuals between 2003 and 2007; see Rosenthal et al. (2003) for trait definitions. The traits addressed in this study were standard length (SL), body depth (BD), sword extension length, dorsal fin width, and vertical bar number. See Figure 7.

A principal components analysis (PCA) was done on the morphometric data set. The first two factors explained 72% of male phenotypic variation; the first principal component loaded positively on body size, body depth, and dorsal fin width, and negatively on sword extension length; the second loaded positively on vertical bar number (table on p. 31). For each of the five populations, I generated five stimuli based on: (1) the mean PC score for both traits; (2) the maximum PC score for PC 1 and the mean for PC 2, (3) the minimum for PC1 and the mean for PC2, (4) the maximum for

PC2 and the mean for PC1 and (5) the minimum for PC2 and the mean for PC1 (Fig. 3). Trait values were calculated by setting PC 1 and 2 to the appropriate value and setting the remaining PC's to zero. See Figures 8a-e and Table 1. Construction of animated stimuli is detailed in Chapter II. Finished stimuli were loaded onto the Monitortrainer utility of the Viewer system for playback to females.

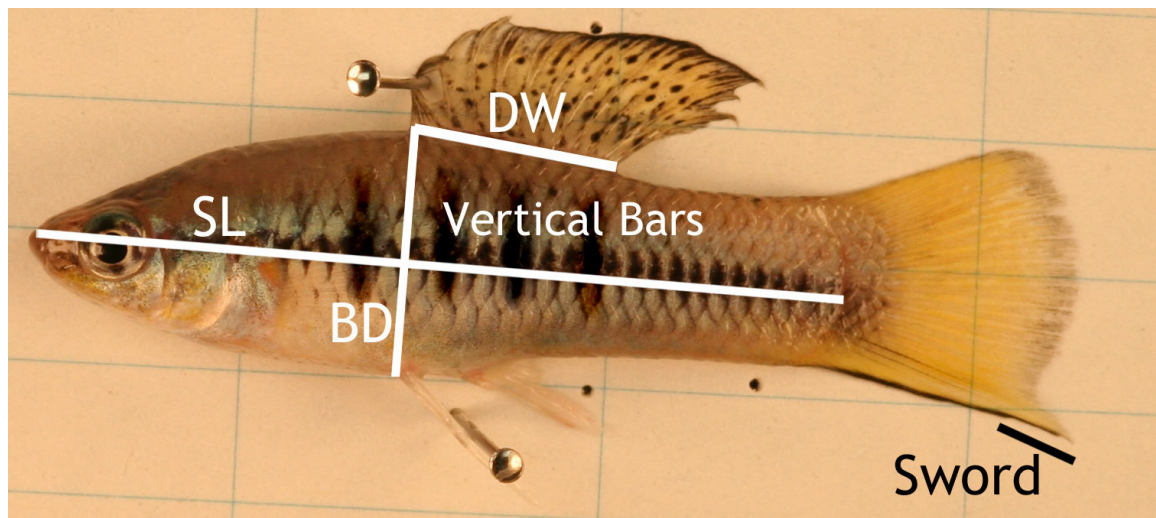


Figure 7 Male Traits

Standard length (SL), body depth (BD), dorsal fin width (DW), vertical bar number and sword length values of parental and hybrid males were measured to determine average values for each population.

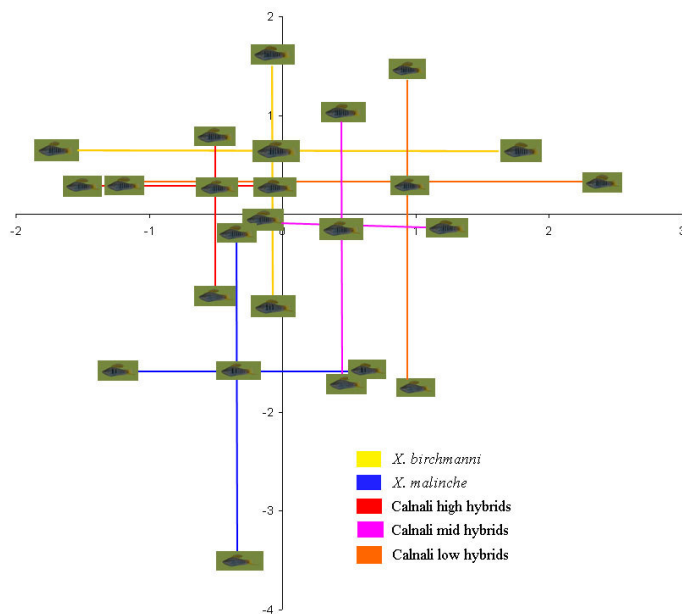


Figure 8a Male Phenotypes in PCA Space (all populations).

Distribution of male phenotypes within PCA space for both parental species and Calnali-high, mid and low populations.

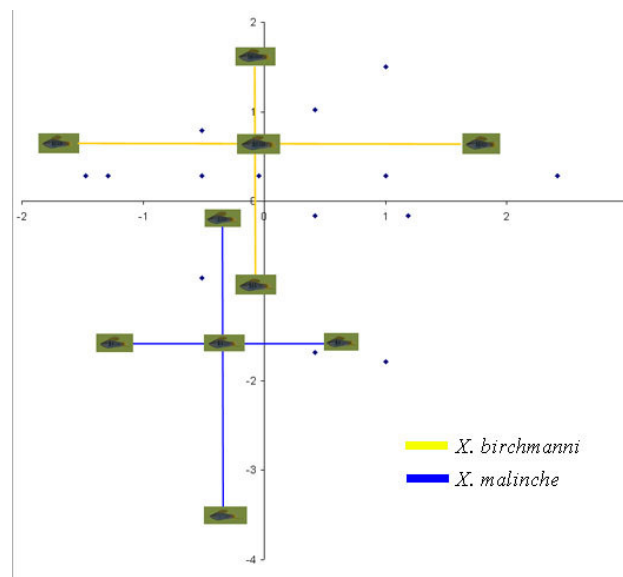


Figure 8b Male Phenotypes in PCA Space (*X. birchmanni* and *X. malinche*).

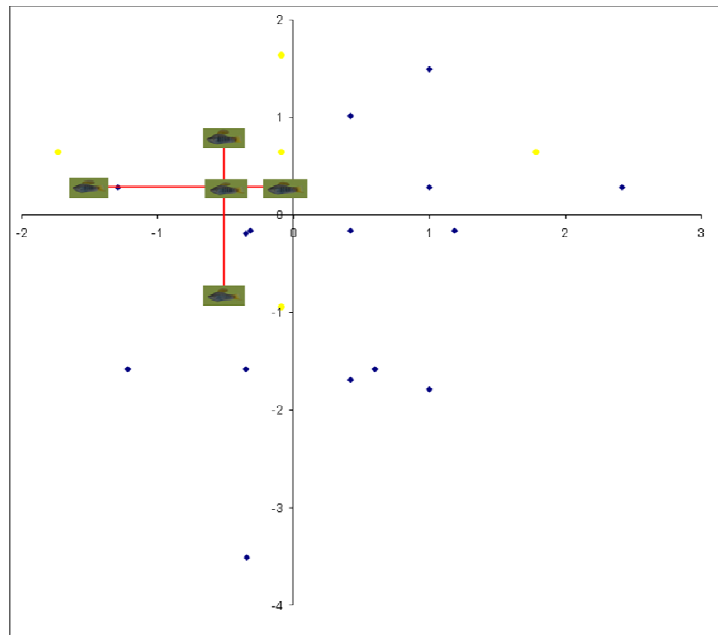


Figure 8c Male Phenotypes in PCA Space (Calnali-high Hybrids).

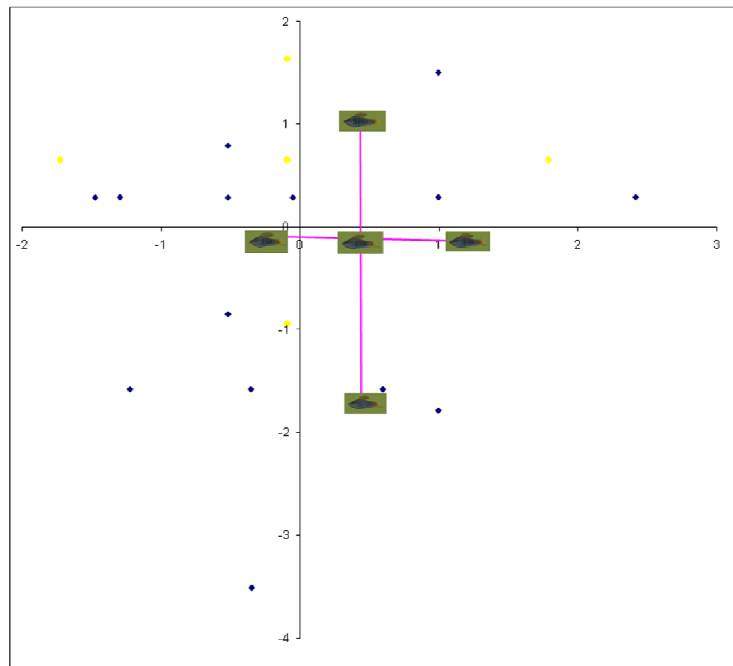


Figure 8d Male Phenotypes in PCA Space (Calnali-mid Hybrids).

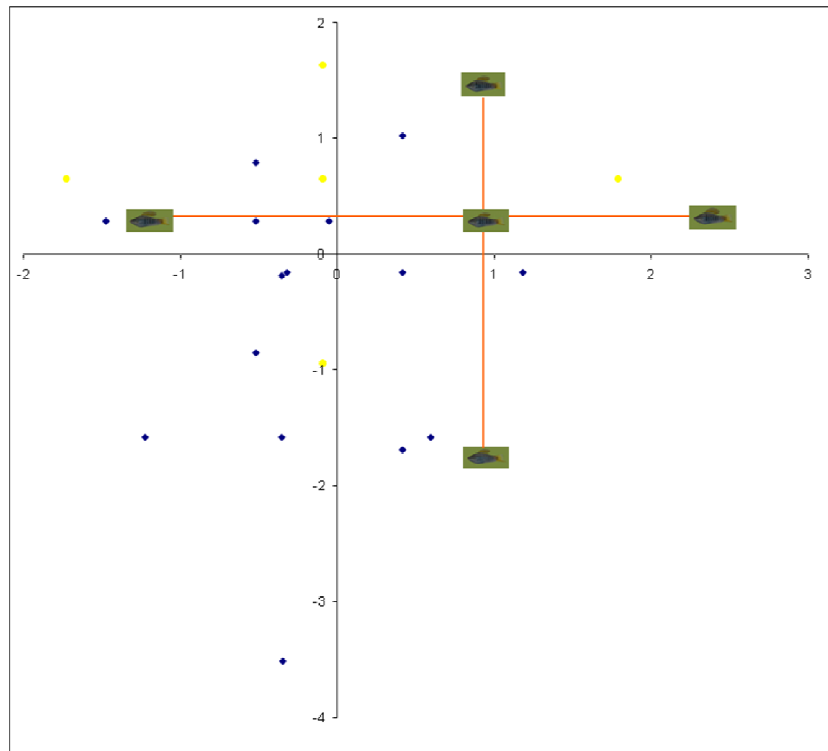


Figure 8e Male Phenotypes in PCA Space (Calnali-low Hybrids).

Table 1 Phenotypic Scores for Each Stimulus

SL indicates standard length (snout to caudal peduncle). Max1 indicates a maximum value for PC1 and zero value for PC2, Min1 indicates a minimum value for PC1 and zero value for PC2, mean indicates zero values for both PC1 and PC2 *etc.*

	X. birchmanni	X. malinche	Cal-hi	Cal-mid	Cal-lo
<i>Max1</i>	FISH1	FISH2	FISH3	FISH4	FISH5
<i>Mean</i>					
<i>bar</i>	6	2	5	5	6
<i>SL</i>	50.6	47.6	43.9	48.9	53.2
<i>Depth</i>	17.6	15.3	14.7	16.5	18.5
<i>Sword</i>	1.2	6.8	2.1	3.2	2.1
<i>Dorsal</i>	15.0	13.1	12.2	14.1	16.0
<i>Min1</i>	FISH6	FISH7	FISH8	FISH9	FISH10
<i>Mean</i>					
<i>bar</i>	6	2	5	5	5
<i>SL</i>	37.2	40.7	38.5	43.2	39.1
<i>Depth</i>	12.1	12.5	12.4	14.2	12.7
<i>Sword</i>	1.3	6.9	2.2	3.3	2.2
<i>Dorsal</i>	9.6	10.3	10.0	11.8	10.3
<i>Max2</i>	FISH11	FISH12	FISH13	FISH14	FISH15
<i>Mean</i>					
<i>bar</i>	8	5	6	7	8
<i>SL</i>	42.8	43.0	41.7	45.1	47.0
<i>Depth</i>	14.8	14.1	14.0	15.5	16.5
<i>Sword</i>	0.0	3.3	0.9	0.3	0.0
<i>Dorsal</i>	12.2	11.7	11.5	13.0	13.9
<i>Min2</i>	FISH16	FISH17	FISH18	FISH19	FISH20
<i>Mean</i>					
<i>bar</i>	3	0	0	0	0
<i>SL</i>	44.6	45.3	42.9	47.0	49.3
<i>Depth</i>	14.4	13.5	13.7	15.0	15.9
<i>Sword</i>	5.3	11.7	5.0	7.1	7.3
<i>Dorsal</i>	12.1	11.5	11.4	12.8	13.7
<i>Mean</i>	FISH21	FISH22	FISH23	FISH24	FISH25
<i>Mean</i>					
<i>bar</i>	6	2	5	5	6
<i>SL</i>	43.5	44.0	42.1	46.0	47.8
<i>Depth</i>	14.7	13.9	13.9	15.3	16.3
<i>Sword</i>	1.2	6.9	2.2	3.3	2.1
<i>Dorsal</i>	12.2	11.6	11.5	12.9	13.8

Female preference tests

Upon arrival from Mexico, females were housed individually in 12L acrylic aquaria, each with a sponge filter at 12:12 (L:D) cycles. Females were fed Tetramin flakes daily as well as freshly hatched brine shrimp at least every other day. Water changes were performed at least once per week to keep each tank clean.

Females were presented with a mean male of her own population and either a mean male of another population or a male from one of four extremes from PCA space of her own population. Stimulus-pair order and side of first presentation were randomized to control for order effects and side biases respectively. Females were tested in cohorts of four on the same stimuli.

Females were presented with two stimulus videos at opposite ends of a 75 cm x 30cm x 30 cm (length x width x height) test tank for 5 minutes. The test tanks were divided lengthwise into three equal sections (left and right “preference” zones and a central “neutral” zone) by lines drawn on the sides of the tank. Animation sequences were played from HP a1710n computers connected to 16” CRT monitors placed at opposite ends of the aquarium, with the screens facing into the tank. Females were individually acclimatized in the test tank for 10 minutes before the start of each test. After the acclimatization period, each female was presented with 10 minutes of monochromatic green screen from both monitors, followed by simultaneous presentation of the 5 minute test stimuli. The female was then presented with 10 minutes of monochromatic green screens, followed by 5 minutes of the same stimulus sets, this time switched between monitors to correct for side bias. Videos were looped during this time.

Her position within the tank was scored by a Biobserve Viewer automated tracking system which permits simultaneous, separate testing of four individuals. Her association time for each stimulus was decided by measuring how much time she spends within the association zones of each stimulus, and preference were determined by measuring with which stimulus she associated most. Trials in which females did not visit both association zones were excluded from analysis. I used net association time (response to population mean – response to other stimulus) for analysis.

Statistical analyses

I evaluated population differences in the strength of preference using a general linear model (GLM) of the effect of species crossed with each net PC score, on net association time. For each population, I then conducted a multiple linear regression of the net difference in principal component scores (population mean stimulus – other stimulus) for both PC 1 and PC 2 on net association time (time with population mean stimulus – time with other stimulus). Because of the relatively low sample size among hybrids, I also conducted the same analysis pooling responses from all three hybrid populations.

For Río Garces *X. birchmanni*, the only population for which significant preferences with respect to PC scores were detected, I subsequently conducted a multiple linear regression of net trait differences on net association time, using each of the five traits (vertical bar number, sword extension length, dorsal fin width, body depth, and body size) used for PCA.

Table 2 Multiple Linear Regression Statistics for Effects of PCA Scores on Net Female Association Time by Population

Significant variables are emboldened.

Species/Population	N	Regression			PC1		PC2	
		R^2	F	p	t	p	t	p
<i>X. birchmanni</i> , Río Garces	86	0.11	5.141	0.008	3.206	0.002	0.3	0.765
<i>X. malinche</i> , Chicayotla	44	0.004	1.394	0.252	0.145	0.886	0.394	0.695
Hybrids, combined	121	0.023	1.394	0.252	-	-	0.542	0.589
Hybrids, Calnali-high	36	0.024	0.412	0.666	0.263	0.794	0.79	0.435
Hybrids, Calnali-mid	33	0.063	1.011	0.376	0.979	0.335	-1.205	0.237
Hybrids, Calnali-low	52	0.056	1.454	0.243	1.556	0.126	0.649	0.519

Table 3 p -values for Fisher's least-significant difference tests comparing preferences for PC1 across populations.

Significant values ($p < .05$) are in bold, marginally nonsignificant values ($.05 < p < .10$) in italics.

	<i>X. malinche</i> , Chicayotla	Hybrids, Calnali-high	Hybrids, Calnali-mid	Hybrids, Calnali-low
<i>X. birchmanni</i> , Río Garces	0.11	0.027	<i>0.085</i>	0.005
<i>X. malinche</i> , Chicayotla		0.348	0.694	0.468
Hybrids, Calnali-high			0.696	0.696
Hybrids, Calnali-mid				0.897

Results

Response rates

X. birchmanni females responded to 57% of trials, *X. malinche* to 30%, Cal-high females to 55%, and both Cal-mid and Cal-low to 56% of trials. A X^2 analysis of the rates of failure (females who do not visit each side of the tank during a trial) between all populations revealed a significant difference between all populations ($X^2 = 15.95$, $p = 0.0036$). The difference was even stronger between the two parental species ($X^2 = 14.85$, $p = 0.0001$). Among hybrid populations, however, this difference disappeared ($X^2 = 1.095$, $p = 0.5783$).

Mating preferences

Preference (relative to the mean stimulus of the females' own population) varied considerably among populations (GLM, $N = 251$, $R^2 = 0.063$) for PC1 ($F = 3.3731$, $p = 0.006$) but not for PC2 ($F = 0.589$, $p = 0.671$). *Post hoc* Fisher's least-significant-differences tests showed that this pattern was largely driven by the Río Garces *X. birchmanni* population (Table 2).

There was a strong effect of PC1 on female response in Río Garces *X. birchmanni* (Table 3). Preferences were not detected for any other population (Table 3). Within *X. birchmanni*, the difference in male traits among stimuli was significantly associated with female preference (multiple linear regression, $N = 86$, $R^2 = 0.178$, $F = 3.456$, $p = 0.007$). With the exception of vertical bar number, all traits were significantly associated with preference (Table 4).

Table 4 Multiple linear regression statistics for effects of individual morphometric variables on net female association time in *X. birchmanni*.

Significant variables are in bold.

Trait	Std. Coefficient	<i>t</i>	<i>p</i>
Vertical bar number	0.307	0.437	0.663
Standard length	-659.976	-2.169	0.033
Body depth	-1698.134	-2.16	0.034
Sword extension length	1.721	2.214	0.03
Dorsal fin width	2336.778	2.163	0.034

CHAPTER IV

CONCLUSIONS

The strong difference between the parental species' response rates may reflect differences in the strength of preference; female *X. malinche* may not switch sides in the trials because they are indifferent between the two stimuli. In addition, differences in response rate may reflect differences in exploratory behavior between the two species, indicating that the two species have evolved behavior differences in response to their natural environments. *X. birchmanni* from the Río Garces spend more time actively swimming out of shelter than *X. malinche* (G. G. Rosenthal, Z. P. Cress, Z. W. Culumber, and C. Wat, unpublished data). A likely explanation for this difference is that *X. birchmanni* habitats are wide, shallow rivers with little or no vegetative cover, while the habitats of *X. malinche* tend to be narrow streams with large amounts of cover and little or no predatory kingfishers. Preliminary analysis of motion-tracking data indicates that *X. birchmanni* females range throughout the open area of the stimulus tank, while *X. malinche* females stay close to the sides, with hybrids exhibiting an intermediate range of spatial patterns.

A fundamental concern is that this could result in artifactual patterns of mating preference; that is, that the apparent lack of mating preference in *X. malinche* is simply due to more timid behavior in a laboratory setting. However, *X. malinche* tested in identical tanks show strong preferences for conspecific olfactory cues (G.G. Rosenthal and H.S. Fisher, unpublished data). Preference differences among responsive

individuals are therefore unlikely to arise merely as an artifact of the laboratory environment.

The stronger preferences found in *X. birchmanni* could help account for phenotypic distributions seen in the wild. Phenotypes characteristic of *X. birchmanni* are introgressing into hybrid populations characterized by predominantly *X. malinche* genomes. A broad range of phenotypes in hybrid populations suggests that hybrids are more permissive and show relaxed sexual selection in regards to visual cues. This could also explain why hybrid populations showed no significant differences in response rates. This may reflect sensory dysfunction in hybrid females, as it has recently been found that *X. birchmanni* / *X. malinche* hybrids show reduced cone opsin gene expression, rendering them essentially colorblind at low levels of light. (Coleman et al., unpublished data).

Female preferences did not covary with male trait distributions. Both hybrids and *X. malinche* females were broadly permissive with respect to male traits, and hybrid females did not show preferences for hybrid phenotypes. These results suggest that variation among populations in hybrid traits is unlikely to be driven by population-specific preference patterns for visual traits. It should be noted, however, that this study presented females with male phenotypes along prevailing axes of covariation. Future studies should address female preferences for individual traits and trait combinations outside the natural range of covariation.

Multivariate preferences in *X. birchmanni* are consistent with previous work using univariate trait manipulations, as described below. This suggests that most

preferences for individual traits are weighted equally by females, with the exception of body size and dorsal fin size.

As it is known that dorsal fin sizes increases allometrically with body size in *X. birchmanni* (Fisher 2006), but that females prefer larger bodies with small dorsal fins (Fisher & Rosenthal 2007), a trade-off is expected between the two traits. My results indicate that females appear to like larger dorsal fins because dorsal fins are correlated with big body size. Independently, however, females do prefer smaller dorsal fins. This shows that females prefer large body size more than they dislike large dorsal fins.

My results also show that females prefer large males with no swords. This is consistent with Wong & Rosenthal's results (2006) that *X. birchmanni* females have secondarily lost their preference for swords and that *X. malinche* females are indifferent to swords.

Regression coefficients were opposite in sign for sword extension length/dorsal fin size and body length/depth. This is consistent with previous studies showing opposing preferences for dorsal fin size and body size (Fisher & Rosenthal 2007) and negative preferences for swords (Wong & Rosenthal 2006).

In *X. birchmanni*, females showed a non-significant positive association with vertical bar numbers. Vertical bars were the only trait that loaded highly onto PC2. This is surprising, given that previous studies in other *Xiphophorus* species (*X. multilineatus*) have shown that females do prefer more vertical bars (Morris et al. 1995). It has also been shown that *X. cortezi* females take into account not just the number of bars, but the

frequency of bars within a bar span (Morris et al. 2001). For this study, I held bar span constant by spacing bars evenly along the body.

Body size and dorsal fin size loaded positively on PC1 and sword extension length loaded negatively on PC1. Thus, *X. birchmanni* preferences are in line with this prevailing axis of trait variation among *X. birchmanni*, *X. malinche* and hybrid populations. Female preferences lie on this one axis of variation, which explains difference in preferences between species.

Recombination of male traits produces hybrid males with transgressive phenotypes not found in parent populations. Hypothetically, some of these males would have unattractive trait combinations, none of the traits females find attractive, many would be no more or less attractive than parental males, and some would be ‘super’ males with combinations of traits so attractive that they will have a mating advantage over their neighbors. It would then be these males who reach new adaptive peaks and pass on their genomes to future generations.

Observations of hybrid swordtails of some populations show a loss of swords within a very short amount of time—around one decade (GG Rosenthal, unpublished data). Knowing that the preference for long swords has secondarily been lost among parental populations, it makes sense that hybrids, who are able to show more extreme phenotypes than parentals, would quickly lose a trait that is sexually selected against. If female preferences are not congruent with hybrid phenotypes, hybrid males, as a whole, will be able to match their phenotypes to female preferences much more rapidly than parentals due to their transgressive properties

Using a novel system in both stimulus construction and experimental design, I have used high-throughput sampling of female preference space to characterize how naturally hybridizing animals respond to multitrait variation in male signals. This approach should prove a useful tool in future studies exploring how multidimensional mate choice influences the dynamics of traits and preferences in natural populations.

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