A COMPARATIVE ANALYSIS OF THE ROLE OF MALE – MALE COMPETITION

IN THE EVOLUTION OF AGGRESSION, VERTICAL BARS AND MALE BODY

SIZE IN THE NORTHERN SWORDTAIL FISHES

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This dissertation entitled

A COMPARATIVE ANALYSIS OF THE ROLE OF MALE – MALE COMPETITION

IN THE EVOLUTION OF AGGRESSION, VERTICAL BARS AND MALE BODY

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BY

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A Comparative Analysis of the Role of Male – Male Competition in the Evolution of

Aggression, Vertical Bars and Male Body Size in the Northern Swordtail Fishes (154pp.)

Director of Dissertation: Molly R. Morris

This dissertation examines components of male-male competition in order to

determine factors that are important in contest dynamics and outcome, as well as the

evolution of particular male traits. Specifically, I use northern swordtail fishes (Pisces:

Poecilliidae, genus *Xiphophorus*) to address the following questions: 1) What role does

size play in conflict resolution; 2) How do males use the signal vertical bars in agonistic

encounters; 3) What was the role of male - male communication in the evolution of the

male trait vertical bars; 4) Are differences in fighting ability and aggression levels

between barred and barless *X. cortezi* males the result of responses to the bars or inherent

properties of being either barred or barless; and 5) Are there relationships between size

and aggression both within and across species.

Results from dyadic contests indicate that competitive ability is largely the result

of an individual's size relative to that of his opponent. However, other factors contribute

to the probability of winning a contest as well. For example, barless *X. cortezi* males

appear to have higher resource holding power (RHP) than same sized barred males, and

this advantage appears to result from overall higher aggression levels. Results from

mirror image stimulation tests indicate that variable bar expression signals aggressive

motivation in most barred species and additionally, signals aggressive intent in barred

species that intensify the expression of the bars prior to biting. Additionally, data

indicate that other factors probably played a larger role in the evolution of the vertical

bars than did male-male competition. However, once males gained the ability to vary the

intensity of the bars, variable bar expression became an important component of

communication in swordtail aggressive interactions. Finally, the relationship between

absolute size and aggression varied within species, suggesting that the costs and benefits

resulting from competition favor higher levels of aggression in larger males in some

species and lower levels of aggression in others. However, there was a positive

relationship between size and aggression across species. While many other factors affect

male size in swordtails, these results suggest that male – male competition is important in

the evolution of male size.

Approved: Molly R. Morris
              Associate Professor of Biology

Dedication

In memory of Dooley.

Friend and family.

5 April 1999 – 9 October 2003

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Table of Contents

List of Tables

List of Figures

GENERAL INTRODUCTION

Ecologists and evolutionary biologists have long recognized the role of male –

male competition in shaping population dynamics. The outcome of agonistic encounters

affects how resources and territories are distributed among individuals, and in many

instances determines which members in the population will breed. There are many

factors that determine when to act aggressively and when to retreat. There are equally as

many factors in determining which of two individuals engaged in a contest will

ultimately become dominant, though the outcome of many interactions is probably

determined by the potential costs that the combatants are able to inflict upon one another

(Enquist and Jakobbson 1986). However, in most species the occurrence of life

threatening injury is small (Enquist and Leimar 1990), suggesting selection favors

behaviors that function to maximize the fitness of the winner while at the same time

minimizing the deleterious affects to the loser (Huntingford and Turner 1987). The focus

of the studies contained within this dissertation is to better understand the factors that

play a role in conflict resolution.

The northern swordtails are an ideal group in which to examine aspects of male-

male competition, the relationship between size and aggression and the role of male –

male interactions in the evolution of the male trait vertical bars as a signal of aggressive

intent. First, most of the relationships in this monophyletic group are supported by data

from several different sources (Rauchenberger et al. 1990, Borowsky et al. 1995, Morris

et al. 2001). Thus, there is a well supported phylogeny (Figure 1) which is essential

when employing comparative analyses. Second, there are a wide range of adult sizes

both within and between species (Figure I.2 – I.4) due to variation at a Y-linked locus, with some species having distinct size classes (Kallman 1989). Sufficient variation in adult sizes, both within and across species, is needed in order to examine the relationships between size and aggression. Third, male swordtail fishes do not exhibit growth after reaching sexual maturity (Kallman 1989), and as a result, age and factors correlated with age (e.g. experience), can be ruled out as confounding the influence of body size. Fourth, there is substantial variation in vertical bars across the species (Figure I.1), as some species have bars, some do not, and some are polymorphic for the bars (Figure I.2 – I.4). In addition, for polymorphic species the relationship between bars and size is variable: in *X. multilineatus*, only the smaller males do not have bars while in *X. cortezi* there is no relationship between size and presence of the bars. And finally, it is possible to measure levels of aggression in these fishes using mirror image stimulation (MIS) tests, measure relative RHP in a series of contests in the laboratory, as well as to manipulate the vertical bar pigment pattern.

Chapter 1 (Moretz 2003) is an in-depth analysis of contest dynamics in one species of swordtail fish, *X. cortezi*. This study utilized a novel contest pairing procedure which paired individuals based on their cumulative contest records. The method allowed me to determine the relationship between size and fighting ability or resource holding power (RHP; Parker 1974), as well examine contest initiation and escalation decision rules. Male size (standard length) proved to be a moderate predictor of an individual's final rank (RHP) but the relationship was not as strong as expected. Individuals changed their contest initiation strategy based on their size relative to that of their opponents, and

contrary to predictions, the smaller of the two males in each contest was more likely to initiate the conflict than was the larger male. The larger of the two males was more likely to win a given contest, however when small males did win, fights lasted longer, suggesting that in some cases smaller males may be able to outlast their opponents.

Chapters 2 (Moretz and Morris 2003) and 3 examine the role of male-male communication in the evolution of the male trait vertical bars. I was interested in determining whether males exhibited differential aggressive responses to the presence or absence of the bars, whether males had the ability to vary the expression of the bars and the timing of intensification in relation to escalation (first bite). I found only moderate congruence between having the trait vertical bars and having a response and found that the trait precedes the evolution of male responses. As a result, I suggest that male-male competition probably did not play a substantial role in the initial evolution of the bars themselves. Likewise, the ability of males to vary the expression of the vertical bars evolved before the response to this signal, suggesting that variable expression was under selection from factors other than male-male competition (e.g. predation). Once present however, variable expression became an important component of communication in swordtail aggressive interactions. In those barred species where intensification occurred after escalation, the bars may provide individuals with information about their opponent's motivational state and willingness to continue fighting. Further modification of the expression of the bars resulted in a shift in the timing of the bar intensification and as a result, a signal that is predictive of biting in those species that express the bars before escalating. In this context, the signal of aggressive intent is completely congruent with

the response: all species with differential aggressive responses to the presence or absence of bars express their bars before biting.

Chapter 4 examines the relative fighting abilities of the two *X. cortezi* bar morphs and whether aggression levels are related to fighting ability. The impetus for this study came from chapters 1 and 2. Examination of data from chapter 1 revealed that barless males were ranked higher than would be expected if size alone determined RHP. In chapter 2, barless males had higher aggression levels than did barred males. By experimentally presenting each male with his opponent in both a barred and barless state, I was able to determine that naturally barless males had a fighting advantage over same sized barred males that was independent of any bar response effects. The greater competitive ability of naturally barless males appears to be related to their higher levels of aggression as contest winners had higher bite frequencies than contest losers. These results suggest that relative levels of aggression are an important factor in contests between opponents closely matched for size.

The final chapter deals with the relationship between size and aggression both within and across the northern swordtails. While many studies have examined the relationship between aggression within species, none have used multiple species as a comparative means to better understand factors that are correlated with aggression within a phylogenetic context. While there are many factors influencing the evolution of male size in swordtails, there was a positive relationship between size and aggression across species, indicating that male-male competition has played an important role in the evolution of male size. However, the relationship between absolute male size and

aggression varied within species. This may indicate that the species are subject to different selection pressures resulting from competition, favoring higher levels of aggression in smaller males in some conditions and higher levels of aggression in larger males in others. Finally, I examined whether selection for large male size has been relaxed in *X. multilineatus* and *X. cortezi* barred males, which respond to the signal vertical bars with reduced aggression. I compared the predicted mean male size of each species resulting from aggression scores in their natural bar state to the predicted mean male size resulting from aggression scores after the vertical bars had been experimentally removed. *Xiphophorus multilineatus* were smaller than expected if size alone predicted aggression levels, possibly because the bars effectively lower the intensity of competition and may reduce the number of fights. However, *X. cortezi* barred males were no smaller than expected, possibly because they compete with the more aggressive barless *X. cortezi* males which do not exhibit a decreased aggressive response to the vertical bars.

Each of these chapters by themselves examines important components of male-male competition. They demonstrate the complexity of but a small part of natural systems in general, and sexual selection specifically. Collectively, these chapters add to a growing body of empirical data that can be used to test current theory and generate new hypotheses, applicable not only to swordtails but to other systems as well.

LITERATURE CITED

Borowsky, R. L., M. McClelland., R. Cheng, and J. Welsh. 1995. Arbitrarily primed DNA fingerprinting for phylogenetic reconstruction in vertebrates: The *Xiphophorus* model. Molec. Biol. Evol. **12**: 1022-1032.

Enquist, M. and S. Jakobbson. 1986. Decision making and assessment in the fighting behaviour of *Nannacara anomala* (Cichlidae, Pisces). Ethology **72**: 143-153.

Enquist, M. and O. Leimar. 1990. The evolution of fatal fighting. Anim. Behav. **39**: 1-9.

Endler, J.A. 1992. Signals, signal conditions and the direction of evolution. *Am. Nat.* **139**, S125-S153.

Huntingford, F.A. and A.K. Turner. 1987. Animal Conflict. Chapman and Hall.

Kallman, K. D. 1989. Genetic control of size at maturity in *Xiphophorus*. In: Ecology and Evolution of Livebearing Fishes (Poeciliidae). (Ed. by G. K Meffe and F.F. Snelson), pp. 163-184. Englewood Cliffs, N.J: Prentice-Hall.

Moretz, J.A. 2003. Aggression and RHP in the northern swordtail fish, *Xiphophorus cortezi*: the relationship between size and contest dynamics in male-male competition. Ethology **109**: 995-1008.

Moretz, J.A. and M.R. Morris. 2003. Evolutionarily labile responses to a signal of aggressive intent. Proc. R. Soc. Lond. B **270**: 2271-2277.

Morris, M.R., de Queiroz, K. and Morizot, D. 2001. Phylogenetic relationships among the Northern Swordtails (*Xiphophorus*) as inferred from allozyme data. Copiea **2001**: 65-81.

Parker, G.A. 1974. Assessment strategy and the evolution of fighting behaviour. J. Theor. Biol. **47**: 223-243.

Rauchenberger, M., K.D. Kallman. and D.C. Morizot. 1990: Monophyly and geography of the Rio Panuco Basin swordtails (genus Xiphophorus) with description of four new species. Am. Mus. Novit. **2975**: 1-41.

Figure I.1 The male trait (phenotype) vertical bars mapped onto the Rauchenberger et al. (1990) tree using parsimony and ordered character state (Moretz and Morris 2003). White branches, no bars; black branches, bars; shaded branches, polymorphic; hatched branches, equivocal.
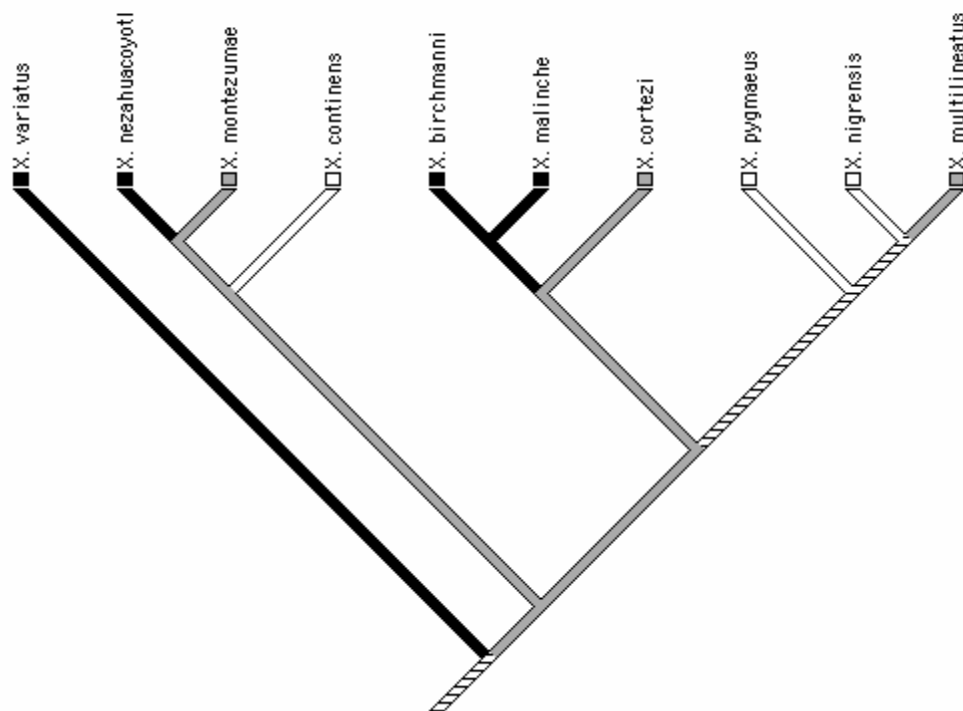
Figure I.2 Swordtail males of the *montezumae* clade (Rauchenberger et al. 1990). A. *X. montezumae*. B. *X. nezahualcoyotl*. C. *X. continens*. Photos courtesy of K. De Queiroz.

A.

B.

C.

Figure I.3 Swordtail males of the *cortezi* clade (Rauchenberger et al. 1990). A. *X. malinche*. B. *X. birchmanni*. C. *X. cortezi*. Photos courtesy of K. De Queiroz.

Figure I.4 Swordtail males of the *pygmaeus* clade (Rauchenberger et al. 1990). A. *X. nigrensis*. B. *X. multilineatus*. C. *X. pygmaeus*. Photos courtesy of K. De Queiroz.

A.

B.

C.

CHAPTER 1

AGGRESSION AND RHP IN THE NORTHERN SWORDTAIL FISH,

XIPHOPHORUS CORTEZI: THE RELATIONSHIP BETWEEN SIZE AND CONTEST

DYNAMICS IN MALE-MALE COMPETITION

ABSTRACT

Current theory predicts that contest outcome, as well as decisions on whether to initiate a

contest, escalate during a contest or retreat are decided by asymmetries in resource

holding potential (RHP) and or expected payoffs between contestants. In this

investigation, dyadic contests were staged between male swordtail fish (*Xiphophorus

cortezi*) where individuals were paired based on cumulative fight records and were

ranked at the end of the trials in order to approximate RHP. Size was the only asymmetry

that I did not attempt to control for and as a result, I was able to determine the

relationships between size, contest initiation, escalation and outcome. Individuals

changed their contest initiation strategy based on their size relative to that of their

opponents, and contrary to predictions, the smaller of the two males in each contest was

more likely to initiate the conflict than was the larger male. However, the larger of the

two males was more likely to win and standard length proved to be a moderate predictor

of an individual's final rank. Regardless of size, initiators faired poorly, winning only

31% of the contests. In instances where the smaller males won the contests, they were no

more likely to initiate the encounter than was the larger male. However when small

males did win, fights lasted longer, suggesting that in some cases smaller males may be able to outlast their opponents.

INTRODUCTION

Male – male aggression plays an important role in natural systems and is one of two key factors in Darwin's (1871) theory of sexual selection. The outcome of agonistic encounters shapes how resources and territories are distributed among individuals, and in many instances determines which members in the population will breed (e.g. Morris et al. 1992). Many encounters include both overt, physical confrontations as well as non-tactile cues through which information regarding variables such as size or aggressive level is signaled (Moyer 1976; Moynihan 1998). It is expected most confrontations will begin with low-level agonistic displays or even non-offensive behavior patterns, often failing to escalate beyond this point (Enquist and Jakobbson 1986). Opponents should base decisions on whether or not to initiate a contest, escalate during the contest or retreat based on information gained before and during the encounter (Hammerstein 1981; Maynard Smith 1982).

One of the foundations of aggression theory is the concept of resource holding power or RHP (Parker 1974). RHP is a measure of an individual's fighting ability, which is itself a combination of characteristics such as body size and weaponry. In animal contests, RHP is also manifested through fighting effort, which can be affected by experience, motivation, prior residence, or other extrinsic factors. Thus, an individual's performance is a combination of its ability to fight as well as the effort that is exerted during the contest. Theory predicts that contest outcome is decided by asymmetries between contestants in RHP (all else being equal) and can be used to predict likely winners (Parker 1974; Maynard Smith and Parker 1976; Hammerstein 1981; Archer

1988). If the asymmetry is large, then contests should be settled quickly, as the relative strength of each opponent should be clear. However, as the difference in RHP between two individuals decreases, the intensity and duration of the contest should increase (Archer 1988; Carpenter 1995) because contestants are less able to accurately assess their strength versus that of their opponents and because their fighting abilities are more evenly matched.

Empirical studies have reported the role of RHP and payoffs in contest outcome, but rarely do studies examine how RHP influences the initiation of contests and if initiation itself affects the outcome of contests. Where experiments have dealt with initiation behaviors, a variety of variables have been identified that explain, in part, general patterns of initiation. Examples of these factors include body size (Robitaille and Bovet 1976; Elwood and Glass 1981; Dowds and Elwood 1983; Mayar and Berger 1992; Figler et al. 1995; Morris et al. 1995), expected payoffs (Dugatkin and Ohlsen 1990; Bautista et al. 1998), motivational state (Cremer and Greenfield 1998), social environment (Bakri and Volpato 1998; Robbins 1999), sex and reproductive status (Guiasu and Dunham 1997 and 1998; Halley and Gjershaug 1998) and residency status (Leimar and Enquist 1984; Figler et al. 1995).

The purpose of this study was three-fold. First, I investigated contest dynamics in order to determine if non-random patterns existed for whether losers or winners initiated contests, escalated during contests or bit more than their opponents. This information is essential in determining the basis for decision rules. Second, I used a novel pairing and ranking procedure to quantitatively approximate RHP. While past studies have focused

on contest outcome and dominance, as well as factors that contribute to an individual's

RHP (e.g. Sneddon et al. 2000), none have attempted to quantitatively measure an

individual's RHP. By using the pairing method presented here, much of the ambiguity

associated with randomly pairing individuals for a specified number of contests is

removed (e.g. there are no individuals with identical win / loss records) and the

performance (RHP) of individuals relative to the performance (RHP) of others is more

clear. Finally, I determined the relationship between RHP and size (standard length, SL).

While it is clear that there are many factors that affect contest outcome, body size is

usually a good predictor of fighting ability (Archer 1988) and can easily be measured. In

addition, there is a large body of data available for comparison as size has historically

been used to predict dominance and contest outcome (Jackson 1988).

METHODS

Study species

Xiphophorus cortezi are small live bearing fish that inhabit pools of streams and

rivers in northern Mexico. Like most other swordtail species observed to date (Franck

and Ribowski 1987; Morris et al. 1992; Morris et al. 1995), *X. cortezi* males are territorial

and will react aggressively towards intruding males both in the field and in staged

contests (Morris pers. com.). Another factor that makes this species suitable for

aggression studies is that growth is determinant and therefore body size cannot be used

by opponents to assess age or experience level. The *X. cortezi* males used in this study

were collected from the Rio Axtla in San Luis Potosi, Mexico and brought back to the

laboratory at Ohio University where they were individually housed in 20L tanks and visually isolated from one another. Fish were fed daily and maintained on a 14h light/ 10h dark photoperiod at a constant room temperature of 22C. Sixteen males were used in the tournament format. An additional 40 males (20 contests) served as an alternate data set. Two weeks prior to the beginning of the contests, the males were anesthetized in order to accurately weigh (WT) and measure each individual. The measurements included standard length (SL = the distance from the tip of the snout to the beginning of the tail fin), total length (TL), total length and sword (TLS) and body depth (DV).

Tournament design and data collection

The contest format was developed in order to assign ranks to individual males quantitatively based on their performance and then use these rankings as an approximation of RHP (relative to other males in the tournament). It functions much like a sport ladder. In the first round of the tournament, sixteen males were randomly assigned to one of eight pairs. In subsequent rounds, fish were randomly paired with other males having the same contest record. For example, at the beginning of round three there were four males that had won both of their previous encounters, eight males that had one win and one loss and four males that were winless. Within each of these three win / loss categories, males were randomly paired with other males belonging to the same category. This pairing procedure was used for each round with the only stipulation being that two males could not be paired together more than once. The experiment consisted of nine rounds, for a maximum of 52 contests. Each contest round was separated by 72

hours, which was long enough to control for possible winner and loser effects (Moretz unpub. data). Individuals were assigned a rank when there were no other males with identical win / loss records and then removed from the tournament. For example, at the end of round four there was only one male with a perfect record (no losses) and he was assigned the highest ranking and removed from the tournament. The procedure of ranking and removing fish continued until round nine, when there were only two males remaining. Within the nine rounds of the tournament there were six win / loss categories that contained an odd number of males and as a result, a male was randomly removed from each of these categories. Thus a sixteen fish tournament results in the ranking of ten individuals. In instances where there are only two males in a particular win / loss category and they have already met, the previous winner receives the higher rank.

There are several benefits gained by using this contest method as compared to either randomly pairing large numbers of males that are only used once or forming random pairs using the same males repeatedly for a specified number of contests. First, this procedure does not rely on having to pair every individual with every other individual in the tournament. This aspect is critical because in these types of tournaments it is possible to complete the study and have individuals with identical contest records, which results in ambiguity in determining the most dominant individuals. Second, at the completion of the tournament there is an unbiased ranking of individuals. These ranks can then be used to investigate the relationship between RHP (as approximated by rank) and the variable of interest (standard length in this study). However, the most important advantage of this method is that it not only identifies the most superior males but more

clearly defines individuals that are intermediate to those that are most highly and lowly ranked. The most dominant individuals and those with the worst performance are the first males to be ranked and removed from the tournament. Those males who are intermediate keep fighting until their contest abilities relative to other males becomes more clear, thus removing the ambiguity one would expect to find when encountering more closely matched individuals. In addition, it is also possible to determine whether behavioral patterns are context dependent or instead, specific to particular males.

Each pair was placed in a 150 L aquarium and separated by an opaque plexi-glass divider for 24 hours. The contest began by removing the divider and allowing the two males to interact. Data were recorded by direct observation and included which male initiated the contest (first display), which male escalated (bit first) and which male won, as well as the elapsed time for each of these events. The total number of bites for each male was also recorded. A male was determined to be the loser when he retreated from his opponent with his dorsal fins lowered and continued to retreat whenever approached. There were no instances of a male signaling defeat and then attacking. Males were visually inspected after each contest and none appeared injured. Likewise, there was no indication that males were overly stressed, as they appeared to behave normally (e.g. they continued feeding) after returning them to their individual tanks.

Twenty additional contests (40 males from the same population) served as an alternate data set and were used to compare patterns of initiation, escalation and outcome to the results obtained from the tournament. Each male was used once and was randomly paired with an opponent. The method of data collection was the same as above.

Statistical analyses

All analyses were performed using the statistical package NCSS (Hintze 2001). Linear regression analysis was used to determine the relationship between size (SL) and rank (RHP). While SL is commonly used as a basis to pair individuals, other morphological components of size have been demonstrated to correlate with dyadic dominance (Beaugrand and Zayan 1985). As such, a regression using stepwise selection was performed to determine the best predictor of rank. To avoid pseudoreplication, I used the trials as the experimental unit in the remaining analyses (see below). Contest durations were log transformed to compare the length of contests of smaller winners versus larger winners. Linear regressions were used to determine the relationship between the size difference of opponents and the total number of bites in a contest, the duration of the contests and the time from initiation to escalation (attack latency). In determining the best predictors of contest outcome, contest dynamics and morphological measurements were analyzed using stepwise logistic regressions (Carpenter 1995; Zucker and Murray 1996).

Some readers may question whether the analyses presented in this manuscript constitute pseudoreplication. Though not a statistician, I do believe the statistical tests presented in this paper were correctly applied and will now give justification for this assertion, leaving each reader to reach his or her own conclusion. First, I agree that any analyses that treated individuals used in multiple trials as the experimental unit would constitute pseudoreplication (notwithstanding appropriate corrections). However, each contest or trial was the statistical unit in both the logistic regressions (predictors of

contest outcome) and in the linear regressions (measures of contest intensity). By treating each contest as the experimental unit, I avoided counting complementary events twice, a practice that would have artificially inflated sample size and resulted in pseudoreplication. For example, there is necessarily a winner and a loser, as well as a larger and smaller male, in each contest. If I were interested in determining whether larger males were more likely to win the trials than smaller males, I might count the number of times each individual won or lost, whether he was the larger or smaller of the two males in each instance and perform some statistical analysis. However, I would be incorrect in doing so because for a particular contest a large male winning is the same as a small male losing, thus my sample size would be artificially inflated by 100%. Each of my analyses avoided this problem. Second, pseudoreplication would also result from contestants being paired together more than once. That was not the case in this study as each trial consisted of a unique pairing. Third, pseudoreplication could also occur if contest dynamics were not context specific but rather an inherent property of the individual. For example, if a few of the smallest individuals always initiated the interactions, those males could unduly influence the correlation between relative size and initiation. That was not the case in this study, as data presented in the results demonstrated that a majority of the males examined switched their initiation strategy based on their size relative to that of their opponent. Finally, the results from the alternate data set where males were used only once matched the results from tournament. While not an argument against pseudoreplication per se, it is an indication that the analysis from my design did not result in spurious results or interpretations.

RESULTS

Contest dynamics

Bouts typically began when one male swam up to his opponent and displayed either vertically in a headstand orientation or laterally. Opponents usually responded by displaying and this continued until one male escalated by biting. Both males continued to display and bite throughout the encounter until one male signaled defeat by lowering his dorsal fin and swimming away.

Of the 51 contests, 47 escalated to biting (in three of which, both individuals recorded the same number of bites). There was one instance where the remaining two individuals in a win / loss category had already met and they were excluded; thus this tournament consisted of 51 contests instead of 52. Fifteen of the 16 males won at least one contest. Fourteen of the 16 males won contests in which they were the larger male while seven males were able to win bouts in which they were the smaller individual. Standard length difference between opponents ranged from 0.39mm to 9.15mm ($\bar{x}$=4.02mm, SD=2.02; range=1-24% of SL). The largest difference that a smaller male overcame to win a contest was 8.72mm (22% of SL). There was no significant difference in mean SL difference between two opponents when smaller males won ($\bar{x}$=3.56mm, SD=1.95) versus when larger males won ($\bar{x}$=4.27mm, SD=2.05, t_{49}= 1.19, p=0.24). The contests ranged in duration from 0.47 minutes to 61.1 minutes ($\bar{x}$=8.01, SD=11.4). The mean duration for contests in which the smaller male won was 13.9 minutes (SD=14.2) versus 8.7 minutes (SD= 15.1) for fights won by the larger male (t_{49}= 3.29, p= 0.001).

There was not a significant relationship between the time from initiation to escalation (attack latency) and the difference in size between two individuals (r^2=0.002, $F_{1,45}$=0.09, p=0.76). Likewise, there was not a significant relationship between the size difference between two opponents and either measure of contest intensity (Figure 1.1); neither the total number of bites in a contest (r^2=0.02, $F_{1,50}$=1.22, p=0.27) nor the duration of the contests (r^2=0.03, $F_{1,50}$=1.32, p=0.26) changed significantly as the size discrepancies between two opponents increased.

The smaller of the two males in each pair was more likely to initiate the contest than was the larger male, doing so in 35 of the 51 contests (Table 1.1). The larger males won 64.7 percent (33 of 51 contests) of the tournament bouts. The smaller of the two males initiated the contest in nine of the 18 encounters that they won. Of the 47 contests where fights escalated to biting, the larger individuals bit first in 20 and the smaller individuals bit first in the remaining 27. Contest losers were more likely to initiate than were winners (35 of 51 contests) but contest winners and losers escalated at almost the same frequency (24 and 23 contests respectively). Likewise, winners bit more in 24 contests and losers bit more in 19 contests.

In order to determine whether males changed their initiation strategy based on their size relative to that of their opponent's and that the observed pattern was not the result of just a few males that always initiated, two contests from the tournament were randomly selected for each male; one in which the male was the smaller of the pair and one in which the male was larger. There were two males that were always larger than their opponents and two males that were always smaller and as such, no conclusions for

relative size and initiation can be drawn. Of the 12 males examined, 10 initiated the contest when they were smaller and failed to initiate when they were the larger of the two males (χ^2=5.33, df=1, p=0.021).

In determining the best predictors of contest outcome, two groups of variables were analyzed; morphological variables (SL, TL, TLS, DV and WT) and behavioral dynamics (initiation, escalation and relative bite frequency). The best relative measurement predictor was TLS, correctly classifying individuals in 66.7% of the trials (χ^2=5.67, df=1, p=0.016). Standard length (SL) correctly classified individuals in 64.7% of the contests (χ^2=4.47, df=1, p=0.035). The only significant behavioral predictor of contest outcome was initiation, correctly classifying losers in 68.7% of the trials (χ^2=7.34, df=1, p=0.007).

Examination of the alternate data set indicated the same patterns of contest dynamics as found in the tournament. The smaller of the two males (χ^2=8.20, df=1, p=0.004) and contest losers (χ^2=5.30, df=1, p=0.021) initiated the contests more than larger males and winners. The larger of the two males was more likely to win the trial (χ^2=10.68 df=1, p=0.001), and there was no difference between contest winners and losers in terms of escalation (χ^2=0.01, df=1, p=0.950) or relative number of bites (χ^2=2.87, df=1, p=0.092). Likewise, neither the smaller nor the larger of the two males was more likely to escalate (χ^2=1.66, df=1, p=0.197) or bite more during the contests (χ^2=1.71, df=1, p=0.191).

RHP and size

There was a significant, positive relationship between SL and RHP (r^2= 0.45, $F_{1,9}$= 6.4, p= 0.035, Figure 1.2). However, the relationship was not as strong as expected if only the influence of size was considered, as there were four individuals that obtained a higher ranking than would be predicted by size alone. This resulted in ten instances where smaller individuals were ranked higher than larger individuals because in some cases the smaller males were ranked higher than multiple larger males (Figure 1.2). Of the five measurements taken prior to the start of the tournament (standard length, total length, total length plus sword, body depth and weight), the best predictor of rank was the DV (body depth) measurement (r^2= 0.65, $F_{1,9}$=15.1, p=0.005). The correlation between SL and DV was 0.94 (p<0.001).

DISCUSSION

Several authors (e.g. Jackson 1990; Bradbury and Vehrencamp 1998) have predicted that the likely winners of contests should initiate the encounter and there is some empirical evidence to support this hypothesis (e.g. Jackson 1988; Jackson 1990; Bekoff and Scott 1989). Why then do the eventual losers (smaller males) more often begin the confrontation in this study (increased aggression in losers or subordinate fish have also been reported for other swordtail species; e.g., Ribowski and Franck 1993; Morris et al. 1995)? While we expect that each individual should try to convey to its opponent that it has superior fighting skills, we should also expect that an individual should refrain from signaling any weakness or their ultimate intentions to retreat

(Maynard Smith and Parker 1976; Krebs and Dawkins 1984). The smaller of the two males (the ultimate losers) might be predicted to escalate if payoff asymmetries were in their favor (Parker 1974; Dugatkin and Ohlsen 1990) or if they incorrectly perceived themselves as the likely winners. In addition, if initiation itself conferred some type of advantage then we might expect those less likely to win to compensate by initiating. However, in this study initiation is correlated with losing and even in those bouts where the smaller of the two males did win they were no more likely to initiate the encounter than were the larger males.

The smaller of the two males might also be expected to initiate some of the contests if RHP assessment is largely inaccurate (Bradbury and Vehrencamp 1998). However, this is unlikely here. If this were the case we would expect to observe a random distribution of initiation events instead of initiation behaviors being largely confined to the ultimate losers in the contests. In fact, there was a tendency of individuals to change their pattern of initiation behaviors that was correlated with their size relative to that of their opponent, indicating that individuals are probably able to assess relative size. Instead, a more likely explanation is that the probability of serious injury during these contests is low and that the cost of initiating the encounter is negligible compared to the possible payoffs (dominance and ownership of the territory). From the viewpoint of the larger males, smaller males may not be viewed as a viable threat, at least not until they behave aggressively or until the value of the resource increases, such as when a female enters the territory. Candolin and Reynolds (2002), for example, reported that dominant European bitterling (*Rhodeus sericeus*) males exhibited

low levels of aggression at the initial stages of territory establishment and only became aggressive towards sneaker males as females began laying eggs. Thus it is possible that because the aquaria used in this study contained no females, larger males were more tolerant of smaller males than they would have been had females been present.

It is also possible that the tolerance of smaller males by larger males is an adaptive response to costs associated with aggression (Candolin and Reynolds 2002). For example, in the field where females are present, aggression against small males may be more costly for the larger males in terms of missed reproductive opportunities while behaving aggressively. Stated another way, territories (even those of low quality) may be inherently more valuable to smaller males, as they are probably more likely to experience greater difficulty obtaining and holding territories than are larger males. Thus they may be more willing to act aggressively as there is a higher benefit to cost ratio than is experienced by the larger males. There are increased risks for dominant males because in essence they have more to lose than subordinate males.

If we expect weaker individuals to try and conceal any actions that might signal any future intentions (submissiveness), then we might also expect weaker individuals to cheat whenever possible by signaling their strength through increased aggression. In this study there was no variation in the types of displays or bites between winners and losers. Thus there are no behaviors that are necessarily correlated with being dominant or large or that would otherwise preclude smaller individuals from performing them. This is in contrast to some other studies that have been able to identify qualitative differences in the behaviors performed by dominant and subordinate individuals. For example, Ribowski

and Franck (1993) found that winners of contests were more likely to perform a greater number of fin grips than were the ultimate losers (though the results were significant, this behavior was only a moderate predictor of contest outcome).

As expected, the larger of the two males in each contest was more likely to win the encounter than was the smaller male and as a result there was a positive relationship between SL and RHP rank. Nevertheless, the smaller males were more successful than I previously hypothesized and were able to obtain RHP rankings that were higher than would be expected if size was the only factor correlated with RHP. This could be the result of inherent asymmetries in motivation or expected payoffs, as well as physiological differences. However it is interesting that when the smaller of the two males did prevail, the contests lasted longer. This may in part explain why there was not a significant relationship between the size difference of pairs and either measure of contest intensity. In addition, the longer contest durations suggest that the smaller males may be able to outlast their larger counterparts. There may be hidden costs to being large; possibly, reduced maneuverability and increased energy expenditure in trying to maneuver. Based on my observations, smaller males may be able to better position themselves to attempt a bite than larger males. I frequently observed large males having to reposition themselves either after a failed bite attempt or after evasive moments by smaller males. Likewise, smaller males seemed more adept at evading than did larger males. Thus, it would seem that per contest, larger males may be positioning and evading more than smaller males and that during extended bouts when there would be a cumulative increase in energy expenditure, smaller males benefit by being more maneuverable, allowing them to

overcome the difference in size (see Halley and Gjershaug, 1998 for a discussion on size, maneuverability and dominance in birds).

It is worth noting that the contest dynamics here are likely not the by-product of the experimental design. When the smaller of the two males won contests, it was not the result of a smaller than average size difference between opponents (there was no statistical difference in the size difference between two opponents when larger males won versus when smaller males won). Also, initiation by eventual contest losers is probably not attributable to or the result of not having ample space to flee. At the end of a contest, losers usually swim to the opposite side of the aquarium and are not actively pursued or harassed by the victor. This option would also then seem to be present at the beginning of a confrontation, as long as one individual acted submissively (fleeing and lowered dorsal fin). Thus there are alternatives to fighting for the smaller of the two males and initiation of contests is not likely the result of a "desperado effect" (Grafen 1987).

This study illustrates the need for a method (such as the one presented here) that provides a quantitative means to evaluate RHP. In both the tournament format and the alternate data set (single, random pairings), it was the smaller of the two males that was more likely to initiate the contest and the larger male that was more likely to win. However, had I only used the data from the method of random pairings, I would have only been able to compare the number of contests where larger individuals won to the number of contests that smaller individuals won, and conclude that larger individuals enjoy a competitive advantage. The tournament format actually allows the investigator to determine the strength of the relationship between size and performance (SL vs. RHP

regression). Likewise, there would have been no way to adequately determine which fish were most dominant, as there would have been large numbers of individuals with similar win / loss records. The tournament format, on the other hand, results in an unambiguous rank for each male and indicates his fighting ability to that of every other male in the experiment. In many systems, the attributes that are important in being competitively superior are readily identifiable: all else equal, size is usually a large determinant. However, by ranking individuals we may gain more insight as we are better able to quantify differences between opponents and quantify the effect that a particular variable of interest has on the interaction between individuals.

LITERATURE CITED

Archer, J. 1988. The Behavioural Biology of Aggression. Cambridge University Press, Cambridge.

Barki, A. and Volpato, G.L. 1998. Early social environment and the fighting behaviour of young *Oreochromis niloticus*. Behavior **135**: 913-929.

Bautista, L.M., Alonso, J.C. and Alonso, J.A. 1998. Foraging site displacement in common crane flocks. Anim. Behav. **56**: 1237-1243.

Beaugrand, J.P. and Zayan, R. 1985. An experimental model of aggressive dominance in Xiphophorus helleri. Behav. Processes **10**: 1-52.

Bekoff, M. and Scott, A.C. 1989. Aggression, dominance and social organization in evening grosbeaks. Ethology **83**: 177-194.

Brabury, J.W. and Vehrencamp, S.L. 1998. Principles of animal communication. Sinauer Associates, Massachusetts.

Candolin, U. and Reynolds, J.D. 2002. Why do males tolerate sneakers? Tests with the European bitterling, *Rhodeus sericeus*. Behav. Ecol. Sociobiol. **51**: 146-152.

Carpenter, G.C. 1995. Modeling dominance: The influence of size, coloration and experience on dominance relations in tree lizards. Herp. Monogr. **9**: 88-101.

Cremer, S. and Greenfield, M.D. 1998. Partitioning the components of sexual selection: attractiveness and agonistic behaviour in male wax moths, *Achroia grisella*. Ethology **104**: 1-9.

Darwin, C. 1871. The Descent of Man and Selection in Relation to Sex. John Murray, London.

Dowds, B.M. and Elwood, R.W. 1983. Shell wars: assessment and the timing of decisions in the hermit crab shell fights. Behaviour **85**: 1-24.

Dugatkin, L.A. and Ohlsen, S.R. 1990. Contrasting asymmetries in value expectation and resource holding power: effects on attack behaviour and dominance in the pumpkinseed fish, *Lepomis gibbous*. Anim. Behav. **39**: 802-804.

Elwood, R.W. and Glass, C.W. 1981. Negotiations or aggression during shell fights of the hermit crab, *Pagurus bernhardus*. Anim. Behav. **29**: 1239-1244.

Enquist, M. and Jakobbson, S. 1986. Decision making and assessment in the fighting behaviour of *Nannacara anomala* (Cichlidae, Pisces). Ethology **72**: 143-153.

Figler, M.H., Twum, M., Finkelstein, J.E. and Peeke, H. 1995. Maternal aggression in the red swamp crayfish, *Procambarus clarkii*: the relation between reproductive status and outcome of aggressive encounters with male and female conspecifics. Behaviour **132**: 107-125.

Franck, D. and Ribowski, A. 1987. Influence of prior agonistic experience on aggression measures in the male swordtail (*Xiphophorus helleri*). Behaviour **103**: 217-240.

Grafen, A. 1987. The logic of divisively asymmetric contests: respect for ownership and the desperado effect. Anim. Behav. **35**: 462-467.

Guiasu, R.C. and Dunham, D.W. 1997. Agonistic interactions in male form II *Cambarus robustus* crayfish and a comparison between male for I and form II intra-form contests. Crustaceana **70**: 721-736.

Guiasu, R.C. and Dunham, D.W. 1998. Inter-form contests in male crayfishes, *Cambarus robustus*. Invert. Biol. **117**: 144-154.

Halley, D.J. and Gjershaug, J.O. 1998. Inter- and intra-specific dominance relationships and feeding behaviour of Golden eagles *Aquila chrysaetos* and Sea Eagles *Haliaeetus albicilla* at carcass. IBIS **140**: 295 – 301.

Hammerstein, P. 1981. The role of asymmetries in animal contests. Anim. Behav. **19**: 193-205.

Hintze, J. 2001. Number Cruncher Statistical Software (NCSS). Kaysville, UT.

Jackson, W.M. 1988. Can individual differences in history of dominance explain the development of linear dominance hierarchies? Ethology **79**: 71-77.

Jackson, W.M. 1991. Why do winners keep winning? Behav. Ecol. Sociobiol. **28**: 271-276.

Krebs, J.R. and Dawkins, R. 1984. Animal signals: mind-reading and manipulation. In: Behavioural Ecology, An Evolutionary Approach (Krebs, J.R. and Davies N.B., eds). Sinauer Associates, Massachusetts, pp 380-402.

Leimar, O. and Enquist, M. 1984. Effects of asymmetries in owner-intruder conflicts. J. Theor. Biol. **111**: 475-491.

Maynard Smith, J. and Parker, G.A. 1976. The logic of asymmetric contests. Anim. Behav. **24**: 159-175.

Maynard Smith, J. 1982. Evolution and Theory of Games. Cambridge University Press, Cambridge.

Mayr, M. and Berger, A. 1992. Territoriality and microhabitat selection in two intertidal New Zealand fish. J. Fish. Biol. 40: 243-256.

Morris, M.R., Batra, P. and Ryan, M.J. 1992. Male-male competition and access to females in the swordtail *Xiphophorus nigrensis*. Copeia **1992**: 980-986.

Morris, M.R., Gass, L. and Ryan, M.J. 1995. Assessment and individual recognition of opponents in swordtails *Xiphophorus nigrensis* and *X. multilineatus*. Behav. Ecol. Sociobiol. **37**: 303-310.

Moyer, K.E. 1976. The psychobiology of aggression. Harper and Row, New York.

Moynihan, M. 1998. The social regulation of competition and aggression in animals. Smithsonian Institution Press, Washington DC.

Parker, G.A. 1974. Assessment strategy and the evolution of fighting behaviour. J. Theor. Biol. **47**: 223-243.

Ribowski, A. and Franck, D. 1993. Demonstration of strength and concealment of weakness in escalating fights of male swordtails (*Xiphophorus helleri*). Ethology **93**: 265-271.

Robbins, M.M. 1999. Male mating patterns in wild multimale mountain gorilla groups. Anim. Behav. **57**: 1013-1020.

Robitaille, J.A. and Bovet, J. 1976. Field observations on the social behaviour of the Norway rat, *Rattus norvegicus*. Biol. Behav. **1**: 289-308.

Sneddon, L.U., Huntingford, A.C. and Orr, J.F. 2000. Weapon strength and competitive success in fights of shore crabs (*Carcinus maenas*). J. Zool. **250**: 397-403.

Zucker, N. and Murray, L. 1996. Determinants of dominance in the tree lizard *Urosaurus ornatus*: The relative importance of mass, previous experience and coloration. Ethology **102**: 812-825.

Table 1.1 Contest dynamics as a function of relative size and outcome. Analyses were performed using logistic regressions with df=1. Parentheses indicate the percent of individuals correctly classified by the model.

	Contest dynamics and relative size *(larger vs. smaller)*	Contest dynamics and outcome *(winner vs. loser)*
Initiate (1st display)	smaller males (68.6) χ^2=7.34, p=0.007	contest loser (68.6) χ^2=7.34, p=0.007
Escalate (1st bite)	no difference (59.6) χ^2=1.83, p=0.179	no difference (53.2) χ^2=0.02, p=0.884
Win	larger male (64.7) χ^2=4.47, p=0.035	NA
Relative # of bites	no difference (62.8) χ^2=2.90, p=0.089	no difference (55.8) χ^2=0.57, p=0.450

Figure 1.1 Measures of contest intensity as a function of the size difference between two opponents. There was not a significant relationship between size difference and either the total number of bites in a contest (Figure 1A; $r^2=0.02$, $F_{1,50}=1.22$, $p=0.27$) or contest duration (Figure 1B; $r^2=0.03$, $F_{1,50}=1.32$, $p=0.26$).
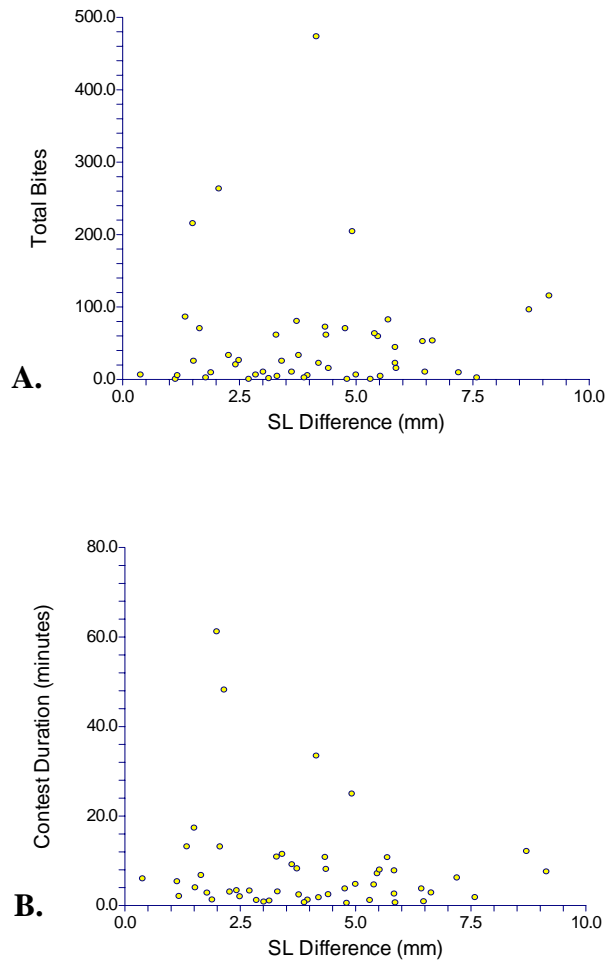
Figure 1.2. Relationship between standard length (mm) and RHP. There were four individuals with higher ranks than comparatively larger individuals. The regression line has the equation RHP= -17.61+0.54SL (r^2= 0.45, $F_{1,9}$= 6.4, p= 0.035).
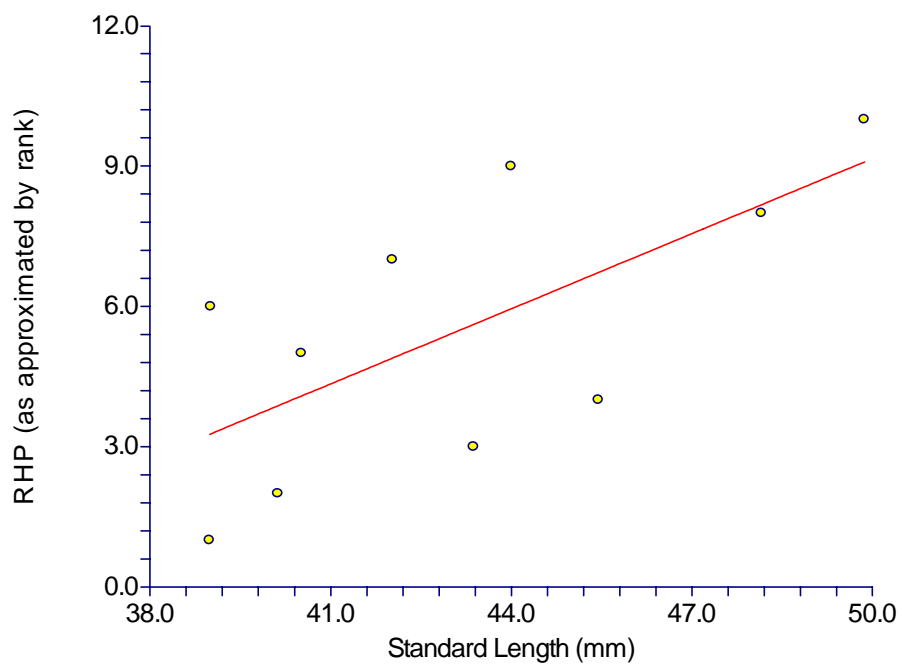
CHAPTER 2

EVOLUTIONARILY LABILE RESPONSES TO A SIGNAL OF AGGRESSIVE

INTENT

ABSTRACT

Males of many swordtail species posses vertical bar pigment patterns that are used both in courtship and agonistic interactions. Expression of the bars may function as a conventional threat signal during conflicts with rival males; bars intensify at the onset of aggression and fade in the subordinate male at contest's end. I used mirror image stimulation and bar manipulations to compare the aggressive responses of the males of four swordtail species to their barred and barless images. I found that having a response to the bars is tightly linked to having genes for bars, while the nature of the response the bars evoked varied across species. Specifically, I report the first known instance where closely related species exhibited differing and contradictory responses to a signal of aggressive motivation. Demonstrating that a signal conveys the same information across species (aggressive intent) while the response to that information has changed among species suggests that the nature of the responses are more evolutionarily labile than the signal.

INTRODUCTION

Historically, animal communication and signal design have been of great interest to biologists, and much research has been focused on the types of signals and the role of signals in aggression and conflict resolution (Parker 1974; Maynard Smith 1982; Enquist 1985). Research has demonstrated that a variety of factors can influence a signal's design such as its physical properties relative to that of its surroundings, its intended audience and other social factors (Endler 1992; Bradbury and Vehrencamp 1998). In particular, the response of the receiver should be an important selective force acting on signal design and use. In order for a signal to be perpetuated, there needs to be coordination between both the signal and receiver, and as such, coevolution between signal and receiver (Alexander 1962; Butlin and Ritchie 1989; Morris and Ryan 1996) as well as the behaviors that link them (Endler 1992). However, the coevolution of signal and receiver response has not always been detected (McKinnon and McPhail 1996; Ryan and Rand 1998; Quinn and Hews 2000), some of which is attributable to differences between sexes (Searcy and Brenowitz 1988; Morris and Ryan 1996). For example, Quinn and Hews (2000) reported behavioral responses to an abdominal patch by males of one species of *Sceloporus* lizard, even though this signal has been evolutionarily lost in this species. Comparative studies of signal and receiver coevolution can be used to reveal the degree to which the signal or the response to that signal can evolve independently, indicating which component is more likely to be under selection outside of the communication system.

I was interested in examining the evolutionary congruence between a sexually selected signal and male response to that signal. The signal of interest was vertical bars (Gordon 1931; Atz 1962), a pigment pattern found throughout swordtails (Figure 2.1) and platyfishes (*Xiphophorus*) and in other poeciliid fishes (e.g., *Heterandria, Phallichthys*). The inheritance of the barring pattern is polygenic for male *X. multilineatus* (Zimmerer and Kallman 1988), a species in which the bars function to deter rival males from territories and attract females (Morris et al. 1995). Males with bars have the ability to intensify their bars or suppress their expression. During aggressive encounters, the bars of *X. multilineatus* males intensify at the onset of the interaction and in conjunction with other cues, fade in the subordinate male at contest's end (Zimmerer and Kallman 1988). Thus, expression of the bars signals aggressive intent in this species and vertical bars are known to indicate aggression in other fish species as well (Hurd 1997a). For vertical bars to function as a conventional threat display (Dawkins 1993), the only cost of the signal would be the need to back up the signal with aggression. While this may be the case, the cost of the bars in relation to predation has not been examined. It is also possible that the bars aid in size assessment, as number of bars (*X. multilineatus*, Zimmerer and Kallman 1988) and total pigment area (*X. cortezi*, Morris et al. 2001a) are both correlated with male size in some species.

I compared the context in which males express their bars as well as the response the bars evoke in male-male interactions across four species of swordtail fishes. Specifically, I wanted to determine if 1) expression of the bars is a good indicator of male aggression across species, 2), whether or not the response to the bars is correlated with

having this pigment pattern and 3) if the aggressive responses, when present, are the same across species. Previous studies that used live males as stimuli in contests (Morris et al. 1995; Morris and Ryan 1996) determined that *X. multilineatus* males responded with decreased aggression towards males with bars as compared to males without bars, but a response to vertical bars was absent in the sister species that lacks bars (*X. nigrensis*). In the current study, I retest *X. multilineatus* and *X. nigrensis* males for response to the bars, but use mirror tests rather than live males. By retesting these two species I can determine if the mirror tests give the same results as tests using live males. I also examine the response to the bars in the smallest size class of *X. multilineatus* males that do not have bars, which has not been previously examined. Responses to the bars for males in this size class will be particularly interesting because even though these small males possess genes for vertical bars, their expression is inhibited by a suppressor gene (Zimmer and Kallman 1988). Finally, I examine and compare the responses to the bars in two additional species that are also closely related (Figure 2.1; Rauchenberger et al. 1990; Morris et al. 2001b); *X. birchmanni*, in which all the males have bars, and *X. cortezi* in which there is a polymorphism in males for bars that is not correlated with size. The inclusion of these two additional species in the analysis allows me to determine if the response to the bars is uniform over a larger representative sample of swordtail species. In addition, I can determine the direction of the responses (increased or decreased aggression) by comparing the level of male aggressive response to that of the most closely related species tested.

METHODS

Study species

I collected adult males of four swordtail species from the Rio Panuco Drainage, Mexico. *Xiphophorus cortezi* (n=17, $\bar{x}$=38.8mm, SD=3.5) males were collected from the Rio Axtla in San Luis Potosi, Mexico. *Xiphophorus multilineatus* (n=12, $\bar{x}$=35.2mm, SD=4.4) males were collected from the Rio Coy and *X. nigrensis* males (n=12, $\bar{x}$=33.8mm, SD=4.9) were collected from the Rio Choy, also both in San Luis Potosi, Mexico. *Xiphophorus birchmanni* males (n=13, $\bar{x}$=45.2mm, SD=6.2) were collected from the Rio Xiliatl in Hidalgo, Mexico. Upon return to the laboratory, the males were measured (standard length, SL) and individually housed in 25L aquaria that were visually isolated from one another. As fish were possibly involved in male-male interactions before being collected, I allowed the males to acclimate for two weeks before testing began in order to control for influence of recent learning experiences. This is sufficient time to remove the effects of prior encounters (Moretz and Morris, unpub. data).

The four species differ with respect to whether or not males possess the vertical bar pigment pattern (Figure 2.1). All *X. birchmanni* males have the vertical bars while all *X. nigrensis* are barless. Males of *X. cortezi* and *X. multilineatus* are polymorphic for this trait. In *X. multilineatus*, only the smallest males lack the bars. Zimmer and Kallman (1988) demonstrated that while males in the smallest size class carry genes for bars, the bars are suppressed by a gene on the Y chromosome. In *X. cortezi,* the presence or absence of bars is not related to male size. Of the males used in this study, nine of the 17 *X. cortezi* males were barless (mean SL barless males = 37.3mm, SD = 3.6; mean SL

barred males = 40.4mm, SD = 2.7; t=-2.01, p=0.06) and only the four smallest males

lacked bars in *X. multilineatus* (mean SL of barless males = 30.6mm, SD = 4.5, mean SL

of barred males = 37.8mm, SD = 1.7; t=-3.95, p=0.003).

Experimental design

Standard mirror image stimulation (MIS) tests were used to determine the

function of the bars by measuring the response of each male to both his barred and

barless image. The testing procedure consisted of attaching a mirror to one end of an

individual's tank and recording the number of displays and the number of bites directed

at the mirror image over a period of five minutes. Interaction time was defined as the

time that an individual interacted with his mirror image by displaying, biting or

swimming back and forth in front of his image. Interaction time also included the time

that an individual simply faced the image within the distance of 10 cm to the mirror,

approximately a quarter of the total length of the tank. Displays were of two types; either

a lateral orientation of the body while quivering or in a vertical headstand position.

These display types are common in actual confrontations (Moretz 2003; Moretz and

Morris pers. obsv). The tests were repeated for each male one week later.

After the initial two mirror tests, I performed a series of manipulations. For those

males with bars, I removed the bars by freeze branding (Raleigh et al. 1973; Figure 2.2)

after anaesthetizing the fish with MS-222. Temporary bars were applied to barless males

using antiseptic dye (Hoefler and Morris 1999; Figure 2.2). In both cases control subjects

were used in order to ensure that the procedures themselves were not altering the

behaviors or compromising the health of the fishes; barless males were painted with water to control for handling and barred males were freeze branded between their bars. In addition, previous studies have demonstrated that neither technique harms the fish or otherwise alters their behaviors (Morris et al. 1995; Hoefler and Morris 1999). Two sets of mirror tests were again conducted with the naturally barred males once their bars had faded (typically two weeks). During this time, the mirror tests were also repeated for the naturally barless males, 30 minutes after being painted.

I used a repeated measures ANOVA to compare the response of individuals to their barred and unbarred images. In order to determine if the time span between the first and second mirror tests, as well as between the third and fourth mirror tests, did not introduce variation into a male's response, I calculated repeatability (Becker 1984; Lessells and Boag 1987) using the between and within group variance from a one way ANOVA to ensure the reliability of this procedure.

RESULTS

The responses of males to their own images in the mirror tests were consistent in all four species examined, as repeatabilities tended to be high for the pre-manipulation and post-manipulation scores (Table 2.1). While results produced from MIS methods have been criticized in studies that relate MIS scores to dominance resulting from dyadic contests (Ruzzante 1992 but see Holtby 1992), one of the useful properties of MIS is that it provides perfect and instantaneous feedback without the confounding factors that can result from using live fish as stimuli (Rowland 1999). Measures were repeatable between

testing periods, indicating that the MIS method used in this study was both appropriate and reliable.

All naturally barred individuals expressed their bars in the mirror tests. Of the eight naturally barred *X. cortezi* males, only one failed to bite at his barred mirror image. The same was true of *X. multilineatus*, with one male of the eight not biting at his mirror image. All *X. birchmanni* males bit at their unmanipulated, barred mirror image. For all three species with naturally occurring bars, males intensified their bars before biting at their mirror images. Therefore, in the context of these tests, expression of the bars is a good indicator of intention to bite for males that have bars in the three barred species. These results match what has been observed in actual male-male contests (Moretz and Morris in review).

Interaction time did not differ for any of the species when presented with their barred versus their barless images (*X. birchmanni*, $F_{3,51}$=1.22, p=0.32; *X. cortezi*, $F_{3,67}$=0.93, p=0.44; *X. multilineatus*, $F_{3,47}$=2.16, p=0.11; *X. nigrensis*, $F_{3,47}$=1.67, p=0.19) nor was there a difference across species in interaction time ($F_{9,215}$=1.27, p=0.27), and as such interaction times were not used in additional analyses. However, the aggressive responses to the bars did vary across species (Table 2.1, Figure 2.2). *X. nigrensis* males did not bite more ($F_{3,47}$=0.33, p=0.82) or display more ($F_{3,47}$=1.09, p=0.37) at their barred image as compared to their barless image. *X. multilineatus* males as a whole bit more at their barless images (Figure 2.3; $F_{3,47}$=9.49, p<0.0005). These results were similar to those previously detected in studies that tested a smaller male in contests with a pair of larger males matched for size, one with bars and one without (Morris et al. 1995; Morris

and Ryan 1996). This suggests that the results of the mirror tests are comparable to the response of males in contests with live males. The response to the bars (number of bites) in *X. multilineatus* was similar for both the naturally barred males (Figure 2.4; $F_{3,31}=6.68$, p=0.002) and the smaller, naturally barless males (Figure 2.4; $F_{3,15}=4.39$, p=0.036). *X. multilineatus* males as a whole also displayed more at their barless images ($F_{3,47}=6.61$, p=0.001). However there was a difference between naturally barred males and naturally barless males in the use of displays; naturally barred males displayed more at their barless image ($F_{3,31}=10.02$, p<0.0005) while the smaller, naturally barless males showed no difference in number of displays at their barred versus barless image ($F_{3,15}=0.59$, p=0.64). As a whole, *X. cortezi* males did not bite more or less at their barred versus their barless mirror images (Figure 2.3; $F_{3,67}=0.50$, p=0.68) and this trend continued for naturally barless males (Figure 2.4; $F_{3,35}=0.09$, p=0.97). However, naturally barred males did exhibit a difference, biting more at their barless images (Figure 2.4; $F_{3,31}=3.20$, p=0.044). As a group, *X. cortezi* males did not differ in the number of displays directed at either their barred or barless images ($F_{3,67}=1.03$, p=0.39) and this trend continued for both naturally barred ($F_{3,31}=1.93$, p=0.16) and naturally barless males ($F_{3,35}=1.48$, p=0.24). *X. birchmanni* males exhibited the opposite response to the bars as measured by number of bites as compared to *X. multilineatus* males and *X. cortezi* barred males, biting more at their barred image as opposed to their barless image (Figure 2.3; $F_{3,51}=15.74$, p<0.00001). However, the number of displays *X. birchmanni* males directed at their mirror image was greater when faced with their barless images as compared to their barred images ($F_{3,51}=7.84$, p=0.0004). Comparing the level of aggression *X. multilineatus* and *X.*

birchmanni males directed at their barless versus barred image to the overall aggressive level of their closest relatives tested in this study, suggests that *X. multilineatus* has decreased aggression towards bars while *X. birchmanni* has decreased aggression towards barless individuals (Figure 2.3).

DISCUSSION

The vertical bar pigment pattern is used as a threat signal by all the males that had bars in the species of swordtail fishes that were tested. All males expressed their bars before biting at their mirror image. Thus, like the auditory conventional signaling of the banded wren (Molles and Vehrencamp 2001), expression of the bars in swordtails is likely to convey short-term information such as motivation, intention and immediate condition. However, the vertical bars should not be considered a "badge of status" or a performance signal (Hurd 1997b) as the bars are not correlated with dominance or fighting ability and are able to be intensified by all barred males, regardless of size or status. Instead, expression and intensification of the bars appears to indicate aggressive motivation in the presence of rival males, and is tightly correlated to having a genotype for bars, even while the nature of the response varies dramatically across species.

By comparing the response of males to their images with and without bars, I could determine if males responded with more aggression, less aggression or were equally aggressive towards individuals with bars as compared to individuals without bars. While comparisons within a species allow us to determine if males respond to the bars or not, it is not possible to know if the response represents an increase in aggression towards one

state or a decrease to the other without a comparison across related species. By comparing the response to an image without bars by *X. multilineatus* males to the overall aggression in *X. nigrensis*, and the response to an image with bars by *X. birchmanni* males to the overall response in *X. cortezi*, it is possible to determine that *X. multilineatus* and *X. birchmanni* have reversed their responses to the bars; *X. multilineatus* has reduced aggression towards males with bars, while *X. birchmanni* has reduced aggression towards males without bars, attacking their barred image with high levels of aggression. I was unable to find other examples in the literature of closely related species exhibiting opposite responses to a signal which appears to convey the same information and suggest that these results demonstrate that the nature of the response is controlled by a mechanism that is more evolutionarily labile than the relationship between owning the signal and male response.

One of the most stunning results was the polymorphic response exhibited by *X. cortezi* males. In this species there are both barred and barless morphs in the population and the presence or absence of bars is not related to body size. From collection records it appears that these two morphs occur in nature with approximately the same frequency (n=68, frequency of barred =52%). Males without bars did not respond to the bars while barred males responded with decreased aggression. There are at least two hypotheses to explain the differences in response between the two morphs. The first focuses on the barless males. It is possible that barless males were not selected to ignore the signal per se, but that the response was lost because it was tightly correlated with the signal and there was selection to lose the signal. Evidence from the current study supports such a

tight correlation between male response and the bars. In addition, Morris et al (2003) found a similar type of relationship between females with bars and their preference for the bars, suggesting that there may be a genetic correlation between response and trait for both males and females. But more importantly, evidence for selection to lose the bars has also been detected in this species. Morris et al (2003) demonstrated that females of this species are polymorphic in their response to the bars, some females preferring males with bars and some females preferring males without bars. The polymorphism in female preference for the bars could be maintaining the polymorphism in males through frequency dependent sexual selection. The second hypothesis focuses on the barred males. Data presented here (Figure 2.4), as well as additional data from staged contests (Moretz 2003; Moretz and Morris in review), indicate that not only are barless males more aggressive than barred males but that barless males consistently win contests over barred males of the same size. It is possible that barred males have been selected to maximize aggression whenever facing a barless opponent in order to compensate for the competitive advantage and higher aggression levels exhibited by barless males.

In *X. multilineatus*, both the naturally barred and barless males responded to the bars with reduced aggression. On the surface, these results do not appear to support the pattern found across the other species, in which response to the bars is tightly correlated with having the bars. However, Zimmerman and Kallman (1988) have demonstrated that the smaller males in *X. multilineatus* do possess genes for bars, but do not express bars because of a suppressor gene. Therefore, the fact that the small barless males in *X. multilineatus* do respond to the bars not only fits the overall pattern that bars and

response are correlated, but suggests that it is the genotype for bars, and not the

phenotype itself, that is correlated with response to bars.

While *X. birchmanni* males fit the pattern of bars and response to bars being

correlated, the nature of their response to the bars was reversed to that detected in males

of the other species with bars; instead of having a response of reduced aggression towards

barred individuals, *X. birchmanni* males reduce their aggression towards barless

individuals. This change in the nature of the response to the bars has occurred between

very closely related species (Figure 2.1) even though the way in which the signal is used

has remained the same. Expression of the bars in *X. birchmanni* is clearly a threat signal

in that all males that expressed their bars bit at their mirror image. The response to a

threat signal is expected to be reduced aggression, as males can use this signal to avoid

the costs of a fight (Maynard Smith and Price 1973; Maynard Smith and Parker 1976).

Compared to the males with bars in *X. cortezi*, *X. birchmanni* males were relatively more

aggressive towards bars as compared to no bars, which is likely to mean more fights, as

males express their bars more often than not in the field (Morris pers. observ.). In

addition, fights between live males in *X. birchmanni* include more aggressive behaviors

(Moretz and Morris, in review) than in *X. cortezi*. One explanation for these results is

that selection to reduce the number and cost of fights seems to be less in *X. birchmanni*

than in other species, which could suggest that costs external to the fights themselves,

like the probability of predation, may be lower in X. *birchmanni* than in the other species.

In *X. birchmanni*, it appears that the vertical bars are a typical example of a

conventional signal; the signal itself is not costly nor is it indicative of an individual's

strength, yet there are indirect costs of expressing the bars, as the bars are more likely to incite rather than reduce aggression in this species. In fact males in this species may be able to avoid physical conflicts with other males by suppressing their bars. Recall that in the MIS tests, *X. birchmanni* males displayed more and bit less at their barless images, suggesting that males are more likely to escalate when faced with an opponent that is willing to escalate. Thus, contests may be avoided if males only express their bars when heightened levels of aggression are necessary (e.g. when two closely matched males encounter one another). In contrast, the same costs associated with signaling and increased aggression do not seem to apply to males of *X. cortezi* and *X. multilineatus*; the signal reduces the number of bites in both species (only barred males in *X. cortezi*). In fact, in the context of aggression only, it would seem that in these species not expressing the bars is more costly than expression of the bars because opponents are likely to respond with increased aggression to an individual that has suppressed this signal in the absence of other context specific (i.e. subordinate) cues.

While I treat the congruence of trait and response as a correlation with a genetic basis, it may be argued that other factors may be responsible for the observed patterns. It is possible for example, that because males were wild caught, some of the observations were the result of learned experiences. I believe this to be unlikely for several reasons. First, this would require that the sampling of individuals was biased in such a manner that both within and across species I collected a disproportionate number of experienced versus inexperienced males (or visa versa). While it is impossible to know the experience levels of the males I collected, records indicate that the individuals used in

this study were representative of the natural populations both in terms of their size ranges and the proportion of barred and barless individuals. Thus, biased sampling is unlikely. Second, the males were isolated for a sufficient length of time to account for recent experiences occurring before testing (Moretz and Morris unpub. data). Finally, measures of MIS repeatability were high for all four species both before and after the bar manipulations. Thus, even between MIS tests there was no evidence that learning affected how each species responded to their mirror images.

In conclusion, this comparative study has revealed a tight correlation between male response and the signal vertical bars both across and within four species of swordtail fishes. The fact that this trait may be genetically correlated with having a response to the trait supports communication theory, suggesting that the coordination between signal and receiver was at some point very important in the evolution of this signal. However, the change in the direction of the response to this signal within one of the species examined suggests that given changes in the selective regime of the communication system, the direction of the response to the signal is more evolutionarily labile and therefore more likely to respond to those changes than the use of the signal itself.

LITERATURE CITED

Alexander, R.D. 1962. Evolutionary change in cricket acoustical communication. Evolution **16**: 443-467.

Atz, J.W. 1962. Effects of hybridization of pigmentation in fishes of the genus *Xiphophorus*. Zoologica **47**: 153-181.

Becker, W.A. 1984. Manual of Quantitative Genetics. Pullman, WA: Academic Enterprises.

Bradbury, J.W. and S.L. Vehrencamp. 1998. Principles of Animal Communication. Sunderland, MA: Sinauer Associates.

Butlin, R.K. and Ritchie, M.G. 1989. Genetic coupling in mate recognition systems: what is the evidence? Biol. J. Linn. Soc. **37**: 237-246.

Dawkins, M.S. 1993. Are there general principles of signal design? Trans. R. Soc. Lond. **340**: 251-255.

Endler, J.A. 1992. Signals, signal conditions and the direction of evolution. Am. Nat. **139**: S125-S153.

Enquist, M. 1985. Communication during aggressive interactions with particular reference to variation in choice of behaviour. Anim. Behav. **33**: 1152-1161.

Hoefler, C.H. and Morris, M.R. 1999. A technique for the temporary application and augmentation of pigment pattern in fish. Ethology **195**: 431-438.

Holtby, L.B. 1992. Through a glass darkly: a response to Ruzzante's reappraisal of mirror image stimulation studies. Can. J. Fish. Aqu. Sci. **49**, 1968-1969.

Gordon, M. 1931. Morphology of the heritable color patterns in Mexican killifish, *Platypoecilus*. Amer. J. Cancer **15**: 732-787.

Hurd, P.L. 1997a. Cooperative signaling between opponents in fish fights. Anim. Behav. **54**: 1309-1315.

Hurd, P.L. 1997b. Is signaling of fighting ability costlier for weaker individuals? J. Theor. Biol. **184**: 83-88.

Lessells, C.M. and P.T. Boag. 1987. Unrepeatable repeatabilities: A common mistake. The Auk **106**: 116-121.

McKinnon, J.S. and J.D. McPhail. 1996. Male aggression and colour divergent populations of the threespine stickleback: experiments with animations. Can. J. Zool. **74**: 1727-1733.

Maynard Smith, J. and G.R. Price. 1973. The logic of animal conflicts. Nature **46**: 15-18.

Maynard Smith, J. and G.A. Parker. 1976. The logic of asymmetric contests. Anim. Behav. **24**: 159-175.

Maynard Smith, J. 1982. Evolution and theory of games. Cambridge: Cambridge University Press.

Meyer, A., J. Morrissey and M. Shartl. 1994. Recurrent evolution of a sexually selected trait in *Xiphophorus* fishes inferred from a molecular phylogeny. Nature **368**: 539-542.

Molles, L.E. and S.L. Vehrencamp. 2001. Songbird cheaters pay a retaliation cost: evidence for auditory conventional signals. Proc. R. Soc. Lond. B **268**: 2013-2019.

Moretz, J.A. 2003. Aggression and RHP in the northern swordtail fish, *Xiphophorus cortezi*: the relationship between size and contest dynamics in male-male competition. Ethology (in press).

Moretz, J.A. and M.R. Morris. In review. Variation in aggression across three species of swordtail fishes: assessing the benefits of mirror tests. Behav. Ecol. Sociobiol.

Morris, M.R., M. Mussel and M.J. Ryan. 1995. Vertical bars on male *Xiphophorus multilineatus*: a signal that deters rival males and attracts females. Behav. Ecol. **6**: 274-279.

Morris, M.R. and M.J. Ryan. 1996. Sexual differences in signal-receiver coevolution. Anim. Behav. **52**: 1017-1024.

Morris, M.R., J.A. Elias and J.A. Moretz. 2001a. Defining vertical bars in relation to female preference in the swordtail fish *Xiphophorus cortezi* (Cyprinodontiformes, Poeciliidae). Ethology **107**: 827-837.

Morris, M.R., de Queiroz, K. and Morizot, D. 2001b. Phylogenetic relationships among the Northern Swordtails (*Xiphophorus*) as inferred from allozyme data. Copiea **2001**: 65-81.

Morris, M. R. Hesselman, L. and Nicoletto, P. 2003. A Polymorphism in female preference for a polymorphic male trait in the swordtail *Xiphophorus cortezi*. Anim. Behav. **65**: 45-52.

Quinn, V.S. and D.K. Hews. 2000. Signals and behavioral responses are not coupled in males; aggression affected by replacement of an evolutionarily lost color signal. Proc. R. Soc. Lond. B **267**: 755-758.

Parker, G.A. 1974. Assessment strategy and the evolution of fighting behaviour. J. Theor. Biol. **47**: 223-243.

Raleigh, R.F., McLearen, J.B. and Groff, D.G. 1973. Effects of topical location branding techniques, and changes in hue on recognition of cold brands in Centrarchid and Salmonoid fish. Trans. Amer. Fish. Soc. **102**: 637-641.

Rauchenberger, M., Kallman, K. D. and Morizot, D. C. 1990. Monophyly and geography of the Rio Panuco Basin swordtails (genus *Xiphophorus*) with description of four new species. Am. Mus. Novit. **2975**: 1-41.

Rowland, W.J. 1999. Studying visual cues in fish behavior: a review of ethological techniques. Envir. Biol. Fish. **56**: 285-305.

Ruzzante, D.E. 1992. Mirror image stimulation, social hierarchies and populational differences in agonistic behaviour: a reappraisal. Can. J Fish. Aqu. Sci. **49**: 1966-1968.

Ryan, M.J. and A.S. Rand. 1998. Evoked vocal response in male tungara frogs: pre-existing biases in male responses? Anim. Behav. **56**: 1509-1516.

Searcy, W.A. and Brenowitz, E.A. 1988. Sexual differences in species recognition of avian song. Nature **332**: 152-154.

Zimmerer, E. J. and Kallman, K. D. 1988. The inheritance of vertical barring (aggression and appeasement signals) in pygmy swordtail *Xiphophorus nigrensis* (Poeciliidae, Teleostei). Copeia **1988**: 299-307.

Table 2.1. Mean displays (d_x), mean bites (b_x) and repeatabilities (r) for males of four swordtail species for both pre- and post- manipulation MIS trials. Parentheses indicate standard error of the mean for each test.

| species | pre-manipulation | | | | post-manipulation | | | |
	mean displays	r	mean bites	r	mean displays	r	mean bites	r
X. birchmanni	d_1=6.9 (1.8)		b_1=27.6 (6.2)		d_1=10.5 (1.7)		b_1=5.8 (1.6)	
all barred	d_2=4.9 (1.1)	0.56	b_2=26.2 (3.8)	0.73	d_2=11.2 (1.4)	0.53	b_2=6.4 (2.1)	0.62
X. cortezi								
barred	d_1=5.1 (1.3)		b_1=16.3 (4.8)		d_1=3.6 (1.4)		b_1=22.3 (5.3)	
	d_2=5.3 (0.7)	0.59	b_2=20.4 (6.1)	0.90	d_2=3.8 (0.6)	0.57	b_2=25.6 (5.2)	0.89
barless	d_1=5.4 (0.9)		b_1=29.4 (4.9)		d_1=6.2 (1.2)		b_1=30.6 (5.5)	
	d_2=5.6 (1.1)	0.55	b_2=31.9 (7.1)	0.70	d_2=5.8 (0.7)	0.51	b_2=33.1 (5.5)	0.61
X. multilineatus								
barred	d_1=1.0 (0.7)		b_1=9.9 (3.5)		d_1=5.0 (1.2)		b_1=19.1 (3.0)	
	d_2=1.0 (0.6)	0.88	b_2=10 (3.5)	0.88	d_2=4.9 (1.2)	0.90	b_2=15.8 (2.0)	0.56
barless	d_1=4.3 (1.4)		b_1=17.8 (7.9)		d_1=2.2 (2.2)		b_1=7.5 (3.1)	
	d_2=2.3 (1.3)	0.67	b_2=23.3 (8.6)	0.93	d_2=2.5 (1.4)	0.81	b_2=7.0 (3.2)	0.57
X. nigrensis	d_1=4.1 (1.1)		b_1=17.4 (4.7)		d_1=4.3 (2.9)		b_1=15.8 (4.5)	
barless	d_2=3.6 (0.7)	0.78	b_2=17.8 (4.9)	0.84	d_2=5.0 (2.2)	0.57	b_2=16.8 (4.7)	0.91

Figure 2.1. The male trait vertical bars mapped onto the Rauchenberger et al. (1990) tree using parsimony. White lines/boxes, no bars; black lines/boxes, bars; shaded lines/boxes, polymorphic. The key above the tree summarizes the responses of naturally barred (top row) and naturally barless (bottom row) male swordtail fishes to the vertical bars (this study, Morris et al. 1995). Black boxes indicate barred male responses; white boxes indicate barless male responses to either bars (black boxes) or no bars (white boxes). The down arrows indicate a decreased response; "none" indicates no difference in response; "na" indicates that this state is not present in a particular species; ?? indicates that the response is not known.
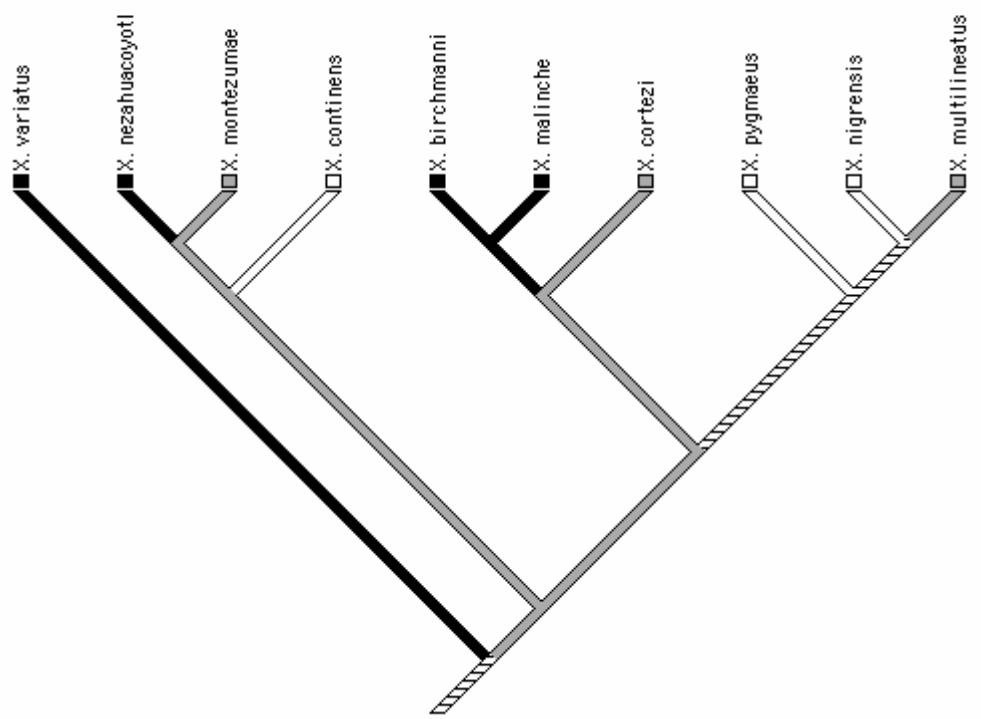
Figure 2.2 Examples of the manipulations used in the MIS tests. (A) Naturally barred *X. multilineatus* male. (B) *X. multilineatus* male with the bars removed. (C) *X. multilineatus* male with artificially applied bars. Photos courtesy of K. De Queiroz.

A.

B.

C.

Figure 2.3. Mean number of bites by males of four swordtail species during five minute MIS trials. P-values were calculated from repeated measures ANOVA. Error bars indicate standard error.
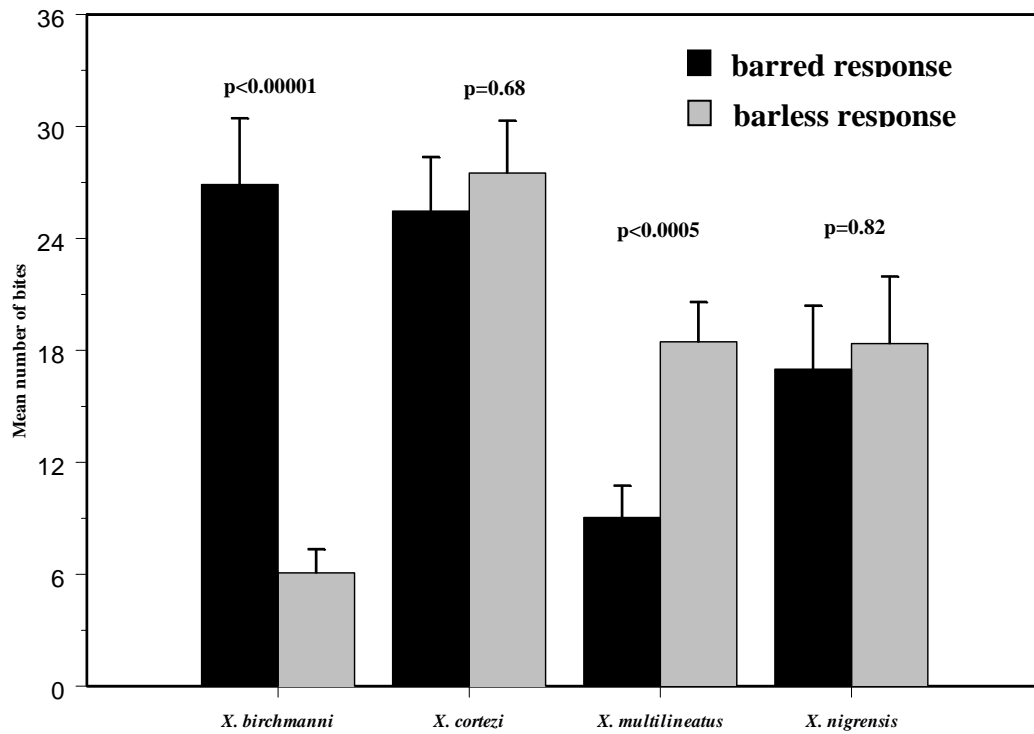
Figure 2.4. Mean number of bites by males of two swordtail species during five minute MIS trials. Individuals have been separated into two groups for each species; naturally barred males and naturally barless males. P-values were calculated from repeated measures ANOVA. Error bars indicate standard error.
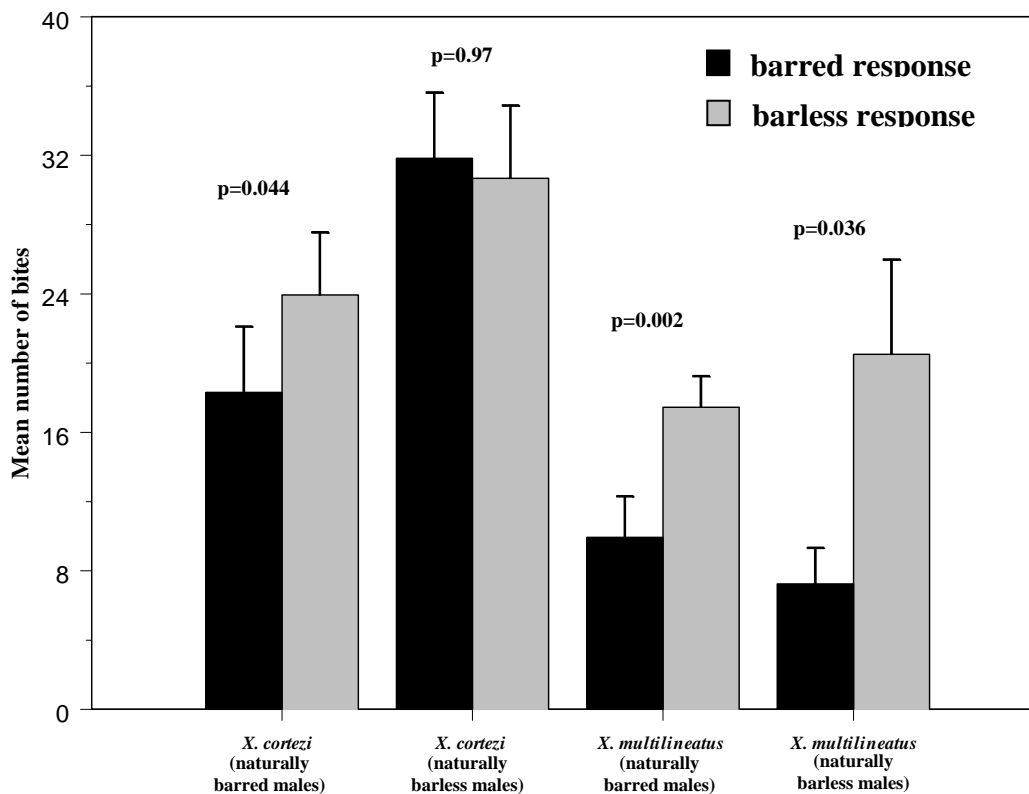
CHAPTER 3

THE EVOLUTION OF A SIGNAL OF AGGRESSIVE INTENT IN NORTHERN
SWORDTAIL FISHES

ABSTRACT

Intra-sexual selection can be an important component in the evolution of male traits. By examining the responses of males to signals used in male-male competition across taxa and within a phylogenetic context, it is possible to examine the evolution of a particular trait as a signal. In northern swordtail fishes, the males of many species posses a vertical bar pigment pattern. In all barred species studied to date, males have the ability to vary the expression of this pigment pattern; bars intensify at the onset of a contest and fade in the losing male at contest's end. In addition, males respond to the presence or absence of the vertical bars with either increased or decreased aggression. In the current study I examined male responses to the bars in all the northern swordtail species as well as an outgroup taxa using mirror image stimulation and bar manipulations. I also examined the ability of males to vary the expression of the bars and the timing of bar intensification in relation to escalation. I found that the bars evolved before the responses to the trait, suggesting that male-male competition probably did not play a substantial role in the initial evolution of the bars. In addition, variable bar expression evolved before the response to this signal, suggesting that the ability to vary bar expression was under selection from factors other than male-male competition. However, once expression of

the bars evolved to be a reliable indicator of the probability that an individual would bite, all males with bars exhibited a response to the bars. These results provide one of the few clear examples supporting the theory for the evolution of a signal from a cue, in which a cue containing information is modified into a reliable predictor of future behaviors.

INTRODUCTION

In many species, male traits often function as signals used in communication. In the context of male-male communication, male traits can be viewed as signals, with other males as the receivers (Boake 1991). Signals can convey information about male dominance status (Lange and Leimar 2003; Hagelin 2002), condition (Hill 1991), motivational state (Bradbury and Vehrencamp 1998) and/or fighting ability (Parker 1974; Ord et al. 2001). While signals differ in their information content, most signals are assumed to be subject to a common set of constraints and selection pressures including physical properties of the environment, intended and unintended receivers and other social factors (Endler 1992; Bradbury and Vehrencamp 1998; Stoddard 1999). However the response of the receiver should be particularly important in the evolution of signals because the signal/response dyad can have fitness consequences for both the sender and receiver (Kodrick-Brown and Brown 1984). Theory suggests that signals are derived from cues, which themselves contain information that may be useful to other individuals. For a cue to become a signal, it must be a reliable predictor of some behavior or characteristic of the sender, as well as have positive fitness consequences for the sender (reviewed in Krebs and Dawkins 1984; Bradbury and Vehrencamp 1998). There also needs to be coordination between signal and receiver and the behaviors that link them in order for the signal to be perpetuated (Alexander 1962; Butlin and Ritchie 1989; Endler 1992).

A powerful method for testing hypotheses about the evolution of signals is to compare signals and responses across species in a phylogenetic context. By doing so it is

possible to examine both the evolution of a particular trait, as well as the evolution of the trait as a signal. The first requires investigating when the trait evolved relative to when the response first appeared, as well as congruence between trait and response. In general, the levels of trait-response congruence (signal-receiver coordination) can be used to assess whether communication during particular interactions was important in the evolution of the trait. Determining how a trait or behavior is modified into a signal can be more difficult. This usually requires knowledge of inadvertent cues as precursors or the modification of behaviors previously associated with some other purpose (including signals in another context).

In the current study, I examined the male trait vertical bars and the aggressive responses to this signal in northern swordtail fishes. This pigment pattern (Gordon 1931; Atz 1962) is found throughout swordtails (Figure 3.1, 3.2) and platyfishes (*Xiphophorus*) and in other poeciliid fishes (Rauchenberger et al. 1990; Moretz and Morris 2003). In all species observed to date, males have the ability to both rapidly intensify and suppress the expression of the bars (Franck 1964; Zimmerer and Kallman 1988; Moretz and Morris 2003). The bars are heritable (Zimmerer and Kallman 1988) and their role has been studied extensively in male - male competition (Morris et al. 1995a & b; Morris and Ryan 1996; Moretz and Morris 2003) and female preference (Morris et al. 1995b; Morris 1998; Morris and Casey 1998; Morris et al. 2001a; Hankinson and Morris 2002; Hankinson and Morris 2003; Morris et al. 2003). Males use this signal both in courtship of females and during aggressive encounters with rival males, where expression of the bars intensifies at

the onset of aggressive behavior and suppression of the bars at the end of a contest

signals subordination.

In a comparative study of two northern swordtail species, Morris and Ryan (1996)

found that the male trait vertical bars and responses by males was congruent across both

species; aggressive responses were present in the species with bars (X. *multilineatus*), but

not in the species without the pigment pattern (*X. nigrensis*). Likewise, in *X. continens*, a

species in which all males are barless, Morris et. al. (in review) demonstrated that the

males of this species lacked an aggressive response to the bars. Moretz and Morris

(2003) examined the responses of males of four swordtail species (*X. birchmanni, X.*

cortezi, X. multilineatus and *X. nigrensis*) and found the same general patterns; only

barred males responded to the bars, though the direction of the responses (either

increased or decreased aggression towards the bars) varied across species. Additionally,

the expression of the bars intensified prior to biting for all four species.

The primary goal of this study was to further examine the evolution of male

responses to the pigment pattern vertical bars within the northern swordtails.

Specifically, I examined the ability of males to vary bar expression, the context in which

bar expression varied and whether males exhibited differential aggressive responses to

the bars. I included the remaining untested northern swordtail species (*X. pygmaeus, X.*

nezahualcoyotl, X. montezumae and *X. malinche*), as well as one species of platyfish (*X.*

variatus) as an outgroup taxa. By comparing the ability of males to manipulate the

expression of the bars across species, as well as the timing of expression in relation to

escalation, I was able to examine the evolution of this trait as a signal of aggressive intent

(future escalation). Determining the levels of trait/response congruence across all the species of northern swordtails, as well as when the response to the bars evolved in relation to the bars themselves, also provides a better understanding of the role of communication in male-male competition in the evolution of this male trait.

METHODS

Study species

The nine species of northern swordtails are divided into three clades (Rauchenberger et al. 1990; Morris et al. 2001b; Figure 3.1). In the *montezumae* clade are the sister species *X. montezumae* and *X. nezahualcoyotl* and a third species, *X. continens*. The *cortezi* clade consists of two sister species, *X. malinche* and *X. birchmanni*, and *X. cortezi*. In the *pygmaeus* clade are *X. nigrensis* and its sister species *X. multilineatus* along with a third species, *X. pygmaeus*.

I collected adult males from the remaining untested northern swordtail species (*X. pygmaeus, X. nezahualcoyotl, X. montezumae* and *X. malinche*) and one closely related species (*X. variatus*; Figure 3.1) from the Rio Pánuco Drainage, Mexico (Table 3.1). Upon return to the laboratory, the males were measured (standard length, SL) and scored for the presence of the vertical bars. The males of each species were individually housed in 25L aquaria that were visually isolated from one another, fed daily and maintained on a 14h light/ 10h dark photoperiod at a constant room temperature of 22C. As fish were possibly involved in male-male interactions before being collected, I allowed the males to acclimate for two weeks before testing began to control for influence of recent learning

experiences. This is sufficient time to remove the effects of prior encounters (Moretz and Morris, unpub. data).

Male responses to vertical bars

Standard mirror image stimulation (MIS) tests were used to determine whether males exhibited differential levels of aggression to the presence or absence of bars by measuring the response of each male to both his barred and barless image. The testing procedure consisted of attaching a mirror to one end of an individual's tank and recording the number of bites directed at the mirror image over a period of five minutes. During each test I also noted if the expression of the bars intensified, and if so, if intensification occurred before or after biting began. Interaction time was defined as the time that an individual interacted with his mirror image by displaying, biting or swimming back and forth in front of his image. Interaction time also included the time that an individual simply faced the image within the distance of 10 cm to the mirror, approximately a quarter of the total length of the tank. Displays were of two types; either a lateral orientation of the body while quivering or in a vertical headstand position. These displays are common in actual confrontations (Moretz 2003; Moretz and Morris pers. obsv). The tests were repeated for each male one week later.

After the initial two mirror tests, a series of manipulations were performed. For those males with bars, the bars were removed by freeze branding the pigmented area (Raleigh et al. 1973) after anaesthetizing the fish with MS-222. Temporary bars were applied to barless males using antiseptic dye (Hoefler and Morris 1999). In both cases

control subjects were used in order to ensure that the procedures themselves were not altering the behaviors or compromising the health of the fishes; barless males were painted with water to control for handling and barred males were freeze branded between their bars. In addition, previous studies have demonstrated that neither technique harms the fish or otherwise alters their behaviors (Morris et al. 1995b; Hoefler and Morris 1999; Moretz and Morris 2003). Two sets of mirror tests were again conducted with the naturally barred males once their bars had faded (typically two weeks). During this time, the mirror tests were also repeated for the naturally barless males, 30 minutes after being painted. A repeated measures ANOVA was used to compare the response of individuals to their barred and unbarred images.

While behavioral biologists have used MIS tests for many years, their use has drawn criticism (Ruzzante 1992 but see Holtby 1992) because often MIS aggression fails to correlate with dominance resulting from dyadic contests (Earley et al. 2000). However, one of the useful properties of MIS is that it provides symmetrical aggression levels and instantaneous feedback, without the confounding factors that can result from using live fish or dummy models as stimuli (Rowland 1999). Moretz and Morris (2003) found this procedure to be a reliable means to assess changes in aggression levels in these fish based on measures of repeatability (Becker 1984; Lessells and Boag 1987). In addition, changes in aggression levels in response to bar manipulations in Moretz and Morris (2003) were similar to those in studies utilizing live males as stimuli (Morris et al. 1995b; Morris and Ryan 1996).

RESULTS

Vertical bars

The species compared differ in whether or not they possessed the vertical bar pigment pattern (Figure 3.1). Bars were present on all males of *X. variatus*, *X. nezahualcoyotl*, *X. birchmanni* and *X. malinche* (this study; Moretz and Morris 2003) whereas all males of *X. continens*, *X. pygmaeus* and *X. nigrensis* were barless (this study; Morris et al. in review; Moretz and Morris 2003). *Xiphophorus montezumae*, *X. cortezi* and *X. multilineatus* were polymorphic for this trait (this study; Moretz and Morris 2003). In *X. multilineatus*, only the smallest males lack the bars. Zimmer and Kallman (1988) demonstrated that while males in the smallest size class carry genes for bars, the bars are suppressed by a gene on the Y chromosome. In *X. cortezi*, the presence or absence of bars is not related to male size (Moretz and Morris 2003) and the same is true of *X. montezumae* males. Of the 14 *X. montezumae* males used in this study, nine were barless (mean SL barless males = 41.5mm, SD = 5.2; mean SL barred males = 45.3mm, SD = 7.0; t=1.17, p=0.26).

In addition to species differing with respect to the presence or absence of bars, the bars themselves also differ across species (Figure 3.2). In both the *pygmaeus* and *cortezi* clades, the bars on barred species are dark black and readily visible, even when males are in a non-aggressive or non-courting state (the bars have not been intensified). In contrast, the bars on *X. montezumae* and *X. nezahualcoyotl* males in the *montezumae* clade are much lighter, both in intensity and color. The bars are also thinner and more deeply set in the body, sometimes making them difficult to see unless they are fully expressed

during aggressive interactions or courtship. The bars on *X. variatus* males are variable between populations (Moretz and Morris pers. obs.). In some populations, the bars are like those found in the *pygmaeus* and *cortezi* clades (dark and readily visible), in others the bars are like those found in the *montezumae* clade (thinner and lighter). In some instances, the bars are almost completely obscured by pigment blotches. The *X. variatus* males used in this study all had thin and lighter bars.

Differential expression of the vertical bars

Barred males of all swordtail species intensified the expression of the bars (Table 3.1). In contrast to naturally barred swordtail males, the bars on *X. variatus* males did not noticeably change in color or intensity either before or after biting had occurred. The timing of intensification also varied across species (Table 3.1). Like all other naturally barred males in the *pygmaeus* and *cortezi* clades (Moretz and Morris 2003), all naturally barred *X. malinche* males intensified their bars before biting at their mirror image. This was not necessarily the case for naturally barred males in the *montezumae* clade. The bars on *X. nezahualcoyotl* males did intensify on naturally barred males during the tests, but did so most often after biting occurred (Table 3.1). The bars on *X. montezumae* males appeared to intensify only after biting had occurred. From a qualitative standpoint, the degree to which the bars intensified in *X. montezumae* males appeared to be less than for males of all other species.

Male responses to vertical bars

Males of all species tested typically displayed at their image before biting, though occasionally some males bit before displaying. In addition, all males of all species interacted with their mirror images regardless of whether they were presented with their barred or barless image. Mean interaction time did not differ for any of the species between when males were presented with their barred versus their barless images (*X. variatus*: $F_{3,39}$= 0.18, p=0.91; *X. montezumae*: $F_{3,55}$= 0.95, p=0.42; *X. nezahualcoyotl*: $F_{3,47}$= 1.24, p=0.31; *X. malinche*: $F_{3,43}$= 1.03, p=0.39; *X. pygmaeus*: $F_{3,35}$= 0.49, p=0.69) nor was there a difference across species in mean interaction time ($F_{12,223}$=0.61, p=0.83). This was also true for previously tested species (Moretz and Morris 2003). As interaction time was uninformative, only the number of bites was used in the subsequent analyses.

Xiphophorus variatus males did not respond to the presence or absence of bars with either increased or decreased levels of aggression ($F_{3,39}$=0.1, p=0.96). *Xiphophorus nezahualcoyotl* males had a tendency to bite more at their barless images, though the increase in aggression when compared to aggression directed at their barred images was not statistically significant (Figure 3.3; $F_{3,47}$=2.70, p=0.061). Like the other naturally barred males in the *pygmaeus* and *cortezi* clades (Moretz and Morris 2003), *X. malinche* males did exhibit a response to the bars, biting more at their barred image as opposed to their barless image (Figure 3.4; $F_{3,43}$=9.81, p=0.0001). *Xiphophorus pygmaeus* males displayed and interacted with their mirror image, however they were not observed biting in either their natural barless state or manipulated barred state. *X. montezumae* males did not exhibit higher levels of aggression in one bar state versus another (Figure 3.3;

$F_{3,55}$=1.23, p=0.31). Like previously examined species that are polymorphic for the vertical bars, naturally barred and barless *X. montezumae* males were also ananlyzed separately; neither *X. montezumae* morph exhibited a response to the bars (Figure 3.4 naturally barred, $F_{3,19}$=0.31, p=0.82; naturally barless; $F_{3,35}$=0.07, p=0.98).

DISCUSSION

The evolution of a signal of aggressive intent

Differential male aggressive responses to the bars did not evolve until after males gained the ability to intensify and suppress their expression (Figure 3.1). Finding that *X. variatus* males did not have the ability to manipulate the expression of their bars was unexpected. However, it is not surprising that they did not exhibit either increased or decreased aggression to the bars, given their inability to change the intensity of their bars. In order for the bars to act as a signal, they would need to indicate either motivational state or male fighting ability and or status. Because the bars do not vary in intensity in *X. variatus*, they are not useful in assessing an opponent's willingness to act aggressively. Likewise, there is no evidence either in this study or from dyadic contests (Moretz and Morris unpublished data) to suggest that dominant or more aggressive males in *X. variatus* differ with respect to bar morphology than less aggressive or subordinate males. Thus, it appears that in *X. variatus* the bars do not act as a signal of aggressive motivation to rival males because presumably the bars do not convey information that could be useful in assessing an opponent's motivational state.

While differential expression of the bars appears to have been an important factor in the evolution of male responses, the ability to vary the expression of the bars evolved before a response to the bars. Two species that do not exhibit an aggressive response to the bars (*X. nezahualcoyotl* and *X. montezumae*) can modify their expression of the bars. Thus, other factors were probably important in males gaining the ability to intensify and suppress the expression of the bars. One possibility is that polymorphisms in female preference (e.g. Morris et al. 2003) may at least be partly involved in the evolution of the ability of males to manipulate the expression of the bars. In the presence of a female polymorphism in which some females prefer males with bars and others prefer males without bars, males that were capable of exhibiting either state should have an advantage as they would be appealing to females with either preference. However, this assumes that males could discriminate between the two types of females and act accordingly. While there is a correlation between having bars in females and a preference for barred males (Morris et al. 2003), it is not yet known if males preferentially express or suppress their bars for either group of females (barred or barless). Likewise, predation pressures could have also played a role in the ability of males to manipulate the expression of their bars; fading them in the presence of predators to avoid detection. While predation in relation to these fish has not been measured, many species occur in clear waters where barred individuals would presumably be more easily detected.

In order for the vertical bars to signal aggressive intent, not only is it necessary to be able to vary the expression of the bars, but also the timing of that expression should be predictive of biting. In this study, the timing of expression of the bars differed between

some of the naturally barred species that had the ability to vary bar intensity (only *X.*

variatus lacked the ability to noticeably manipulate expression of the bars). The trait is

likely to signal the willingness to escalate to biting in only the naturally barred males of

the *pygmaeus* and *cortezi* clades, as only males from these two clades intensified the bars

before biting (this study; Moretz and Morris 2003; Table 3.1). In contrast, intensification

of the bars in *X. montezumae* and *X. nezahualcoyotl* males usually occurred after biting

had already begun and as such, could not be used by opponents to assess another male's

willingness to escalate to biting. It is still possible however, that darkening of the bars

conveys some information, possibly current motivational state and the willingness to

continue with agonistic interactions. While I did not examine this directly, I do know

from dyadic contests that in all northern swordtail species (but not in *X. variatus*) the bars

fade in the loser at the end of a contest, and in conjunction with other visual cues signal

subordination (Morris 1995b; Moretz 2003; Moretz and Morris unpublished data). Thus,

at the least, the vertical bars signal current motivation in all naturally barred species

(except *X. variatus*) and future intentions to escalate to biting in all naturally barred males

that had a response to the bars (barred males in the *cortezi* and *pygmaeus* clades).

However I find no evidence that the expression of bars function as a badge of status or a

performance signal (Hurd 1997), as all naturally barred swordtail males, regardless of

size or status, were able to intensify their expression.

Together as a whole, these results provide one of the few clear examples of how a

cue evolves into a signal. In the ancestral condition, the vertical bars may have helped

individuals assess size (bar number and total pigmented area is correlated with size in

some species, Zimmerer and Kallman 1998; Morris et al. 2001) or may have been useful in attracting females. However they were probably not useful in assessing the aggressive state of rival males. In the ancestor to the northern swordtail clade, selection favored males having the ability to vary the expression of the bars, and though this was probably the result of factors other than male-male competition, once expression was varied in the context of male contests, variable expression of the bars was probably useful in assessing the motivational state of an opponent. The shift from a motivational cue to a signal predictive of a specific behavior occurred once the timing of the expression was modified such that it always occurred before biting; expression of the bars evolved into a reliable signal of aggressive intent.

Male responses to vertical bars

Only those species that used the bars as a signal of future escalation exhibited differential aggressive responses to the bars, and parsimony indicates that male responses to the bars evolved independently in two instances (Figure 3.1). A response to the bars evolved first in the ancestor to the *cortezi* clade, in which all males exhibited a response except for barless *X. cortezi* males, and a second time in the ancestor to *X. multilineatus*, in which all males respond to the bars with decreased aggression. However, it has been previously demonstrated that how traits are defined and interpreted can drastically affect the conclusions drawn from studies utilizing multiple species for comparisons (Wiens and Morris 1996). By categorizing any change in aggression levels as a response to the bars, I am ignoring the fact that the species are responding very differently to the vertical

bars. *Xiphophorus cortezi* barred males respond to an image without bars with less aggression than the overall levels of aggression of *X. cortezi* barless males. A similar comparison of *X. multilineatus* males to its sister species *X. nigrensis*, makes it possible to determine that *X. multilineatus* males also respond to the bars with decreased levels of aggression (Moretz and Morris 2003). In contrast, by comparing the responses of *X. birchmanni* and *X. malinche* to their closest relative, *X. cortezi*, it is possible to determine that the responses of these two species are opposite from *X. cortezi* and *X. multilineatus*; males behave less aggressively towards their barless images and attack their barred images with higher levels of aggression. In this sense, *X. birchmanni* and *X. malinche* may not exhibit a response to the bars per se, but rather a response to barless stimuli. If the direction of the responses are considered to indicate different traits (or states of the same trait), responses to the bars have evolved separately three times instead of two; a decreased response to barred stimuli in the ancestor to *X. cortezi*, a decreased response to barless stimuli in the ancestor to *X. birchmanni* and *X. malinche* and a decreased response to barred stimuli in the ancestor to *X. multilineatus*.

Theory predicts that the response to a threat signal should be reduced aggression as individuals can use this information to assess a competitor's current motivational state and avoid a potentially costly fight (Maynard Smith and Price 1973; Maynard Smith and Parker 1976). However this was not always the case as both *X. birchmanni* and *X. malinche* males were less aggressive towards their barless images as compared to their barred images, while only naturally barred *X. cortezi* males and *X. multilineatus* males responded to the bars with decreased aggression. There are several possible explanations

why *X. malinche* males and *X. birchmanni* males exhibited lower levels of aggression in the absence of the vertical bars. First, as fading the bars occurs in the losers of contests and contest losers cease aggressive behaviors (Moretz and Morris unpublished data), individuals not expressing their bars may not be viewed as a threat. In other words, males may refrain from or reduce aggressive behaviors towards males that are not acting aggressively, thereby avoiding the potential costs associated with prolonged escalated conflicts. A somewhat contradictory explanation may be that costs associated with aggressive interactions are less in these two species compared to *X. cortezi* and *X. multilineatus*. For example, costs external to the fights themselves could be less, such as the probability of predation, and as a result selection for reducing the number of fights has been relaxed. Finally, it is possible that the males of these two species do not recognize barless males as being conspecifics. Unlike *X. cortezi* and *X. multilineatus* males which frequently come in contact with naturally barless males, *X. malinche* and *X. birchmanni* males are barred. Therefore, vertical bars could indicate a conspecific and barless male may not be viewed as a rival.

Evolution of vertical bars

Signal – receiver coordination is thought to be an important component in the evolution of particular male traits. In general, the levels of congruence between trait and response can reveal whether the responses were an important selective factor in the evolution of the trait, as well as the degree to which trait and response have co-evolved. The current study indicates that differential male aggressive responses to the presence or

absence of the bars did not play a role in the initial evolution of this pigment pattern or the variable expression of the pigment pattern. Both the bars and the ability to vary their intensity evolved before the male responses (Figure 3.1). This suggests that male-male competition played little, if any, role in the initial evolution of male vertical bars. However, the context in which the bars were expressed was tightly correlated with having an aggressive response and the strong correlation between having the trait and having a response in the *cortezi* and *pygmaeus* clades suggests male aggression has helped maintain the presence of this trait in these species. Note that this study primarily focused on differential aggressive responses to the bars. Therefore, it is not possible to completely rule out male-male competition as a contributing factor in the evolution of the vertical bars. It is possible that the bars also aid individuals in assessing the size of an opponent. In *X. multilineatus* the numbers of bars are correlated with male size (Zimmerer and Kallman 1998) and in *X. cortezi* total pigmented area is correlated with male size (Morris et al. 2001). Thus the number of bars may provide information that individuals could use in determining whether to attack an opponent, or flee and reduce the likelihood of a potentially costly fight.

Many male signals have dual receivers and as a result are affected by both components of sexual selection (Tinbergen 1953). In the case of the northern swordtails, evidence suggests that female preference may have played a larger role in the evolution of the vertical bars than has male-male competition. In all species observed to date, at least a portion of females have demonstrated a preference for bars when the trait is present (*X. multilineatus*, Morris et al 1995b; *X. cortezi*, Morris et. al 2003). Likewise,

when the preference for no bars is present the male trait is absent (*X. pygmaeus*; Hankinson and Morris 2002, 2003) and in no species have females exhibited indifference; females either prefer males with bars or males without bars. This is not to suggest that congruence between female preference and male trait has always been detected. There are two instances where female preference for the bars is present but the male trait has been evolutionarily lost. In *X. continens*, females exhibit a polymorphic preference (Morris et al. in review) and in *X. nigrensis* (Morris and Ryan 1996), females prefer males with bars, though in neither species are there barred males. The loss of this trait could be the result of several factors including population bottlenecks, founder events and competition leading to character displacement (Morris et al. in review). Another possibility is that predation may have contributed to the loss of the vertical bars, as my observations suggest that barred males are more visible in their natural environment than barless males. More importantly however, while I do not yet know if females have a pre-existing bias and prefer the trait before it evolved, female preference for vertical bars precedes male responses to the vertical bars (*X. variatus*, Morris unpublished data). Thus given that the male trait is present only when females have a preference for the trait, females are never indifferent, the trait is absent when females prefer barless males and female preference precedes male response, it seems likely that female preference was more influential in the evolution of the vertical bars than was male-male competition.

In summary, I examined the evolution of a signal of aggressive intent. There was only moderate congruence between having the trait vertical bars and having a response

and I found that the trait precedes the evolution of male responses. As a result, I suggest that male-male competition probably did not play a substantial role in the initial evolution of the bars themselves. Likewise, the ability of males to vary the expression of the vertical bars evolved before the response to this signal, suggesting that variable expression was under selection from factors other than male-male competition (e.g. predation). Once present however, variable expression became an important component of communication in swordtail aggressive interactions. In those barred species where intensification occurred after escalation, the bars may provide individuals with information about their opponent's motivational state and willingness to continue fighting. Further modification of the expression of the bars resulted in a shift in the timing of the bar intensification and as a result, a signal that is predictive of biting in those species that express the bars before escalating. In this context, the signal of aggressive intent is completely congruent with the response: all species with differential aggressive responses to the presence or absence of bars express their bars before biting.

LITERATURE CITED

Alexander, R.D. 1962. Evolutionary change in cricket acoustical communication. Evolution **16**: 443-467.

Atz, J.W. 1962. Effects of hybridization of pigmentation in fishes of the genus *Xiphophorus*. Zoologica **47**: 153-181.

Becker, W.A. 1984. Manual of Quantitative Genetics. Pullman, WA: Academic Enterprises.

Boake, C.R. 1991. Coevolution of senders and receivers of sexual signals: genetic coupling and genetic correlations. Trends. Ecol. Evol. **6**: 225-227.

Bradbury, J.W. and S.L. Vehrencamp. 1998. Principles of Animal Communication. Sunderland, MA: Sinauer Associates.

Butlin, R.K. and M.G. Ritchie. 1989. Genetic coupling in mate recognition systems: what is the evidence? Biol. J. Linn. Soc. **37**: 237-246.

Earley, R.L., Y. Hsu and L.L. Wolf. 2000. The use of standard aggression testing method to predict combat behaviour and contest outcome in *Rivulus marmoratus* dyads (Teleostei: Cyprinodontidae). Ethology **106**: 743-761.

Endler, J.A. 1992. Signals, signal conditions and the direction of evolution. Am. Nat. **139**: S125-S153.

Franck, D. 1964. Vergleichende Verhaltensstudien and Lebendegebarenden Zahnkarpfen der Gattung *Xiphophorus*. Zoo. Jahrb. Physiol. Bd. **71**: 117-170.

Gordon, M. 1931. Morphology of the heritable color patterns in Mexican killifish, Platypoecilus. Amer. J. Cancer **15**: 732-787.

Hagelin, J.C. 2002. The kinds of traits involved in male-male competition: a comparison of plumage, behavior and body size in quail. Behav. Ecol. **13**: 32-41.

Hankinson, S.J. and M.R. Morris. 2002. Sexual selection and species recognition in the pygmy swordtail, Xiphophorus pygmaeus: conflicting preferences. Behav. Ecol. Sociobiol. **51**: 140-145.

Hankinson, S.J. and M.R. Morris. 2003. Avoiding a compromise between sexual selection and species recognition: female swordtail fish assess multiple species-specific cues. Behav. Ecol. **14**: 282-287.

Hill, G.E. 1991. Plumage coloration is a sexually selected indicator of male quality. Nature **350**: 337-339.

Hoefler, C.H. and M.R. Morris. 1999. A technique for the temporary application and augmentation of pigment pattern in fish. Ethology **195**: 431-438.

Holtby, L.B. 1992. Through a glass darkly: a response to Ruzzante's reappraisal of mirror image stimulation studies. Can. J. Fish. Aqu. Sci. **49:** 1968-1969.

Hurd, P.L. 1997. Is signaling of fighting ability costlier for weaker individuals? J. Theor. Biol. **184**: 83-88.

Kodrick-Brown, A. and J.H. Brown. Truth in advertising: the kinds of traits favored in sexual selection. Am. Nat. **124**: 309-323.

Krebs, J.R. and R. Dawkins. 1984. Animal signals: Mind-reading and manipulation. In Behavioural Ecology: an evolutionary approach (J.R. Krebs and N.B. Davies, eds.), pp.380-402. Sunderland, MA: Sinauer Associates.

Lange, H. and O. Leimar. 2003. The function of threat display in wintering great tits. Anim. Behav. **65**: 573-584.

Lessells, C.M. and P.T. Boag. 1987. Unrepeatable repeatabilities: A common mistake. The Auk **106:** 116-121.

Maynard Smith, J. and G.R. Price. 1973. The logic of animal conflicts. Nature **46**: 15-18.

Maynard Smith, J. and G.A. Parker. 1976. The logic of asymmetric contests. Anim. Behav. **24**: 159-175.

Moretz, J.A. 2003. Aggression and RHP in the northern swordtail fish, *Xiphophorus cortezi*: the relationship between size and contest dynamics in male-male competition. Ethology **109**: 995-1008.

Moretz, J.A. and M.R. Morris. 2003. Evolutionarily labile responses to a signal of aggressive intent. Proc. R. Soc. Lond. B **270**: 2271-2277.

Morris, M.R., L. Gass, and M.J. Ryan. 1995a. Assessment and individual recognition of opponents in swordtails *Xiphophorus nigrensis* and *X. multilineatus*. Behav. Ecol. Sociobiol. **37**: 303-310.

Morris, M.R., M. Mussel and M.J. Ryan. 1995b. Vertical bars on male *Xiphophorus multilineatus*: a signal that deters rival males and attracts females. Behav. Ecol. **6**: 274-279.

Morris, M.R. and M.J. Ryan. 1996. Sexual differences in signal-receiver coevolution. Anim. Behav. **52**: 1017-1024.

Morris, M.R. 1998. Female preference for trait symmetry in addition to trait size in swordtail fishes. Proc. R. Soc. Lond. B **1399**: 907-911.

Morris, M.R. and K. Casey. 1998. Female swordtail fish prefer symmetrical sexual signal. Anim. Behav. **55**: 33-39.

Morris, M.R., J.A. Elias and J.A. Moretz. 2001a. Defining the sexually selected male trait vertical bars in relation to female preference in the swordtail fish *Xiphophorus cortezi*. Ethology **107**: 827-837.

Morris, M.R., K. De Queiroz and D.C. Morizot. 2001b. Phylogenetic relationships among populations of northern swordtails (*Xiphophorus*) as inferred from allozyme data. Copeia **2001**: 65-81.

Morris, M.R., P.F. Nicoletto and E. Hesselman. 2003. A polymorphism in female preference for a polymorphic male trait in the swordtail fish *Xiphophorus cortezi*. Anim. Behav. **65**: 45-52.

Morris, M.R., J.A. Moretz, K. Farley and P. Nicoletto. (In review). The role of sexual selection in the loss of sexually selected traits in the swordtail fish *Xiphophorus continens*.

Ord, T.J., D.T. Blumstein and C.S. Evans. 2001. Intrasexual selection predicts the evolution of signal complexity in lizards. Proc. R. Soc. Lond. B **268**: 737-744.

Parker, G.A. 1974. Assessment strategy and the evolution of fighting behaviour. J. Theor. Biol. **47**: 223-243.

Raleigh, R.F., J.B. McLearen and D.G. Groff. 1973. Effects of topical location branding techniques, and changes in hue on recognition of cold brands in Centrarchid and Salmonoid fish. Trans. Amer. Fish. Soc. **102**: 637-641.

Rauchenberger, M., K.D. Kallman. and D.C. Morizot. 1990: Monophyly and geography of the Rio Panuco Basin swordtails (genus Xiphophorus) with description of four new species. Am. Mus. Novit. **2975**: 1-41.

Rowland, W.J. 1999. Studying visual cues in fish behavior: a review of ethological techniques. Envir. Biol. Fish. **56**: 285-305.

Ruzzante, D.E. 1992. Mirror image stimulation, social hierarchies and populational differences in agonistic behaviour: a reappraisal. Can. J Fish. Aqu. Sci. **49**: 1966-1968.

Stoddard, P.K. 1999. Predation enhances complexity in the evolution of electric fish signals. Nature **400**: 254-256.

Tinbergen, N. 1953. Social Behaviour in Animals. London: Methuen.

Wiens, J.J. and M.R. Morris. 1996. Character definitions, sexual selection and the evolution of swordtails. Am. Nat **147**: 866-869.

Zimmerer, E. J. and K.D. Kallman. 1988. The inheritance of vertical barring (aggression and appeasement signals) in pygmy swordtail *Xiphophorus nigrensis* (Poeciliidae, Teleostei). Copeia **1988**: 299-307.

Table 3.1. Sample size, mean standard length (SL; mm), the ability to vary bar expression and the percentage of barred individuals intensifying expression of the bars before biting for males of 10 species of *Xiphophorus* fishes. Taxa have been grouped by clade (Rauchenberger et al. 1990). Parentheses indicate standard deviation of the mean male size for each species; na indicates not applicable. Superscripts refer to this and previous studies: 1, this study; 2, Morris et al. in review; 3, Moretz and Morris 2003.

| clade | males | | variable bar | % of males intensifying |
species	N	SL (SD)	expression	before escalation
outgroup				
X. variatus [1]	10	33.1 (3.3)	no	na
montezumae				
X. montezumae [1]	14	42.8 (5.9)	yes	0
X. nezahualcoyotl [1]	12	36.3 (4.2)	yes	25
X. continens [2]	12	25.3 (1.6)	na	na
cortezi				
X. malinche [1]	11	38.7 (4.1)	yes	100
X. birchmanni [3]	13	45.2 (6.2)	yes	100
X. cortezi [3,4]	17	38.8 (3.5)	yes	100
pygmaeus				
X. nigrensis [3,5]	12	33.8 (4.9)	na	na
X. multilineatus [3,5]	12	35.2 (4.4)	yes	100
X. pygmaeus [1,6]	9	22.8 (0.6)	na	na

Figure 3.1. The male trait (phenotype) vertical bars mapped onto the Rauchenberger et al. (1990) tree using parsimony (modified from Moretz and Morris 2003). Character state is ordered such that transition from barless to barred (or visa versa) first requires a polymorphic state. White branches, no bars; black branches, bars; shaded branches, polymorphic; hatched branches, equivocal. The key above the tree summarizes the aggressive responses of naturally barred (top row) and naturally barless (bottom row) male swordtail fish to the vertical bars (this study; Morris et al. 1995, Moretz and Morris 2003, Morris et al. in review). The down arrows indicate lower aggression to either barred (black boxes) or barless (white boxes) mirror images; none indicates no difference in response; na indicates that the state is not present in a given species.
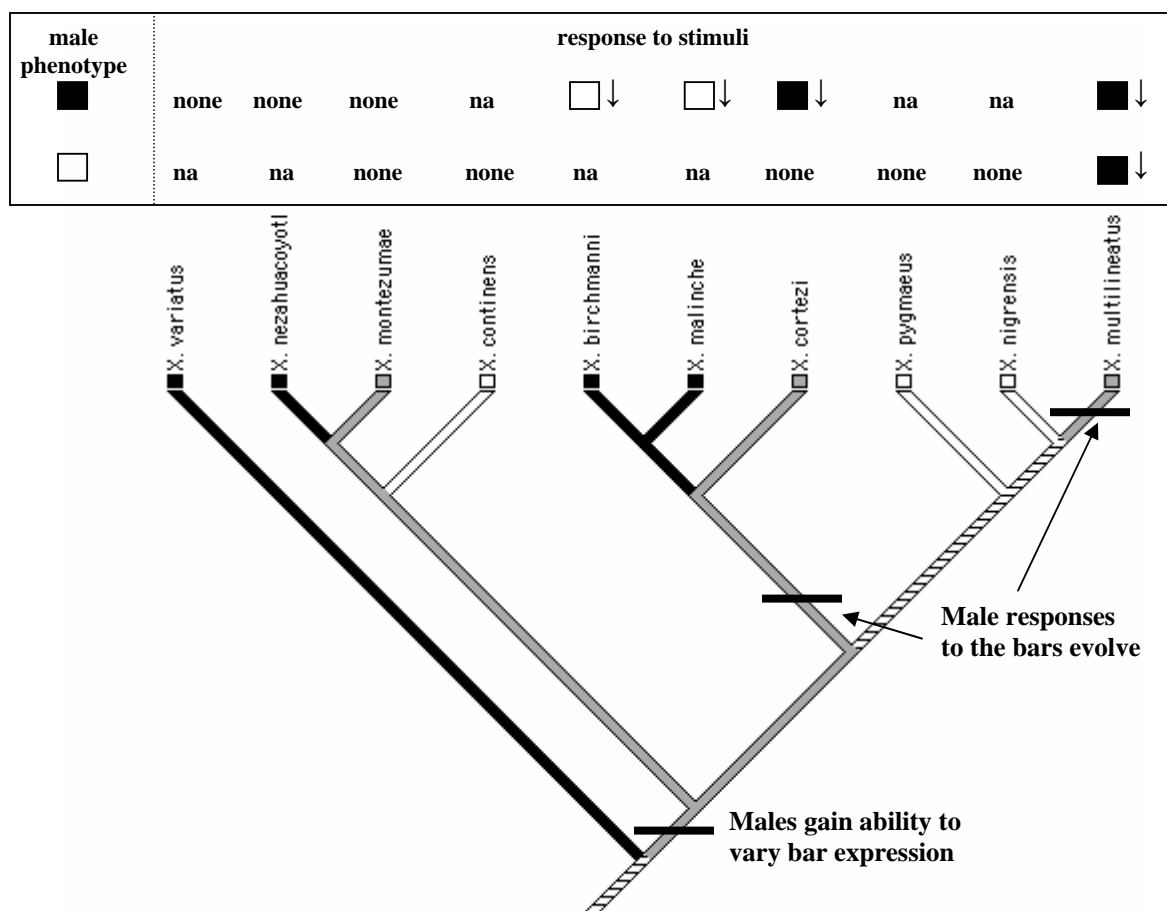
Figure 3.2. Vertical bars on a *X. cortezi* male (A) and *X. nezahualcoyotl* male (B). The bars on both males are in their normal state (neither intensified nor suppressed). Note that in (B) the bars are thinner, not as dark and appear to be "set" deeper within the body. Photos courtesy of K. De Queiroz.

A.

B.

Figure 3.3. Mean number of bites by nine species of swordtail males during five minute MIS trials (this study; Moretz and Morris 2003, Morris et al. in review). Dashed lines within the graph separate clades. P-values were calculated from repeated measures ANOVAs. Error bars indicate standard error. *Xiphophorus pygmaeus* males were not observed biting (NA) at their mirror images in either bar state.
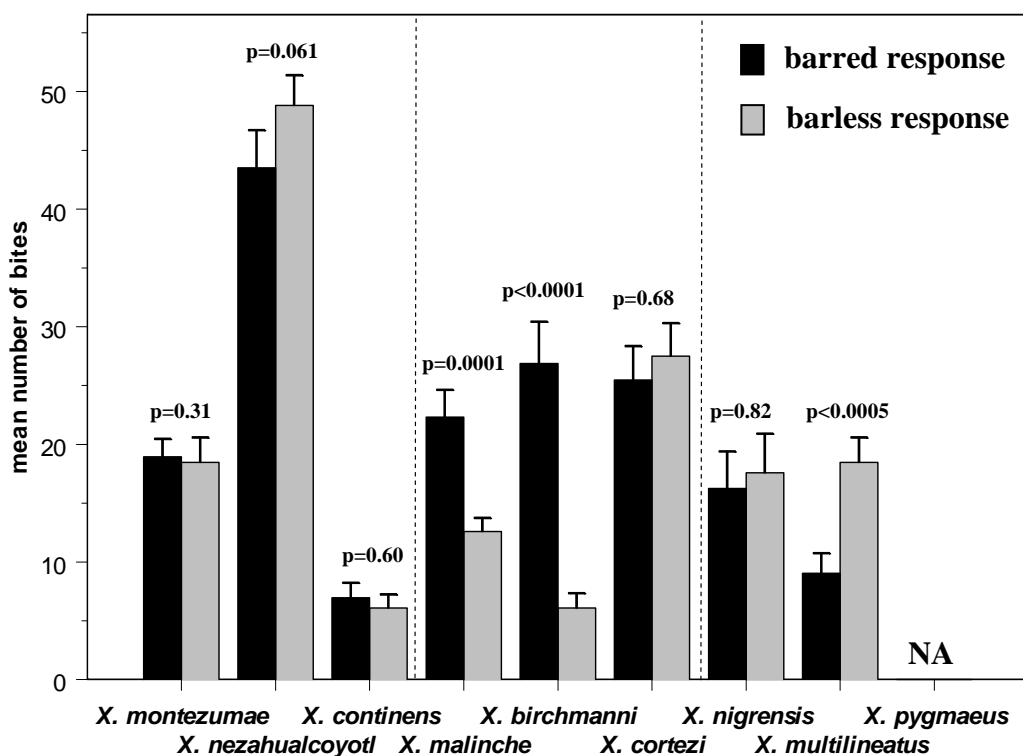
Figure 3.4. Mean number of bites by males of three swordtail (polymorphic) species during five minute MIS trials (this study; Moretz and Morris 2003). Individuals have been separated into two groups for each species: naturally barred males and naturally barless males. P-values were calculated from repeated measures ANOVAs. Error bars indicate standard error.
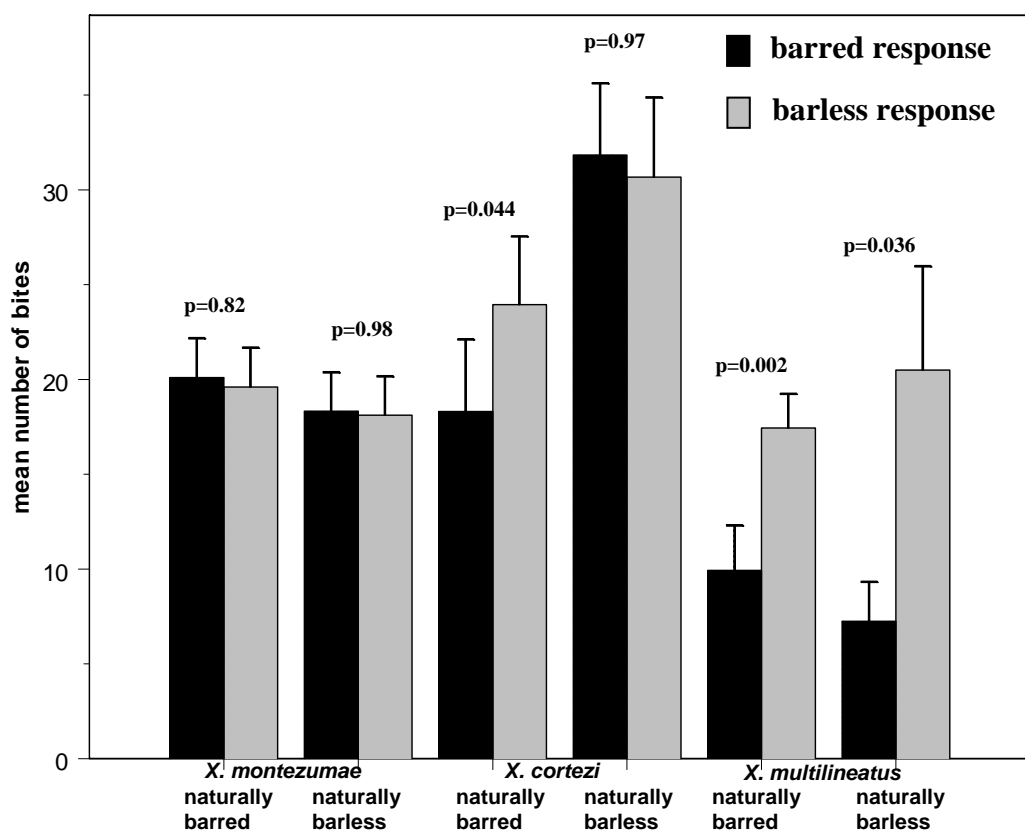
CHAPTER 4

AGGRESSION AND FIGHTING ABILITY ARE CORRELATED IN THE SWORDTAIL FISH *XIPHOPHORUS CORTEZI*: THE ADVANTAGE OF BEING BARLESS

ABSTRACT

Males of the swordtail fish *Xiphophorus cortezi* are polymorphic for the pigment pattern vertical bars. Barred and barless males occur with approximately the same frequency and there is no significant difference in size between the two morphs. Males that possess the bars have the ability to both intensify and suppress their expression and this signal is used in both courtship of females and in male – male interactions. Previous studies indicate that barless males are more aggressive than barred males, only barred morphs respond to the bars and the response to the bars is reduced aggression. In this study I matched barred and barless males for size and paired them in dyadic contests in order to determine if either morph was more dominant and if so, if dominance was the result of higher aggression levels. I found that barless males had higher bite frequencies and were able to win a majority of the contests while barred males consistently escalated to biting first, even though in most cases they were ultimately the losers. In order to determine whether the observed aggression levels and dominance were inherent to being barless or a consequence of responses to the bars themselves, the dyads were re-paired once after barless males were given temporary bars and once after barred males had their

bars removed. Thus, each morph encountered his opponent in both a barred and barless state. Regardless of bar state, naturally barless males continued to be more aggressive and more dominant than their barred counterparts. In addition, naturally barred males only won contests in which they bit more. These results indicate that for this species, aggression is an important component of winning contests when opponents are roughly the same size. As a result, naturally barless males as a whole appear to have higher resource holding potential (RHP) than naturally barred males of the same size because of their greater aggression levels.

INTRODUCTION

Aggression is thought to be an important component of fighting ability, and yet few studies have demonstrated that more aggressive males are more dominant (e.g. Ribowski and Franck 1993). One inherent problem in studying the relationship between aggression and dominance is that the expression of aggression is context specific. The relative size of one's opponent (e.g. Mayar and Berger 1992; Morris et al. 1995a), prior experience (e.g. Beaugrand et al. 1991; Beaugrand et al. 1996) and value of the contested resource (e.g. Dugatkin and Ohlsen 1990) are but a few of the factors known to influence how aggressive a male will be in a contest. Thus, in order to understand how aggression contributes to resource holding power (RHP) and contest outcome, asymmetries between opponents need to either be held constant or manipulated in such a way that they can be investigated independently of their effects.

Polymorphisms in color patterns and coloration are found in many groups of vertebrates and their evolution, function and form have been studied extensively. While some of the variation within species is due to differences between sexes or age classes, much of the observed variation in color patterns can be found among males. Differences in male coloration are important in aggressive interactions in a range of taxa, including birds (Lemel and Wallin 1993; Pryke et al. 2002), lizards (Carpenter 1995; Baird et al. 1997) and fishes (Borowsky 1973; Martin 1977; Horth 2003) and may signal an individual's resource holding power (RHP), social rank, motivational state or overall condition. In fishes, male coloration generally falls into one of two broad groups based on whether colors or color patterns can be quickly varied in response to context. In the

first group are polychromatic species, whose males are "fixed" to a particular color morph. A classical example is that of the Midas Cichlid, *Theraps* (*Cichlasoma*) *citrinellum*, where both grey and gold morphs exist, the later of which tend to be more aggressive and dominant (Barlow 1973; Barlow 1983a & b; Barlow 1994). The second group consists of species whose males are able to rapidly change colors or color patterns. The ability to darken the body or parts of the body is commonly used as a signal in agonistic encounters and can signal either subordination (e.g. oscar cichlid *Astronotus ocellatus*, Beeching 1995; juvenile atlantic salmon *Salmo salar*, O'Connor et al. 1999) or dominance (e.g. juvenile guppies *Poecilia reticulata*, Martin and Hengstebeck 1981). Likewise, intensification of body coloration can be used to signal aggressive intent and conversely, suppression of coloration can be used to signal defeat or subordination (Moretz and Morris 2003).

In swordtail fishes (*Xiphophorus*), aggression in relation to color morphs has been examined in three previous studies. In the first, Heuts and Nijman (1998) examined the aggressive behaviors of two color breeds (red and black) resulting from hybridization of *X. maculatus* and *X. helleri*. In contests, red male morphs were more likely to attack first and show first signs of dominance than were black morphs. Similar results were reported by Franck et al. (2003) in which they found that *X. helleri* males with a red lateral stripe were dominant over same sized and larger *X. helleri* males with a black or brown lateral stripe. Finally, Kingston et al. (2003) examined *X. pygmaeus* males from three different natural populations. Males of this species are either one of two morphs; blue or gold. While females preferred blue males in two of the three populations examined, gold

morphs were more successful in agonistic encounters and also pursued females more aggressively than did blue morphs.

The males of *Xiphophorus cortezi* are polymorphic for vertical bars, a pigment pattern found throughout swordtails (Rauchenberger et al. 1990; Moretz and Morris 2003) and platyfishes. Unlike *X. multilineatus* in which only the smallest males lack bars, there is no correlation between size and having bars in *X. cortezi,* and the two morphs appear to occur with approximately the same frequency in nature (Morris et al. 2003). The bars on males that have this pigment pattern are usually visible when males are in their resting state. However, barred males have the ability to intensify the expression of the bars as well as suppress their expression, and use this signal during courtship with females and in contests with rival males. Because the vertical bars can be easily manipulated in the laboratory, it is possible to distinguish between differences in aggression resulting from differential responses to the bars and inherent properties of being either naturally barred or barless.

The evolution of the vertical bars may have been affected by both female choice and male-male competition. Females in this species have demonstrated a polymorphism in preference for the presence or absence of the bars (Morris et al. 2003), a preference for bar symmetry (Morris 1998; Morris and Casey 1998; Merry and Morris 2001), a preference for bar number (Morris 1998) and a preference for bar frequency (Morris et al. 2001). Likewise, *X. cortezi* males use the vertical bars in aggressive encounters much like other swordtail species (e.g. Zimmerer and Kallman 1988; Moretz and Morris 2003); males intensify the expression of the bars at the onset of an aggressive encounter and bars

fade in the subordinate male at contest's end. In both staged contests and mirror image stimulation trials using males of this and several other swordtail species, intensification of the bars precedes aggressive behavior, and signals aggressive intent (Moretz and Morris 2003; unpublished data).

The purpose of this study was three-fold. First, I wanted to determine whether one morph was more dominant than the other when matched for size in staged contests and if dominance was related to aggression levels. This is important in establishing whether there is a link between dominance and aggression. Such a link, if correlated with being either naturally barred or barless, would indicate that size for size, one morph would have higher RHP than the other. Second, I wanted to determine whether contest dynamics were affected by the presence or absence of bars. Manipulating the presence of the bars on both morphs allowed me to establish whether initiation and escalation decision rules were affected by the presence or absence of bars, or whether the decision rules were correlated with a particular morph and not context dependent. Finally, I was interested in determining whether relative aggression levels were context dependent or an inherent property of being naturally barred or barless. By manipulating the bars and presenting each male with his opponent in a different context, it was possible to determine whether contest outcome and aggression levels are the result of aggressive responses to the bars or whether one morph has an advantage that is independent of bar state.

The impetus for these questions came from two previous studies. In the first, Moretz (2003) used a novel contest pairing procedure to obtain an approximation of RHP

for males relative to other males in a tournament. Revaluation of these data indicate that

of the males that were ranked higher than would be expected if size alone explained RHP,

most were barless (Figure 4.1). Additionally, while contest dynamics in the previous

study were reported in terms of smaller males versus larger males and winners versus

losers, there was not a comparison of contest dynamics of barred males versus barless

males. The current study allows for this comparison while controlling for the

confounding effects of relative size. In the second study, Moretz and Morris (2003)

found that only naturally barred males responded to the bars, and they did so with

decreased aggression towards their barred mirror image. Even though barred males were

more aggressive towards their barless mirror images than their barred mirror images,

naturally barless males exhibited higher levels of aggression than did barred males in

either state.

METHODS

Study species

Xiphophorus cortezi are small live-bearing fish that inhabit pools of streams and

rivers in northeastern Mexico. The *X. cortezi* males used in this study were collected

from the Rio Axtla in San Luis Potosi, Mexico over a period of three years (June 1999,

March 2000 and December 2001). At the end of each collection trip males were brought

back to the laboratory at Ohio University where they were individually housed in 20L

tanks and visually isolated from one another. Fish were fed daily and maintained on a

14h light/ 10h dark photoperiod at a constant room temperature of 22C. Two weeks prior

to the beginning of the contests, males were anesthetized in order to accurately weigh and measure each individual (SL, standard length).

Experimental design

Sixteen pairs of males (1 barred, 1 barless male) were matched for size (SL). The size difference between the opponents ranged from 0.1 mm to 0.7 mm ($\bar{x}$=0.38, SD=0.17). Each pair was placed in a 150 L aquarium and separated by an opaque plexi-glass divider for 24 hours. The contest began by removing the divider and allowing the two males to interact. Data were recorded by direct observation and included which male initiated the contest (first display), which male escalated (first bite) and which male won. The total number of bites for each male was also recorded and bite frequency was calculated as bites / minute (bpm). A male was determined to be the loser when he retreated from his opponent with his dorsal fins lowered and continued to retreat whenever approached. There were no instances of a male signaling defeat and then attacking. Males were visually inspected after each contest and none appeared injured. Likewise, there was no indication that males were overly stressed, as they appeared to behave normally (e.g. they continued feeding) after returning to their individual tanks.

There were three phases to the contests. The first phase consisted of contests between pairs in their natural bar state (naturally barless vs. naturally barred). After this first phase, I performed a series of manipulations. The second phase took place three weeks later, which was long enough to control for winner and loser effects, as well as opponent recognition from the previous encounter (Moretz unpublished data). The same

males were re-paired after temporary bars were applied to the naturally barless males

using antiseptic dye (Hoefler and Morris 1999). To control for possible handling effects,

the naturally barred males were pseudo-painted using water instead of the dye. The

number of bars painted on each barless male matched the number of bars on his naturally

barred opponent (min=4, max=8). The contests were performed 30 minutes after the

manipulations and the same data were collected as described above. In the third phase, I

removed the bars of naturally barred males by freeze branding the pigmented area with

dry ice (Raleigh et al. 1973) after anaesthetizing the fish with MS-222. To control for

possible effects associated with branding the fish, naturally barless males were also

freeze branded. Three weeks later, the males were then re-paired with the same males for

the final round of contests and the same data were recorded as described above. In

addition to the above controls, previous studies have demonstrated that neither technique

harms the fish or otherwise alters their behaviors (Morris et al. 1995b; Hoefler and

Morris 1999; Moretz and Morris 2003). Likewise, visual inspections of the fish did not

reveal any obvious injuries or changes in behaviors.

Statistical analyses

All analyses were performed using the statistical package NCSS (Hintze 2001).

In determining whether barred or barless males were more likely to initiate the contests,

escalate first in the contests or win more contests, logistic regressions were used where

the trials were the experimental units (Carpenter 1995; Zucker and Murray 1996).

Likewise, logistic regressions were used to analyze the same aspects of contest dynamics

as above but in terms of contest winners and losers. I used repeated measures ANOVAs to determine if either barred or barless males had higher bite frequencies and if either contest winners or losers had higher bite frequencies. Repeated measures ANOVAs were also used to determine if contest bite frequencies varied across the three experimental phases.

RESULTS

Bouts typically began when one male approached his opponent and displayed either vertically in a headstand orientation or laterally. If the male was naturally barred, his bars either intensified prior to his displaying or during his displays. Opponents usually responded by displaying and this continued until one male escalated by biting. All contests escalated to biting. Both males continued to display and bite throughout the encounter until one male signaled defeat by lowering his dorsal fin and swimming away. If the losing male was naturally barred, his bars also faded during this time. There were no observable differences between naturally barred and barless males in terms of the types of behaviors or displays used in any of the contests.

Phase 1 – both males in their natural bar state

Barless males won 12 of the 16 contests against barred males (Table 4.1). Neither barred nor barless males were more likely to initiate the encounter (logistic regression: χ^2=0.29, df=1, p=0.59, 56.3% correct model classification), however barred males were more likely to escalate first (Table 4.1). Contest winners bit more in more contests than

did losers (χ^2=12.77, df=1, p=0.0005, 87.5%), though winners were neither more likely

to initiate (χ^2=2.76, df=1, p=0.097, 68.8%) nor escalate (χ^2=2.35, df=1, p=0.126, 68.8%)

than were contest losers.

Phase 2 – artificial bars applied to naturally barless males, barred males in their natural state

Naturally barless continued to win more contests when given artificial bars,

winning two more contests (14 of 16) against naturally barred males in phase 2 than in

phase 1 (Table 4.1). Of the four barred contest winners in phase 1, only one was

successful in phase 2. There was one barred male that won for the first time in phase 2.

As in phase 1, neither barred nor barless males were more likely to initiate the encounter

(χ^2=0.25, df=1, p=0.61, 56.3%). However, unlike in phase 1 where barred males were

more likely to escalate first, neither morph was more likely to escalate first in phase 2

(Table 4.1). Like phase 1, winners were neither more likely to initiate (χ^2=3.06, df=1,

p=0.08, 68.8%) nor escalate (χ^2=0.02, df=1, p=0.89, 62.5%) than were contest losers.

Contest winners bit more in a greater proportion of contests than did losers (χ^2=9.71,

df=1, p=0.002, 81.3%).

Phase 3 – bars removed from naturally barred males, barless males in their natural state

Naturally, barless males won 13 of the 16 contests against barred males with their

bars removed (Table 4.1). Of the three naturally barred contest winners, only one won in

both the previous two phases, while one was a previous winner in phase 1. The

remaining naturally barred winner won for the first time in phase 3. As in the two

previous phases, neither barred nor barless males were more likely to initiate the

encounter (χ^2=0.01, df=1, p>0.99, 50%). However, as in phase 1 but not phase 2, the

naturally barred males were more likely to escalate first (Table 4.1). Winners bit more in

more contests than did losers (χ^2=6.90, df=1, p=0.009, 81.3%), though contest winners

were neither more likely to initiate (χ^2=0.25, df=1, p=0.61, 56.3%) nor escalate first in

more contests (χ^2=0.91, df=1, p=0.34, 62.5%) than contest losers.

Across phase analysis

Contest intensity as a whole, measured by the mean bite frequency of each pair,

did not vary across phases (phase 1: $\bar{x}$ = 10.4 bpm, SE=1.16; phase 2: $\bar{x}$ = 8.7 bpm,

SE=0.65; phase 3: $\bar{x}$ = 11.6 bpm, SE=0.98; $F_{2,47}$ = 2.76, p=0.08). Naturally barless males

had higher bite frequencies than naturally barred males regardless of treatment (Figure

4.2; $F_{1,95}$ = 15.61, p<0.0004; morph by phase interaction $F_{2,95}$ = 0.31, p=0.74) and

frequency of bites was not affected by treatment for either morph (Figure 4.2; barless,

$F_{2,47}$ = 3.04, p=0.06; barred, $F_{2,47}$ = 1.23, p=0.31). Contest winners had higher bite

frequencies than contest losers regardless of phase (Figure 4.3; $F_{1,95}$ = 20.67, p<0.0001;

outcome by phase interaction $F_{2,95}$ = 0.30, p=0.73). For all three phases, barred males

only won contests in which they had the higher bite frequency and there was a significant

difference in mean bite frequency difference between two opponents when naturally

barred males won ($\bar{x}$=0.49 bpm difference, SE=0.009) versus when naturally barless

males won ($\bar{x}$=2.84 bpm difference, SE=0.27; $t_{44.9}$ = 8.19, p<0.00001) which suggest that

even small differences in aggression may be in important in deciding contest outcome and lends further support for higher aggression levels in naturally barless males.

DISCUSSION

Naturally barless males had a clear advantage over naturally barred males and were able to win a majority of the contests regardless of whether the signal vertical bars had been experimentally added or removed. As the bar manipulations did not affect contest outcome (naturally barless males won a significant proportion of contests in all three phases), it would appear that the superior fighting abilities of naturally barless males are an inherent property of being barless, and not the result of responses to the bars themselves. Size for size, naturally barless males as a whole appear to have higher RHP than naturally barred males and the fighting advantage enjoyed by naturally barless males seems to be related in part to their higher aggressive state. Much like the results reported by Moretz and Morris (2003) where males were presented with both their barred and barless mirror images, in this study, naturally barless males had higher bite frequencies than naturally barred males regardless of treatment. Likewise, in this study barred males only won contests in which they were more aggressive, though occasionally losing encounters in which they bit more than their opponent. As contests are usually decided by asymmetries in RHP (Parker 1974; Maynard Smith and Parker 1976; Hammerstein 1981; Archer 1988), the deciding factor in these trials, where the confounding aspect of relative size has been removed, seems to be differences in aggression levels. However, aggression and contest outcome or dominance are not always correlated (e.g. Fitzgerald

and Kedney 1987) and typically, an organism's size relative to that of its opponent is an important factor in winning or losing a fight (Archer 1988). In *X. cortezi*, when opponents are not matched for size, contest outcome is largely decided by size asymmetries (Moretz 2003). In addition, there is no correlation between winning or losing a fight and aggression levels, or being larger or smaller than an opponent and aggression levels (Moretz 2003). Thus in this species, body size is the most important factor in the ability of males to obtain and defend territories, while relative aggression levels are an important factor when the size difference between two individuals is negligible.

An obvious question would be why selection would favor increased or decreased aggression in one morph and not the other. There are at least two, non-mutually exclusive hypotheses to account for the differences in aggression levels between the two natural bar types. The first focuses on selection for reduced aggression in barred males. As there is a correlation between having the genotype for the bars and having a response to the bars (Moretz and Morris 2003), and barred males in this species respond to the bars with decreased aggression, it is possible that their overall levels of aggression have been affected as well. In other words, their maximum aggression levels, which are exhibited when encountering barless males, may be limited as a result of decreased aggression toward barred males. This scenario necessarily assumes that there is a tradeoff between decreased aggression towards barred males on the one hand and increased aggression towards barless opponents on the other hand. Thus, selection for decreased aggression in response to the bars may have reduced their overall aggression levels, requiring that the

benefits resulting from decreased aggression towards barred males override any costs that result from being less aggressive than barless males. However, it is also possible that lower overall aggression levels in barred males may not be the result of a tradeoff but rather selection for lower overall aggression levels as well. This implies that the benefits of decreased aggression in response to the bars are also gained as a result of lower overall aggression levels. The second hypothesis focuses on selection due to the aggressive responses of other males. Barless males may be more aggressive because regardless of their opponent, there is no inhibition of aggression. Barless males either face other barless males which have high aggression levels or barred males who behave more aggressively towards barless males than barred males. As such, selection may have favored increased aggression in this morph as compared to barred males. Likewise, if there is a tradeoff for barred males between reduced aggression in response to the bars and maximum aggression levels, barless males would not be subject to this tradeoff, as barred males do not exhibit decreased aggression to the bars.

Neither naturally barred nor naturally barless males were more likely to initiate the contests, though naturally barless males had overall higher bite frequencies. However, naturally barred males were more likely to escalate first against barless opponents (phase 1 and in phase 3). In phase 2, where both males possessed bars, neither of the two morphs was more likely to escalate first. These results bring to attention two important points. The first is that there is a clear decision rule for the naturally barred males to escalate sooner whenever encountering a barless male, which resulted in barred males escalating before barless males in phases 1 and 3. This response seems to be both

inherent to being naturally barred, as only the barred males demonstrated a tendency to escalate first, and context dependent as naturally barred males changed their escalation strategy based on the presence or absence of bars on their opponent. The second point seems counterintuitive: the less aggressive (in terms of bite frequencies) of the two morphs is in essence behaving more aggressively by biting first. The status-signaling hypothesis predicts quite the opposite (Rohwer 1975). Fundamentally, this hypothesis proposes that coloration can accurately convey information about rank or dominance and that this information can be used by subordinate individuals to avoid potentially costly interactions (Rohwer 1982; Senar and Camerino1998), thus naturally barred males should avoid contests with barless males. Naturally barless males on the other hand, might be predicted to escalate first if males with greater fighting ability have less to gain by avoiding confrontations (Hurd 1997; but see Maan et al. 2001). However, in some instances it is the likely losers of contests that are expected to escalate first. In the Just and Morris (2003) model, an evolutionary stable strategy (ESS) is possible where males with lesser RHP initiate escalation, and the aggressive behaviors of likely losers are a direct consequence of moderate differences in RHP. In this model, if likely winners can count on likely losers to either escalate or retreat, then it may pay the probable winner to leave the initiative to the likely loser in the hope that he may retreat (possibly by mistake), in which case a costly fight would be avoided. Alternatively, as contests with these fish do not result in injury and naturally barred males are able to win occasionally, it may pay probable losers to ignore the apparent fighting asymmetries and test their opponents (Grafen 1987).

Bite frequencies as a whole, as well as bite frequencies of the two morphs individually, did not vary significantly across the three contest phases. However, there was a trend towards decreased biting in the second phase where both opponents were barred (naturally and through manipulations; Figure 4.2). We should expect that bite frequencies would be lower in phase 2 for the naturally barred males since it has been demonstrated that barred males respond to the bars with decreased aggression in this species (Moretz and Morris 2003) as well as in another swordtail species, *X. multilineatus* (Morris et al. 1995b, Moretz and Morris 2003). Likewise, because barless males do not respond to the bars with either increased or decreased aggression (Moretz and Morris 2003), bite frequencies for naturally barless males should have remained near constant. There are likely two related explanations for the observed, albeit non-significant, decrease in aggression in phase 2. The first is a response to the bars with reduced aggression by the naturally barred males to the artificial bars. Though not significant, naturally barred males hade higher bite frequencies in phase 1 (by almost one bite per minute) and phase 3 (by one bite per minute) than in phase 2, where naturally barless males exhibited artificial bars. Thus it seems possible that for the naturally barred males, aggression levels were inhibited by the presence of artificial bars on naturally barless males. The second factor may be a response of the naturally barless males to the reduced aggression levels exhibited by the barred males. As barless males do not respond to the bars themselves (Moretz and Morris 2003), it seems likely that their decreased levels of aggression in phase 2 are a direct effect of decreased aggression by barred males. In other words, it would appear that individuals shift their levels of aggressiveness based, in

part, on the aggressive levels of their opponent. It is also possible that the decreased aggression levels were the result of handling each individual (from painting), though this seems unlikely. Males were still interactive and previous studies using the painting method indicate that neither aggression levels nor behaviors were affected.

In the swordtail *X. pygmaeus*, both male aggressive behavior and female mating preferences appear important in maintaining the blue/gold polymorphism found in males (Kingston et al. 2003). Gold males are more successful in agonistic encounters than are blue males, and gold males actively pursue females to a greater extent than do blue males (possibly resulting in more forced copulations). The advantage of gold males appears to be offset by female preference for blue males, though other factors such as predation and selection on traits correlated with color morph may also play a role in the maintenance of this polymorphism (Kingston et al. 2003). The maintenance of the polymorphism in *X. cortezi* is less clear. In the absence of female mating preferences, a correlation between male competitive advantage and reproductive success would favor barless males, due to their higher levels of aggression and dominance. However females are polymorphic in their preference, with some females having a preference for barred males and others having a preference for barless males (Morris et al. 2003). While female preference likely plays a role in the maintenance of the bar polymorphism, it probably does not offset the competitive advantage of barless males to the extent that female preference in *X. pygmaeus* offsets the competitive advantage of gold males. As a result, it seems likely that other factors also play a role in the maintenance of the bar polymorphism in *X. cortezi*. For example, increased aggression may have additional costs, such as increased

mortality as a result of higher hormone levels (e.g. reduced immune system response, Alfredo et al. 1996), an increased risk of injury or increased predation (e.g. Jakobsson et al. 1995) resulting from higher levels of conspicuous behaviors (increased number of contests). Thus, the benefits associated with dominance may be offset by potential costs, leaving neither morph with a clear advantage due to male-male competition. However, relative predation and injury rates in the field between the two morphs are not known, and further studies are needed in order to fully understand the maintenance of this polymorphism.

In summary, I matched barred and barless males for size and paired them in dyadic contests. I found that naturally barless males had higher bite frequencies and were able to win a majority of the contests while barred males consistently escalated to biting first, even though in most cases they were ultimately the losers. In cases where barred males were able to win the contests, they had higher aggression levels than their barless opponents. Taken together, these results indicate that in this species, aggression is an important factor of contest outcome when opponents are evenly matched for size. Regardless of bar treatment, naturally barless males continued to be more aggressive and more dominant than their barred counterparts suggesting that higher aggression levels and dominance appear to be an inherent property of being barless rather than context resulting from responses to the bars themselves. As a result, naturally barless males as a whole appear to have higher RHP than naturally barred males of the same size because of their greater aggression levels. While relative size is a determining factor of contest

outcome in many systems, and as such often correlated with RHP, aggression levels may

also be an important component of an individual's RHP.

LITERATURE CITED

Archer, J. 1988. The Behavioural Biology of Aggression. Cambridge University Press, Cambridge.

Alfredo, S., J.P Veiga, J. Martin, P. Lopez, M. Abelenda and P. Marisa. 1996. The cost of producing a sexual signal: testosterone increases the susceptibility of male lizards to ectoparasitic infestation. Behav. Ecol. **7**: 145-150.

Baird, T.A., S.F. Fox and K.J. McCoy. 1997. Population differences in the roles of size and coloration in intra- and intersexual selection in the collared lizard, *Crotaphytus collaris*: influence of habitat and social organization. Behav. Ecol. **8**: 506-517.

Barlow, G.W. 1973. Competition between color morphs of the polychromatic Midas cichlid *Cichlasoma citrinellum*. Science **179**: 806-807.

Barlow, G.W. 1983a. Do gold Midas cichlid fish win fights because of their color, or because they lack normal coloration? Behav. Ecol. Sociobiol. **13**: 197-204.

Barlow, G.W. 1983b. The benefits of being gold: behavioral consequences of polychromatism in the Midas cichlid *Cichlasoma citrinellum*. Environ. Biol. Fishes **8**: 253-247.

Barlow, G.W. and P. Siri. 1994. Polychromatic midas cichlids respond to dummy opponents: color, contrast and context. Behaviour **130**: 77-112.

Beaugrand J., C. Goulet and D. Payette. 1991. Outcome of dyadic conflict in male green swordtail fish, *Xiphophorus helleri*: effects of body size and prior dominance. Anim. Behav. **41**: 417-424.

Beaugrand J., D. Payette and C. Goulet. 1996. Conflict outcome in male green swordtail fish: interaction of body size, prior dominance/subordination experience, and prior residency. Behav. **133**: 303-319.

Beeching, S.C. 1995. Colour pattern and inhibition of aggression in the cichlid fish *Astronotus ocellatus*. J. Fish Biol. **47**: 50-58.

Borowsky, R. 1973. Relative size and the development of fin coloration in *Xiphophorus variatus*. Physiol. Zool. **46**: 22-28.

Carpenter, G.C. 1995. Modeling dominance: The influence of size, coloration and experience on dominance relations in tree lizards. Herp. Monogr. **9**: 88-101.

Dugatkin L.A. and S.R. Ohlsen. 1990. Contrasting asymmetries in value expectation and resource holding power: effect on attack behaviour and dominance in the pumpkinseed sunfish, *Lepomis gibbosus*. Anim. Behav. **39**: 802-804.

Fitzgerald, G.J. and G.I. Kedney. 1987. Aggression, fighting and territoriality in sticklebacks: three different phenomena? Biol. Behav. **12**: 186-195.

Franck, D., A. Muller and N. Rogmann. 2003. A colour and size dimorphism in the green swordtail (population Jalapa): female mate choice, male-male competition, and male mating strategies. Acta Ethol. **5**: 75-79.

Grafen, A. 1987. The logic of divisively asymmetric contests: respect for ownership and the desperado effect. Anim. Behav. **35**: 462-467.

Hammerstein, P. 1981. The role of asymmetries in animal contests. Anim. Behav. **19**: 193-205.

Heuts, B.A. and V. Nijman. 1998. Aggressive behaviour of two swordtail colour breeds (*Xiphophorus*, Poeciliidae) in a prior residence situation. Behav. Processes **43**: 251-255.

Hintze, J. 2001. Number Cruncher Statistical Software (NCSS). Kaysville, UT.

Hoefler, C.H. and M.R. Morris. 1999. A technique for the temporary application and augmentation of pigment pattern in fish. Ethology **195**: 431-438.

Horth, L. 2003. Melanic body colour and aggressive mating behaviour are correlated traits in male mosquitofish (*Gambusia holbrooki*). Proc. R. Soc. Lond. B **270**: 1033-1040.

Hurd, P.L. 1997. Is signaling of fighting ability costlier for weaker individuals? J. Theor. Biol. **184**: 83-88.

Jakobsson, S., O. Brick and C. Kullberg. Escalated fighting behavior incurs increased predation risks. Anim. Behav. **49**: 235-239.

Just, W. and M.R. Morris. 2003. The Napoleon complex: why smaller males pick fights. Evol. Ecol. **17**: 509-522.

Kingston, J.J., G.G. Rosenthal and M.J. Ryan. 2003. The role of sexual selection in maintaining a color polymorphism in the pygmy swordtail, *Xiphophorus pygmaeus*. Anim. Behav. **65**: 735-743.

Lemel, J. and K. Wallin. 1993. Status signaling, motivational condition and dominance: an experimental study in the great tit, *Parus major*. Anim. Behav. **45**: 549-558.

Mann, M.E., G.C. Ton, G. Wittenberg and J. Wittenberg. 2001. Escalated fighting despite predictors of conflict outcome: solving the paradox in a South American cichlid fish. Anim. Behav. **62**: 623-634.

Martin, R.G. 1977. Density dependent aggressive advantage in melanistic male mosquitofish (*Gambusia holbrooki*). Florida Scientist **40**: 393-399.

Martin, D. and M.F. Hengstebeck. 1981. Eye color and aggression in juvenile guppies, *Poecilia reticulata*. Anim. Behav. **29**: 325-331.

Maynard Smith, J. and G.A. Parker. 1976. The logic of asymmetric contests. Anim. Behav. **24**: 159-175.

Mayr M. and A. Berger. 1992. Territoriality and microhabitat selection in two intertidal New Zealand fish. J. Fish. Biol. **40**: 243-256.

Merry, J.W. and M.R. Morris. 2001. Preference for symmetry in swordtail fish. Anim. Behav. **61**: 477-479.

Moretz, J.A. 2003. Aggression and RHP in the northern swordtail fish, *Xiphophorus cortezi*: the relationship between size and contest dynamics in male-male competition. Ethology **109**: 995-1008.

Moretz, J.A. and M.R. Morris. 2003. Evolutionarily labile responses to a signal of aggressive intent. Proc. R. Soc. Lond. B **270**: 2271-2277.

Morris, M.R., L. Gass and M.J. Ryan. 1995a: Assessment and individual recognition of opponents in swordtails *Xiphophorus nigrensis* and *X. multilineatus*. Behav. Ecol. Sociobiol. **37**: 303-310.

Morris, M.R., M. Mussel and M.J. Ryan. 1995b. Vertical bars on male *Xiphophorus multilineatus*: a signal that deters rival males and attracts females. Behav. Ecol. **6**: 274-279.

Morris, M.R. 1998. Female preference for trait symmetry in addition to trait size in swordtail fishes. Proc. R. Soc. Lond. B **1399**: 907-911.

Morris, M.R. and K. Casey. 1998. Female swordtail fish prefer symmetrical sexual signal. Anim. Behav. **55**: 33-39.

Morris, M.R., J.A. Elias and J.A. Moretz. 2001. Defining the sexually selected male trait vertical bars in relation to female preference in the swordtail fish *Xiphophorus cortezi*. Ethology **107**: 827-837.

Morris, M.R., P.F. Nicoletto and E. Hesselman. 2003. A polymorphism in female preference for a polymorphic male trait in the swordtail fish *Xiphophorus cortezi*. Anim. Behav. **65**: 45-52.

O'Connor, K.I., N.B. Metcalfe and A.C. Taylor. 1999. Does darkening signal submission in territorial contests between juvenile Atlantic salmon, *Salmo salar*? Anim. Behav. **58**: 1269-1276.

Parker, G.A. 1974. Assessment strategy and the evolution of fighting behaviour. J. Theor. Biol. **47**: 223-243.

Pryke, S.R., S. Andersson, J.M. Lawes and S.E. Piper. 2002. Carotenoid status signaling in captive and wild red-collared widowbirds: independent effects of badge size and color. Behav. Ecol. **13**: 622-631.

Raleigh, R.F., J.B. McLearen and D.G. Groff. 1973. Effects of topical location branding techniques, and changes in hue on recognition of cold brands in Centrarchid and Salmonoid fish. Trans. Amer. Fish. Soc. **102**: 637-641.

Rauchenberger, M., K.D. Kallman and D.C. Morizot. 1990. Monophyly and geography of the Rio Panuco Basin swordtails (genus *Xiphophorus*) with description of four new species. Am. Mus. Novit. **2975**: 1-41.

Ribowski A. and D. Franck. 1993. Subordinate swordtail males escalate faster than dominants: a failure of the social conditioning process. Aggressive Behav. **19**: 223-229.

Rowher, S.A. 1975. The social significance of avian winter plumage variability. Evolution **29**: 593-610.

Rowher, S.A. 1982. The evolution of of reliable and unreliable badges of fighting ability. Amer. Zool. **22**: 531-546.

Senar, J.C. and M. Camerino. 1998. Status signaling and the ability to recognize dominants: an experiment with siskins (*Carduelis spinus*). Proc. R. Soc. Lond. B **265**: 1515-1520.

Zimmerer, E. J. and K.D. Kallman. 1988. The inheritance of vertical barring (aggression and appeasement signals) in pygmy swordtail *Xiphophorus nigrensis* (Poeciliidae, Teleostei). Copeia **1988**: 299-307.

Zucker, N. and L. Murray. 1996: Determinants of dominance in the tree lizard
Urosaurus ornatus: The relative importance of mass, previous experience and
coloration. Ethology **102**: 812-825.

Table 4.1. Number of first escalations and contests won by natural bar morphs for each of the three experimental phases. Analyses were performed using logistic regressions with df=1. Model % indicates the percent of individuals correctly classified by the logistic regression model. In phase 1, both males are in their natural bar state. In phase 2, temporary bars have been applied to the naturally barless males. In phase 3, the bars have been removed from the naturally barred males.

	escalation					contests won				
phase	barred	barless	χ^2	p	model %	barred	barless	χ^2	p	model %
1	13	3	6.90	0.009	81.3	4	12	4.19	0.04	75.0
2	7	9	0.29	0.59	56.3	2	14	12.17	0.001	87.5
3	12	4	4.56	0.03	75.0	3	13	6.90	0.009	81.3

Figure 4.1. Modified from Moretz (2003). Relationship between standard length (mm) and RHP. Of the four males with higher ranks than comparatively larger individuals, three were barless. Pluses (+) indicate naturally barred males while minuses (-) indicate naturally barless males.
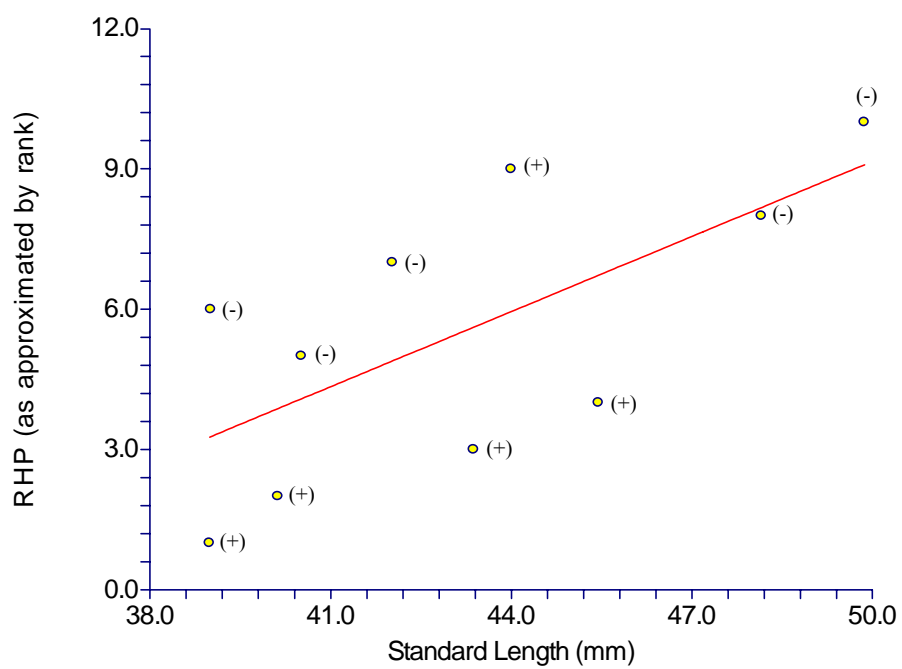
Figure 4.2. Mean bite frequency for naturally barred and naturally barless *X. cortezi* males in matched size contests across three experimental conditions (phases). In phase 1, both males are in their natural bar state. In phase 2, temporary bars have been applied to the naturally barless males. In phase 3, the bars have been removed from the naturally barred males. Error bars indicate standard error.
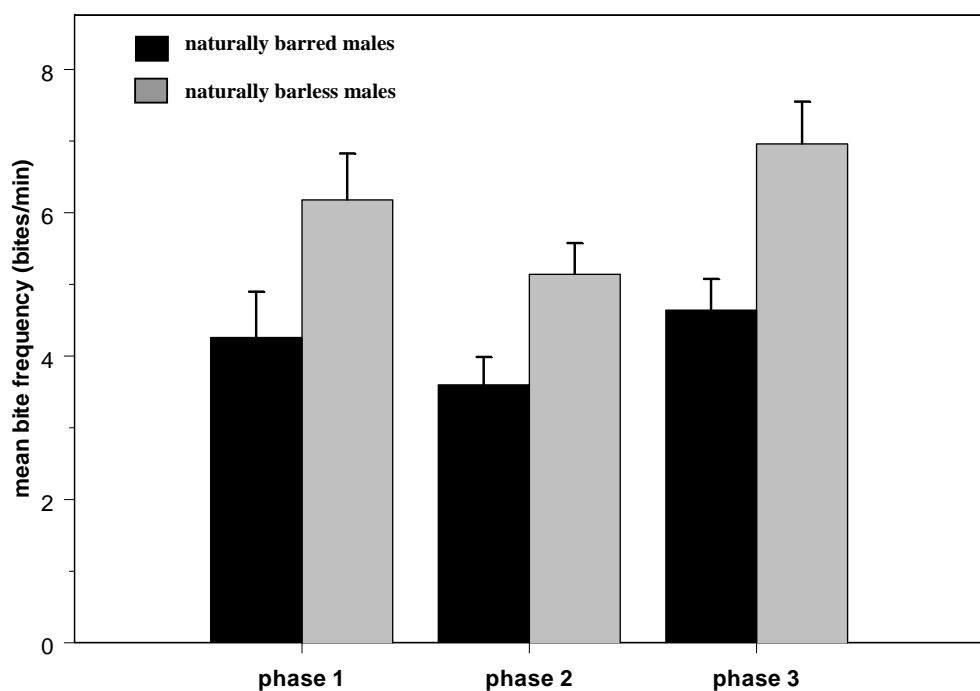
Figure 4.3. Mean bite frequency for contest winners and losers of sized matched *X. cortezi* males across three experimental conditions (phases). In phase 1, both males are in their natural bar state. In phase 2, temporary bars have been applied to the naturally barless males. In phase 3, the bars have been removed from the naturally barred males. Error bars indicate standard error.
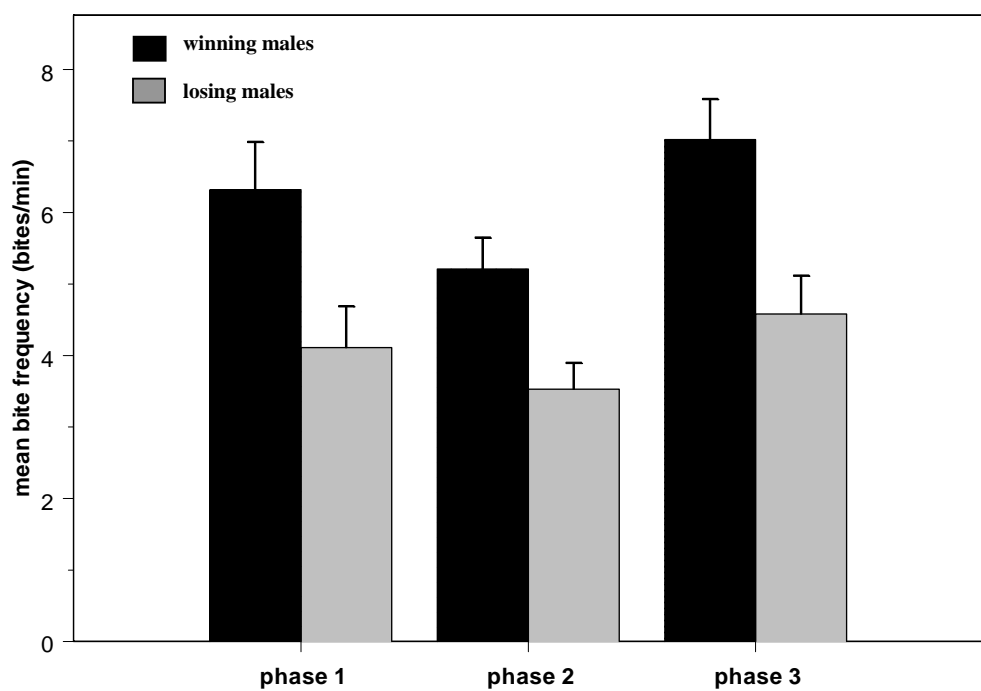
CHAPTER 5

A COMPARATIVE ANALYSIS OF THE RELATIONSHIP BETWEEN SIZE AND

AGGRESSION IN MALE NORTHERN SWORDTAIL FISHES

ABSTRACT

It is often useful to examine factors that are important in male – male interactions in order

to determine what role competition plays in the evolution of specific male traits. As

aggression functions exclusively in male- male competition, traits that are correlated with

aggression are likely affected by selection resulting from competition. In the current

study, I examined the evolutionary relationship between male size and aggression in the

northern swordtail fishes. I used phylogenetically independent contrasts of male size and

aggression scores from mirror image stimulation to determine whether there was a

relationship across species. While there are many factors influencing the evolution of

male size in swordtails, I found a positive relationship between size and aggression across

species, indicating that male-male competition has played an important role in the

evolution of male size. However, the relationship between absolute male size and

aggression varied within species. This may indicate that the species are subject to

different selection pressures resulting from competition, favoring higher levels of

aggression in smaller males in some conditions and higher levels of aggression in larger

males in others. Finally, I examined whether selection for large male size has been

relaxed in *Xiphophorus multilineatus* and *X. cortezi* barred males, which respond to the

signal vertical bars with reduced aggression. I compared the predicted mean male size of each species resulting from aggression scores in their natural bar state to the predicted mean male size resulting from aggression scores after the vertical bars had been experimentally removed. *Xiphophorus multilineatus* were smaller than expected if size alone predicted aggression levels, possibly because the bars effectively lower the intensity of competition and may reduce the number of fights. However, *X. cortezi* barred males were not smaller than expected, possibly because they compete with the more aggressive barless *X. cortezi* males which do not exhibit a decreased aggressive response to the vertical bars.

INTRODUCTION

Access to limited resources is often gained by males through direct competition with one another. Typically, male conflicts are settled by differences in resource holding power (RHP; Parker 1974; Maynard Smith and Parker 1976). If dominant males have higher mating success due to male-male competition, then traits that are correlated with dominance (or RHP) should be adaptive. While many factors are known to influence the outcome of male agonistic encounters (e.g. residency status, Leimar and Enquist 1984; payoff asymmetries, Dugatkin and Ohlsen, 1990; prior experience, Hsu and Wolf 1999), all else equal, relative size and outcome are often correlated. Larger males tend to be more dominant than comparatively smaller males because size is often related to strength or fighting ability (Jackson 1988; Maier 1998) or correlated with specialized weaponry (e.g. Hughes 1996). If larger males are more dominant and as a result are able to increase their reproductive success (e.g. Morris et al. 1992), then selection, in the absence of counter prevailing influences, should result in larger body size (Andersson 1994). Likewise, as some level of aggression is important in defending resources (Fitzgerald and Kedney 1987), as the importance of male-male competition in reproductive success increases, so should levels of overall aggression. Thus, across closely related organisms, a positive relationship between species size and aggression levels may indicate that male – male competition has played an important in role in the evolution of male size.

In the current study I investigated the evolutionary relationship between aggression and body size across the nine species of northern swordtail fishes and one closely related species used as an outgroup. While other studies have looked at the

relationship between absolute male size and aggression within a single species (e.g. Mathis and Britzke 1999), none have determined it there is an evolutionary relationship between male size and aggression across species. While it is clear that there are many factors influencing the evolution of male size in swordtails (e.g. female preference, Ryan et al. 1990; predation, Ryan et al. 1992), aggression functions exclusively in male-male competition, and a significant relationship between size and aggression across species would suggest that male-male competition plays an important role in the evolution of male body size.

I was also interested in determining whether selection for large male size has been relaxed in two swordtail species where the response to a signal of aggressive intent is reduced aggression. Both *X. multilineatus* and *X. cortezi* are polymorphic for the male trait vertical bars, though in *X. multilineatus* only the smallest males lack this pigment pattern while in *X. cortezi* there is not a correlation between having the bars and male size. Males that have this signal use it both in courtship of females and during aggressive encounters with rival males, where intensification of the bars signals aggressive intent and suppression of the bars at the end of a contest signals subordination (Morris et al. 1995; Moretz and Morris 2003). In *X. multilineatus*, all males respond to the bars with reduced aggression (Morris et al. 1995; Morris and Ryan 1996; Moretz and Morris 2003) while in *X. cortezi*, only barred males exhibit a reduced aggressive response (Moretz and Morris 2003). As the bars function as a signal that effectively lowers the intensity of competition, it is possible that selection for larger male size has been relaxed.

The northern swordtail fishes (*Xiphophorus*) are an ideal group for studying the evolutionary relationship between size and aggression. First, most of the relationships in this monophyletic group are supported by data from several different sources (Rauchenberger et al. 1990, Borowsky et al. 1995, Morris et al. 2001). Therefore, there is a well supported phylogeny for this group (Figure 5.1), which is vital to comparative analyses. Second, there are a wide range of adult sizes both within and between species due to variation at a Y-linked locus, with some species having distinct size classes (Kallman 1989). Third, male swordtail fishes do not exhibit growth after reaching sexual maturity (Kallman 1989), and as a result, it is possible to determine relationships between size and aggression, independent of confounding factors such as age. In addition, most species of swordtail fishes will act aggressively towards intruding males (Franck and Ribowski 1987; Morris et al. 1992; Morris et al. 1995, Moretz 2003). Equally important however, there is empirical evidence that supports the hypothesis of an evolutionary link between aggression levels and male size across species. In *X. pygmaeus*, large male size has been evolutionarily lost. Males of this species do chase one another but escalation to biting is infrequent, if it occurs at all (Hankinson and Morris 2002; Kingston et al. 2003; chapter 3). Levels of aggression are also relatively low in another swordtail species with small male size, *X. continens* (Morris et al. in review; chapter 3). In contrast, the males of other larger swordtail species escalate to biting frequently and have higher overall aggression levels (e.g. Morris et al. 1995; Moretz 2003). Thus, it at least seems plausible that larger species may be more aggressive than comparatively smaller ones.

METHODS

Study species

I collected adult males of all nine northern swordtail species and one closely

related species (Table 1; Figure 1) from the Rio Pánuco Drainage, Mexico. Upon return

to the laboratory, males were measured (standard length) and individually housed in 25L

aquaria that were visually isolated from one another. Fish were fed daily and maintained

on a 14h light/10h dark photoperiod at a constant room temperature of 22C. As fish were

possibly involved in male-male interactions before being collected, I allowed the males to

acclimate for two weeks before testing began in order to control for influence of recent

learning experiences. This is sufficient time to remove the effects of prior encounters

(Moretz and Morris unpublished data).

Male aggression levels

Standard mirror image stimulation (MIS) tests were used to determine the

aggression level of each male. The testing procedure consisted of attaching a mirror to

one end of an individual's tank and recording the number of bites directed at the mirror

image over a period of five minutes. Interaction time was defined as the time that an

individual interacted with his mirror image by displaying, biting or swimming back and

forth in front of his image. Interaction time also included the time that an individual

simply faced the image within 10 cm of the mirror, approximately a quarter of the total

length of the tank. The tests were repeated for each male one week later. Aggression

scores in this study include data from Moretz and Morris (2003). However, in most instances the sample size for each species is larger in the current study.

While behavioral biologists have used MIS tests for many years, their use has drawn criticism (Ruzzante 1992 but see Holtby 1992) because often MIS aggression fails to correlate with dominance resulting from dyadic contests (Earley et al. 2000). However, one of the useful properties of MIS is that it provides symmetrical aggression levels and instantaneous feedback, without the confounding factors that can result from using live fish or dummy models as stimuli (Rowland 1999). Moretz and Morris (2003) found this procedure to be a reliable means to assess aggression levels in these fish based on measures of repeatability (Becker 1984; Lessells and Boag 1987). In addition, changes in behavior in response to bar manipulations in Moretz and Morris (2003) were in the same direction (reduced aggression) as studies utilizing live males (Morris et al. 1995; Morris and Ryan 1996).

Phylogenetic and statistical analyses

Data analyses were performed with the statistical package NCSS (Hintze 2004). The relationship between size and aggression was evaluated within species using linear regressions. Regressions using phylogenetic independent contrasts (Felsenstein 1985; Figure 5.1) were used to examine the evolutionary relationship between size and aggression across species. Branch lengths were calculated from cytochrome B sequences (Dries unpublished data) using PAUP (Swofford 2002), and constrained to the Rauchenberger et al. (1990) phylogeny. Polytomies were resolved by assigning those

branches a length of 0.00001. Standardized independent contrasts were calculated using COMPARE (Martins 2003). In order to test whether the decreased aggression in response to the bars exhibited by *X. multilineatus* males and *X. cortezi* barred males resulted in mean sizes that were smaller than expected, I re-examined the relationship between size and aggression across species, omitting the data for *X. multilineatus* and *X. cortezi*. I then used the resulting regression equation and the higher aggression scores for *X. multilineatus* and *X. cortezi* resulting from the bar removal (data from Moretz and Morris 2003) to predict the mean male size for *X. multilineatus* and *X. cortezi* barred males. I compared their predicted mean sizes to their actual mean sizes using a paired t-test. As *X. cortezi* barless males do not respond to the bars, they were not considered in the analyses.

RESULTS

All males of all species interacted with their mirror images, though the total time males spent interacting with their mirror images differed across species ($F_{9,161}$=8.27, $p<0.0001$; Table 5.1). *Xiphophorus pygmaeus* males interacted with their mirror images, however in no instance were they observed biting (previous studies indicate that aggression between males is largely confined to chasing, Hankinson and Morris 2002; Kingston et al. 2003). As interaction time differed across species, aggression scores were standardized and calculated as bites per minute (bpm = total bites/interaction minutes). There were significant differences in mean size ($F_{9,161}$=30.65, $p<0.0001$; Table 5.1) and aggression levels ($F_{9,161}$=10.55, $p<0.0001$; Table 5.1) across species, indicating that there

was sufficient variation to examine the evolutionary relationship between size and aggression.

The species differed in terms of whether there was a significant relationship between size and aggression within individual species. In six species there was no relationship between size and aggression (Table 5.2). In the remaining three species that bit at their mirror images, the males of one species exhibited a positive relationship between size and aggression and two exhibited a negative relationship between size and aggression (Table 5.2).

There was a significant, positive relationship across species between the standardized contrasts of mean species size and mean species aggression levels (r^2=0.53, $F_{1,8}$=8.02, p=0.025; Figure 5.2). There was also a significant positive relationship when analyzing the species means themselves (r^2=0.59, $F_{1,9}$=11.41, p=0.01; Figure 5.3) and the slopes of the two lines were not different (t_{15}=1.75, p=0.10). A regression of the standardized contrasts for mean species size and size range indicated that as the mean size increased so did the variability in size within species (r^2=0.85, $F_{1,8}$=39.4, p=0.0004).

In order to test whether *X. multilineatus* and *X. cortezi* barred males were smaller than expected as a result of their decreased aggression to the vertical bars, contrasts were recalculated using mean species size and mean aggression levels from all species except these two (r^2=0.61, $F_{1,6}$=7.73, p=0.039). As the equation from the resulting regression using the contrasts is not suitable for predicting mean size, a regression using the raw data (species means) was used and compared to the equation using the contrasts. The equation from the raw data also indicated a significant, positive relationship between size

and aggression (r^2=0.63, $F_{1,7}$=10.09, p=0.019), and the slopes of the two lines did not

differ (t_{11}=1.68, p>0.05). In order to test whether the equation would yield accurate

predictions of actual male size, the original mirror aggression scores (this study) were

entered into the equation in order to generate predicted male sizes. These were then

tested against the actual male sizes. In neither case did the equation adequately predict

mean male size (*X. multilineatus*, t_{16}=5.22, p<0.0001; *X. cortezi*, t_{22}=2.20, p=0.04). This

is not surprising given the amount of unexplained variation in the relationship between

size and aggression. However, it is still possible to test whether males of these two

species are smaller than expected by scaling the results to the equation. In doing so, male

sizes generated from the mirror scores can be viewed as the actual scores if aggression

perfectly predicted size and compared to the male sizes resulting from the mirror scores

in which the vertical bars have been experimentally removed. In this case it is also

necessary to balance the sample sizes such that only males are considered for which

mirror tests have been conducted both before and after the bars were removed. These

data are from Moretz and Morris (2003) and thus contain only a subset of the *X. cortezi*

(n=8) and *X. multilineatus* (n=12) males included in this study. The results from the

equation indicate that *X. multilineatus* males were smaller than expected (t_{11}=4.01,

p=0.002) but *X. cortezi* males were no smaller than would be expected if aggression alone

predicted size (t_7=0.97, p=0.37).

DISCUSSION

Levels of male – male competition are inherently difficult to measure because of the many complex factors that affect competition itself (e.g. availability of territories and mates) as well as the number of ways that organisms deal with those factors (e.g. alternative reproductive strategies). Instead, it is often useful to examine factors that are important in male – male competition in order to determine what role competition plays in the evolution of specific male traits. As aggression functions exclusively in male-male competition, traits that are correlated with aggression are likely affected by selection pressures resulting from competition. In this study there was a positive relationship between size and aggression across species, suggesting that male-male competition has played an important role in the evolution of male body sizes. Likewise, there were differences in mean size and aggression levels across species, suggesting that the species have experienced different levels of competition and selection pressures, and have evolved different methods of dealing with male-male competition.

At the present, it would appear that competition is most intense in the larger species as these tend to be more aggressive than comparatively smaller species. As in many systems, relative size in swordtails is important in determining the outcome of contests and as such, selection should favor increasingly larger size. However, a variety of strategies for reducing aggression levels and competition have evolved within swordtails, suggesting that at some point male-male competition in these species may have been more intense. For instance, males of two species use the signal vertical bars in contests and aggression levels are lower when the vertical bars are present than when this

signal is removed (Morris et al. 1995; Morris and Ryan 1996; Moretz and Morris 2003).

Other species have distinct size classes and males of the smallest size classes neither

guard females nor a territory, but instead adopt a sneak chase tactic to copulate with

females (Ryan and Causey 1989; Zimmerer and Kallman 1989). In both cases, it is

possible that intense competition resulted in selection favoring behaviors that reduced

direct male-male competition. However it is also possible that the strategies for reducing

aggression levels were not the result of competition directly, but from other selection

pressures such as predation. Likewise, what appear to be strategies for reducing

aggression may instead be the result of more intense selection for some other

characteristic; for example, stronger selection for successful copulations with

uncooperative females than selection for controlling access to mates (Bisazza et al. 2000;

Magurran and Macias Garcia 2000).

While there was a positive relationship between size and aggression across

species, the relationship between absolute size and aggression differed within species.

Differences within species may signal relative differences in costs associated with being

more aggressive or inherent differences between small and large males in the value of a

contested resource. In contests between evenly sized individuals, smaller males might be

expected to have higher levels of aggression when the risk of injury increases with the

absolute size of the opponent. Thus large males would benefit from being less aggressive

because of the increased risk of injury, while small males could behave more

aggressively, as they would be less likely to sustain substantial injury from their opponent

than would larger males. Another simple explanation for a negative relationship between

size and aggression is that in systems where size is a major determinant of contest outcome, larger males may not need to assert aggression as often as smaller males (Hagelin 2002). Smaller males might also be expected to be more aggressive than larger males if there is an inherent asymmetry in the value of the contested resource that favors smaller males. For instance, territories may be more valuable to smaller males because they probably have greater difficulty in obtaining them than larger males and as a result are more aggressive because of their greater motivation. However others have suggested that larger males actually have more to gain, and as a result exhibit higher levels of aggression because they may be more likely to successfully defend the territory over a longer period of time than would smaller males (Mathis and Simons 1994; Mathis and Britzke 1999). Likewise, the cost of contests themselves may be more energetically costly for smaller males if body size is correlated with energy reserves, in which case we might predict higher aggression levels in larger males (Mathis and Britzke 1999).

Within species relationships do not necessarily impact the relationship across species. In the current study, only *X. multilineatus* exhibited a positive relationship between absolute size and aggression, while there was a negative relationship in both *X. cortezi* and *X. nigrensis*. Mathematically it would be possible for each species to have a negative relationship between size and aggression and still have a positive relationship across species, so long as larger species had higher mean levels of aggression. Thus studies of male-male competition across species are useful in identifying evolutionary patterns resulting from correlated traits, while studies that focus on the relationships

within species can suggest differences in the relative costs of aggression for smaller and larger males.

Selection for larger male size may be relaxed in *X. multilineatus*, a species that responds to the vertical bars with decreased aggression. Recall that in this species, that the predicted mean size resulting from aggression scores in their natural state was less than the predicted mean size resulting from aggression scores after their bars had been removed. While relative size is usually the determining factor of contests in this species (unpublished data), selection for increased opponent assessment so that contests can be settled with relatively less aggression may be more intense than selection for larger male size. In *X. multilineatus*, the number of vertical bars is correlated with male size (Zimmerer and Kallman 1998) and as a result could aide individuals in assessing the size of an opponent. Likewise, the expression of the bars is intensified prior to biting and could signal willingness of an individual to escalate to biting (Moretz and Morris 2003). Thus, the bars may provide information that individuals could use in determining their fighting ability and or motivational state relative to that of their opponent's, thereby reducing the number and intensity of fights.

Unlike *X. multilineatus*, reduced aggression in response to the vertical bars is not linked with decreased size in barred *X. cortezi* males. In this species, predicted mean size resulting from aggression scores in their barred state (lower aggression) did not differ from predicted mean size resulting from aggression scores after their bars had been removed (higher aggression). Even though the bars may provide the same opportunities for increased opponent assessment as in *X. multilineatus*, barred males must still compete

with barless males, which are more aggressive and lack the same response to the bars that barred males exhibit (Moretz and Morris 2003). Relative size is an important component of contest outcome in *X. cortezi* (Moretz 2003), though when matched for size, contest winners tend to have higher aggression levels than their opponents (chapter 4). In essence, barred males may be at a competitive disadvantage because they are less aggressive than their barless counterparts. Given their lower levels of aggression, we might predict that barred males should be larger than barless males. However, my collection records indicate that barred and barless morphs occur with roughly the same frequency (Morris et al. 2003) and are approximately the same size (unpublished data). This suggests that in this species, factors other than size and aggression are important in gaining access to mates and other resources.

In summary, I found a significant relationship between size and aggression across species. While many other factors affect male size, these results suggest that male – male competition is one of the important factors in the evolution of male size. In addition, the relationship between size and aggression varied within species. This may indicate that the species face different selection pressures as a consequence of competition, resulting in costs and benefits that favor higher levels of aggression in larger males in some conditions and lower levels of aggression in others. Finally, selection for larger male size may be relaxed in *X. multilineatus* as a result of a signal that reduces aggression in contests between males. However, *X. cortezi* barred males were not smaller than expected even though they also exhibit decreased aggression to the vertical bars, possibly because they must compete with the more aggressive barless *X. cortezi* males.

LITERATURE CITED

Andersson, M. 1994. Sexual Selection. Princeton University Press.

Becker, W.A. 1984. Manual of Quantitative Genetics. Pullman, WA: Academic
    Enterprises.

Bisazza, A., S. Manfredi and A. Pilastro. 2000. Sexual competition, coercive mating and
    mate assessment in the one-sided livebearer, *Jenynsia multidentata*: are they
    predictive of sexual dimorphism? Ethology **106**: 961-978.

Borowsky, R. L., M. McClelland., R. Cheng, and J. Welsh. 1995. Arbitrarily primed
    DNA fingerprinting for phylogenetic reconstruction in vertebrates: The
    Xiphophorus model. Molec. Bio. Evol. **12**: 1022-1032.

Carpenter, G.C. 1995. Modeling dominance: The influence of size, coloration and
    experience on dominance relations in tree lizards. Herp. Monogr. **9**: 88-101.

Dugatkin, L.A. and S.R. Ohlsen. 1990. Contrasting asymmetries in value expectation
    and resource holding power: effects on attack behaviour and dominance in the
    pumpkinseed fish, *Lepomis gibbous*. Anim. Behav. **39**: 802-804.

Earley, R.L., Y. Hsu and L.L. Wolf. 2000. The use of standard aggression testing
    method to predict combat behaviour and contest outcome in *Rivulus marmoratus*
    dyads (Teleostei: Cyprinodontidae). Ethology **106**: 743-761.

Felsenstein, J. 1985. Phylogenies and the comparative method. Am. Nat. **125**: 1-15.

FitzGerald, G.J. and G.I. Kedney. 1987. Aggression, fighting and territoriality in
    sticklebacks: three different phenomena? Biology of Behav. **12**: 186-195.

Franck, D. and A. Ribowski. 1987. Influence of prior agonistic experience on
    aggression measures in the male swordtail (*Xiphophorus helleri*). Behaviour **103**:
    217-240.

Hagelin, J.C. 2002. The kinds of traits involved in male-male competition: a comparison
    of plumage, behavior and body size in quail. Behav. Ecol. **13**: 32-41.

Hankinson, S.J. and M.R. Morris. 2002. Sexual selection and species recognition in the
    pygmy swordtail, Xiphophorus pygmaeus: conflicting preferences. Behav. Ecol.
    Sociobiol. **51**: 140-145.

Hintze, J. 2004. Number Cruncher Statistical Software (NCSS). Kaysville, UT.

Holtby, L.B. 1992. Through a glass darkly: a response to Ruzzante's reappraisal of mirror image stimulation studies. Can. J. Fish. Aqu. Sci. **49**, 1968-1969.

Hsu, Y. and L.L. Wolf. 1999. The winner and loser effect: integrating multiple experiences. Anim. Behav. **57**: 903-910.

Hughes, M. 1996. Size assessment via a visual signal in snapping shrimp. Behav. Ecol. Sociobiol. **38**:51-57.

Jackson, W.M. 1988. Can individual differences in history of dominance explain the development of linear dominance hierarchies? Ethology **79**: 71-77.

Kallman, K. D. 1989. Genetic control of size at maturity in *Xiphophorus*. In: Ecology and Evolution of Livebearing Fishes (Poeciliidae). (Ed. by G. K Meffe and F.F. Snelson), pp. 163-184. Englewood Cliffs, N.J: Prentice-Hall.

Kingston, J.J., G.G. Rosenthal and M.J. Ryan. 2003. The role of sexual selection in maintaining a color polymorphism in the pygmy swordtail, *Xiphophorus pygmaeus*. Anim. Behav. **65**: 735-743.

Leimar, O. and Enquist, M. 1984. Effects of asymmetries in owner-intruder conflicts. J. Theor. Biol. **111**: 475-491.

Lessells, C.M. and P.T. Boag. 1987. Unrepeatable repeatabilities: A common mistake. The Auk **106:** 116-121.

Magurran, A.E. and C. Macias Garcia. 2000. Sex differences in behaviour as an idirect consequence of mating system. J. Fish Biol. **57**: 839-857.

Maier, R. 1998. Comparative animal behavior: and evolutionary and ecological approach. Needham Heights: M.A.

Mathis, A. and R.R. Simons. 1994. Size-dependent responses of resident male red-backed salamanders to chemical stimuli from conspecifics. Herpetologica **50**: 335-344.

Mathis, A. and E. Britzke. 1999. The roles of body size and experience in agonistic displays of the ozark zigzag salamander, *Plethodon Angusticlavius*. Herpetologica **55**: 344-352.

Martins, E. P. 2003. COMPARE, version 4.5. Computer programs for the statistical analysis of comparative data. Distributed by the author at http://compare.bio.indiana.edu/. Department of Biology, Indiana University, Bloomington IN.

Maynard Smith, J. and Parker, G.A. 1976. The logic of asymmetric contests. Anim. Behav. **24**: 159-175.

Meyer, A., J. Morrissey, and M. Shartl. 1994. Recurrent evolution of a sexually selected trait in *Xiphophorus* fishes inferred from a molecular phylogeny. Nature **368**: 539-542.

Moretz, J.A. 2003. Aggression and RHP in the northern swordtail fish, *Xiphophorus cortezi*: the relationship between size and contest dynamics in male-male competition. Ethology **109**: 995-1008.

Moretz, J.A. and M.R. Morris. 2003. Evolutionarily labile responses to a signal of aggressive intent. Proc. R. Soc. Lond. B **270**: 2271-2277.

Morris, M.R., Batra, P. and Ryan, M.J. 1992. Male-male competition and access to females in the swordtail *Xiphophorus nigrensis*. Copeia **1992**: 980-986.

Morris, M.R., M. Mussel and M.J. Ryan. 1995. Vertical bars on male *Xiphophorus multilineatus*: a signal that deters rival males and attracts females. Behav. Ecol. **6**: 274-279.

Morris, M.R. and M.J. Ryan. 1996. Sexual differences in signal-receiver coevolution. Anim. Behav. **52**: 1017-1024.

Morris, M.R., K. De Queiroz and D.C. Morizot. 2001. Phylogenetic relationships among populations of northern swordtails (*Xiphophorus*) as inferred from allozyme data. Copeia **2001**: 65-81.

Morris, M.R., P.F. Nicoletto and E. Hesselman. 2003. A polymorphism in female preference for a polymorphic male trait in the swordtail fish *Xiphophorus cortezi*. Anim. Behav. **65**: 45-52.

Morris, M.R., J.A. Moretz, K. Farley and P. Nicoletto. In review. The role of sexual selection in the loss of sexually selected traits in the swordtail fish *Xiphophorus continens*.

Parker, G.A. 1974. Assessment strategy and the evolution of fighting behaviour. J. Theor. Biol. **47**: 223-243.

Rauchenberger, M., K.D. Kallman and D.C. Morizot. 1990. Monophyly and geography of the Rio Panuco Basin swordtails (genus *Xiphophorus*) with description of four new species. Am. Mus. Novit. **2975**: 1-41.

Rowland, W.J. 1999. Studying visual cues in fish behavior: a review of ethological techniques. Envir. Biol. Fish. **56**: 285-305.

Ruzzante, D.E. 1992. Mirror image stimulation, social hierarchies and populational differences in agonistic behaviour: a reappraisal. Can. J Fish. Aqu. Sci. **49**: 1966-1968.

Ryan, M.J. and B.J. Causey. 1989. "Alternative" mating behavior in the swordtails Xiphophorus nigrensis and Xiphophorus multilineatus. Behav. Ecol. Sociobiol. **24**: 341-348.

Ryan, M. J., D. Hews, and W.E. Wagner Jr., 1990. Sexual selection on alleles that determine body size in the swordtail *Xiphophorus nigrensis*. Behav. Ecol. Sociobiol. **26**:231-237.

Ryan, M. J., C.M. Pease, and M.R. Morris 1992. A genetic polymorphism in the swordtail Xiphophorus nigrensis: Testing the predictions of equal fitnesses. Am. Nat. **139**: 21-31.

Swofford, D.L. 2002. PAUP: Phylogenetic analysis using parsimony,4th edition (4.0 beta 10). Sinauer Associates, Sunderland, Mass.

Zimmerer, E. J. and K. D. Kallman. 1988. The inheritance of vertical barring (aggression and appeasement signals) in pygmy swordtail *Xiphophorus nigrensis* (Poeciliidae, Teleostei). Copeia **1988**: 299-307.

Zimmerer, E. J. and K. D. Kallman. 1989. Genetic basis for alternative reproductive tactics in the pygmy swordtail, *Xiphophorus nigrensis*. Evolution **43**: 1298-1307.

Table 5.1. Sample size, mean standard length (SL), SL range, mean mirror interaction time and mean bite rate (bpm) for each species. Taxa have been grouped by clade (Rauchenberger et al. 1990). Values in parentheses indicate standard deviation.

clade species	n	SL (mm)	SL range	interaction (min)	bpm
outgroup					
X. variatus	10	33.1 (3.3)	10.1	9.94 (0.09)	3.81 (2.00)
montezumae					
X. montezumae	17	41.4 (6.3)	21.1	9.96 (0.06)	4.05 (1.59)
X. nezahualcoyotl	22	36.6 (5.1)	17.9	9.99 (0.06)	7.10 (3.30)
X. continens	19	25.3 (1.3)	6.4	7.63 (1.90)	1.32 (1.03)
cortezi					
X. malinche	11	38.7 (4.1)	13.6	9.94 (0.11)	4.48 (2.15)
X. birchmanni	15	46.2 (7.7)	25.4	9.16 (1.17)	5.41 (3.29)
X. cortezi	23	38.6 (4.2)	16.4	9.73 (0.64)	5.38 (3.38)
pygmaeus					
X. nigrensis	19	32.1 (5.2)	17.6	9.34 (1.10)	3.97 (3.22)
X. multilineatus	17	35.0 (4.3)	16.6	9.04 (1.92)	2.07 (1.75)
X. pygmaeus	9	22.8 (0.6)	1.5	8.19 (1.65)	0.00 (0.00)

Table 5.2. The relationship between size (SL) and aggression (bpm) within each species. Taxa have been grouped by clade (Rauchenberger et al. 1990). NS denotes no significant relationship; NA indicates that no biting was observed.

clade species	relationship	r^2	F_{df}	p
outgroup				
X. variatus	NS	0.14	$F_{1,9}=1.29$	p=0.29
montezumae				
X. montezumae	NS	0.02	$F_{1,16}=0.23$	p=0.64
X. nezahualcoyotl	NS	<0.01	$F_{1,21}=0.004$	p=0.95
X. continens	NS	0.03	$F_{1,18}=0.54$	p=0.47
cortezi				
X. malinche	NS	0.02	$F_{1,10}=0.19$	p=0.67
X. birchmanni	NS	0.18	$F_{1,14}=2.89$	p=0.11
X. cortezi	negative	0.18	$F_{1,22}=4.57$	p=0.04
pygmaeus				
X. nigrensis	negative	0.47	$F_{1,18}=15.08$	p=0.001
X. multilineatus	positive	0.37	$F_{1,16}=8.83$	p=0.01
X. pygmaeus	NA	NA	NA	NA

Figure 5.1. The northern swordtail phylogeny (Rauchenberger et al. 1990). Numbers
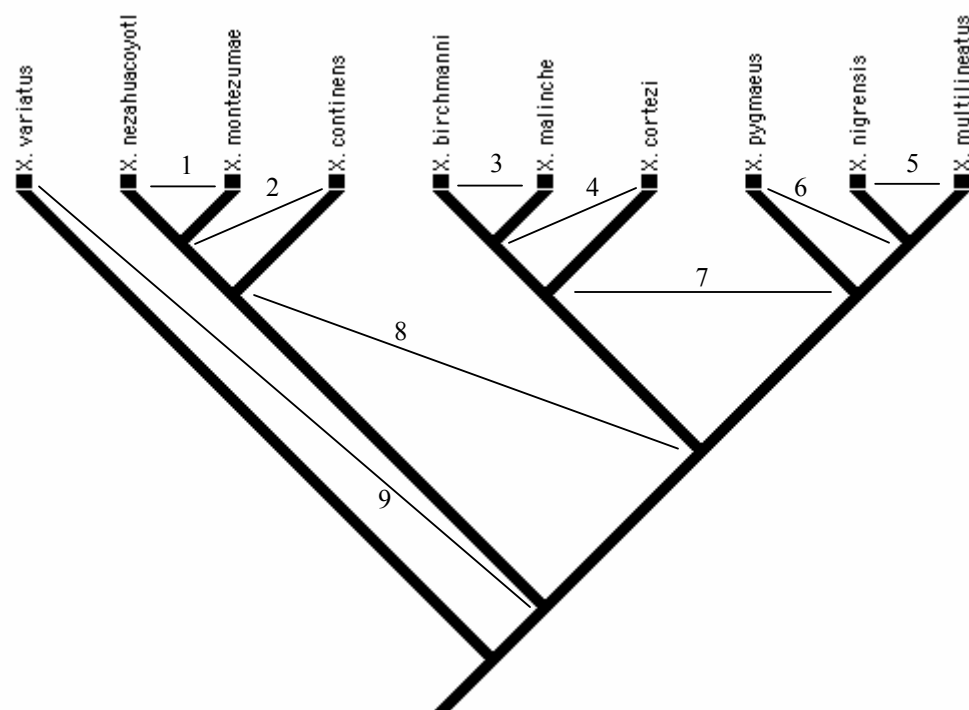correspond to contrasts used in the phylogenetic analysis.

Figure 5.2 Bites per minute contrasts regressed against standard length contrasts for nine nodes of the *Xiphophorus* fish phylogeny. Numbers designate the contrasts in Figure 5.1. The regression line has the equation bpm contrast= 2.50+0.25SL contrast (r^2=0.53, $F_{1,8}$=8.02, p=0.025).
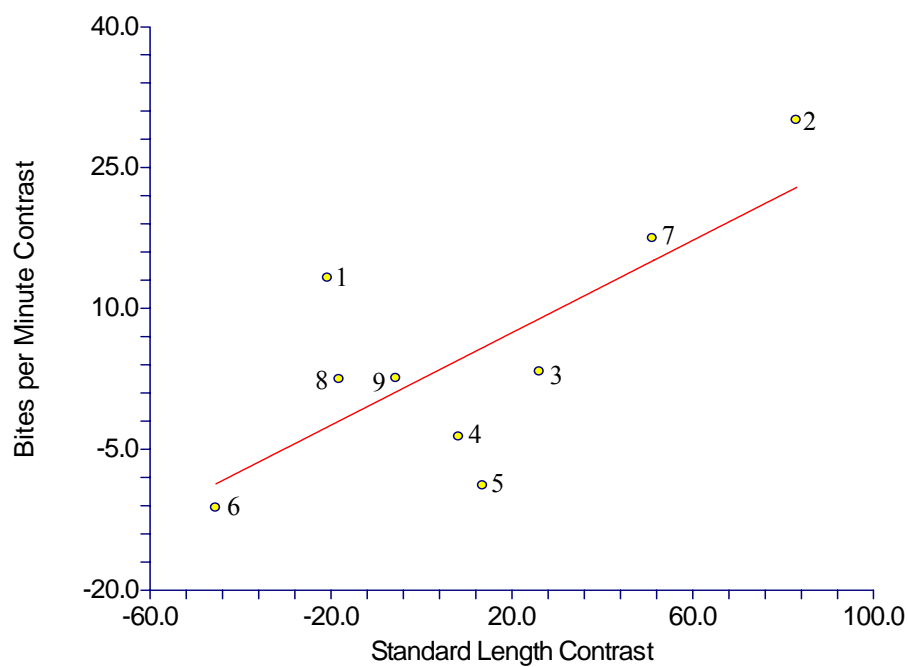
Figure 5.3 Bites per minute regressed against standard length for ten species of *Xiphophorus* fishes. The regression line has the equation bpm= -4.24+0.23SL (r^2=0.59, $F_{1,9}$=11.41, p=0.01).