

Evolutionarily labile responses to a signal of aggressive intent

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Males of many swordtail species possess 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. We 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. We 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, we 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.

Keywords: aggression; conventional signals; *Xiphophorus*; signal–receiver coevolution

1. 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 & Vehrencamp 1998). In particular, the response of the receiver should be an important selective force acting on signal design and use. 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 & Ritchie 1989; Morris & Ryan 1996) as well as the behaviours that link them (Endler 1992). However, the coevolution of signal and receiver response has not always been detected (McKinnon & McPhail 1996; Ryan & Rand 1998; Quinn & Hews 2000), some of which is attributable to differences between sexes (Searcy & Brenowitz 1988; Morris & Ryan 1996). For example, Quinn & Hews (2000) reported behavioural 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.

We were 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 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 & 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 & 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 the number of bars (*X. multilineatus*, Zimmerer & Kallman 1988) and the total pigment area (*X. cortezi*, Morris *et al.* 2001a) are both correlated with male size in some species.

We 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, we wanted to determine if:

- (i) expression of the bars is a good indicator of male aggression across species;
- (ii) whether or not the response to the bars is correlated with having this pigment pattern; and
- (iii) 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 & Ryan 1996) determined that *X. multilineatus* males responded with decreased aggression towards males with bars as compared to males

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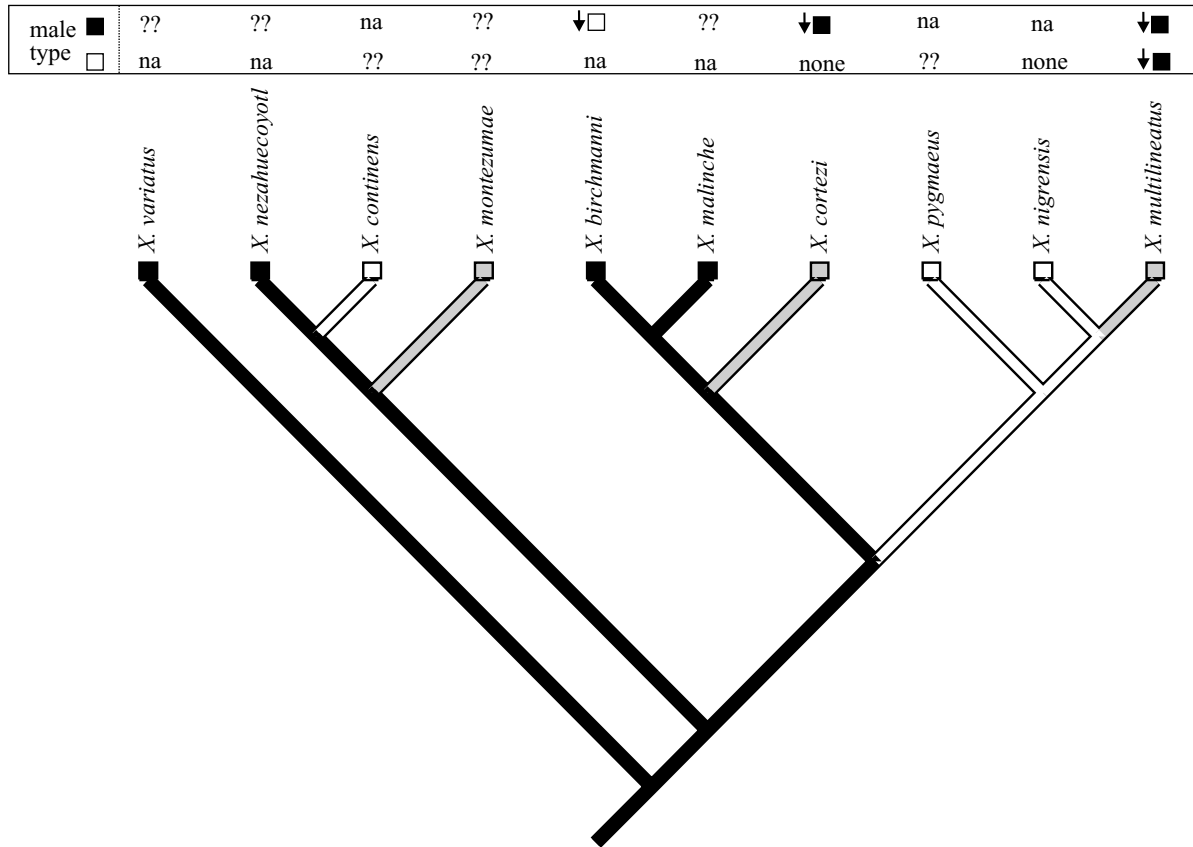


Figure 1. The male trait vertical bars mapped onto the Rauchenberger *et al.* (1990) tree using parsimony. White lines/boxes, no bars; black lines/boxes, bars; shaded lines/boxes, polymorphic. The box above the tree summarizes the responses of male swordtail (*Xiphophorus*) fishes to the vertical bars (this study, Morris *et al.* 1995). Black boxes indicate barred male responses while white boxes indicate barless male responses to either bars (black boxes) or no bars (white boxes). '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.

without bars, but a response to vertical bars was absent in the sister species that lacks bars (*X. nigrensis*). In the current study, we 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 we can determine if the mirror tests give the same results as tests using live males. We 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 (Zimmerer & Kallman 1988). Finally, we examine and compare the responses to the bars in two additional species that are also closely related (figure 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 our analysis allows us to determine if the response to the bars is uniform over a larger representative sample of swordtail species. In addition, we 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.

2. MATERIAL AND METHODS

(a) Study species

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

The four species differ with respect to whether or not males possess the vertical bar pigment pattern (figure 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. Zimmerer & 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 out of the 17 *X. cortezi* males were barless (mean SL barless males = 37.3 mm, s.d. = 3.6; mean SL barred males = 40.4 mm, s.d. = 2.7; $t = -2.01$, $p = 0.06$) and only the four smallest males lacked bars in *X. multilineatus* (mean SL of barless males = 30.6 mm, s.d. = 4.5, mean SL of barred males = 37.8 mm, s.d. = 1.7; $t = -3.95$, $p = 0.003$).

(b) 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 5 min. The 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. The interaction time also included the time that an individual simply faced the image within the distance of 10 cm to the mirror, approximately one-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 (J. A. Moretz and M. R. Morris, personal communication). The tests were repeated for each male one week later.

After the initial two mirror tests, we performed a series of manipulations. For those males with bars, we removed the bars by freeze branding (Raleigh *et al.* 1973) after anaesthetizing the fishes with MS-222. Temporary bars were applied to barless males using antiseptic dye (Hoeffler & Morris 1999). In both cases control subjects were used to ensure that the procedures themselves were not altering the behaviours 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 fishes nor otherwise alters their behaviours (Morris *et al.* 1995; Hoeffler & 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 min after being painted.

We used a repeated measures ANOVA to compare the response of individuals to their barred and unbarred images. 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, we calculated the repeatability (Becker 1984; Lessells & Boag 1987) using the between and within group variance from a one-way ANOVA to ensure the reliability of this procedure.

3. 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 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 fishes as stimuli (Rowland 1999). Our measures were repeatable between testing periods, indicating that the MIS method used in this study was both appropriate and reliable.

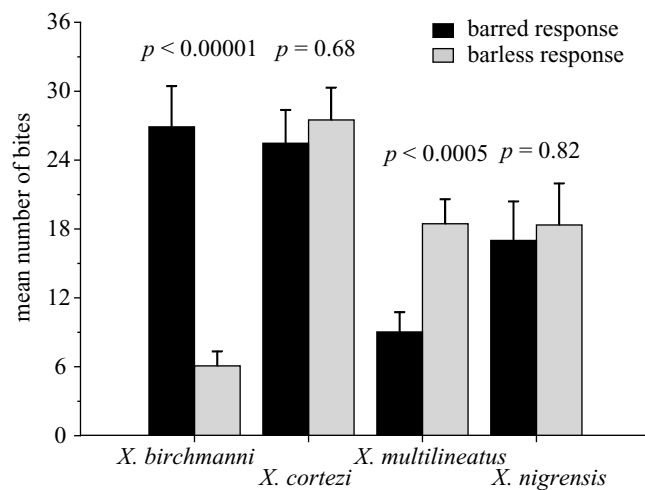
All naturally barred individuals expressed their bars in the mirror tests. Out of the eight naturally barred *X. cortezi* males, only one failed to bite at his barred mirror image. The same was true of *X. multilineatus*, with one male of the eight not biting at his mirror image. All *X. birchmanni* 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, the 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 & Morris 2003).

The 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 ($F_9 = 1.27$, $p = 0.27$), and as such interaction times were not used in additional analyses. However, the aggressive responses to the bars did vary across species (table 1, figure 2). *Xiphophorus 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. *Xiphophorus multilineatus* males as a whole bit more at their barless images (figure 2; $F_{3,47} = 9.49$, $p < 0.0005$). 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 & 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 3; $F_{3,31} = 6.68$, $p = 0.002$) and the smaller, naturally barless males (figure 3; $F_{3,15} = 4.39$, $p = 0.036$). *Xiphophorus 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 the 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; $F_{3,67} = 0.50$, $p = 0.68$) and this trend continued for naturally barless males (figure 3; $F_{3,35} = 0.09$, $p = 0.97$). However, naturally barred males did exhibit a difference, biting more at their barless images (figure 3; $F_{3,31} = 3.20$, $p = 0.044$). 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 ($F_{3,35} = 1.48$, $p = 0.24$). *Xiphophorus birchmanni* males exhibited the opposite

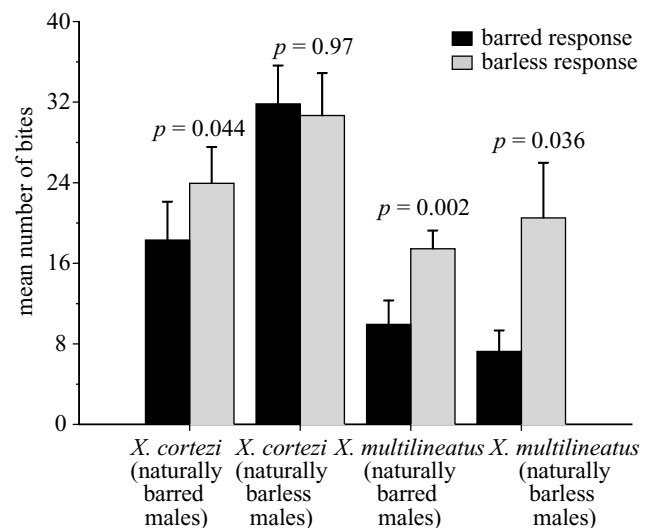
Table 1. Mean displays (d_x), mean bites (b_x) and repeatabilities (r) for males of four swordtail species for both pre- and post-manipulation MIS trials.

(The parentheses indicate the 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
<i>Xiphophorus birchmanni</i>	$d_1 = 6.9$ (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
<i>X. cortezi</i>								
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
<i>X. multilineatus</i>								
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)		$b_1 = 7.3$ (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
<i>X. nigrensis</i>								
barless	$d_1 = 4.1$ (1.1)		$b_1 = 17.4$ (4.7)		$d_1 = 4.3$ (2.9)		$b_1 = 15.8$ (4.5)	
	$d_2 = 3.6$ (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. Mean number of bites by males of four swordtail species during five minute MIS trials. p -Values were calculated from repeated measures ANOVA. The error bars indicate the standard error.

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; $F_{3,51} = 15.74$, $p < 0.00001$). 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).

Figure 3. Mean number of bites by males of two swordtail species during 5 min 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. The error bars indicate the standard error.

4. 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 we tested. All males expressed their bars before biting at their mirror image. Thus, like the auditory conventional signalling of the banded wren (Molles & 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, the 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, we 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. We were 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 our 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 3), as well as additional data from staged contests

(Moretz 2003; Moretz & Morris 2003), 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 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, Zimmerer & Kallman (1988) have demonstrated that the smaller males in *X. multilineatus* do possess genes for bars, but do not express bars owing to 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 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 & Price 1973; Maynard Smith & Parker 1976). Compared to the males with bars in *X. cortezi*, *X. birchmanni* males were relatively more aggressive towards bars compared to no bars, which is likely to mean more fights, as males express their bars more often than not in the field (M. R. Morris, personal communication). In addition, fights between live males in *X. birchmanni* include more aggressive behaviours (Moretz & Morris 2003) 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). By contrast, the same costs associated with signalling 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 we 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 our observations were the result of learned experiences. We believe this to be unlikely for several reasons. First, this would require that our sampling of individuals was biased in such a manner that both within and across species we collected a disproportionate number of experienced versus inexperienced males (or vice versa). While it is impossible to know the experience levels of the males we collected, our 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 (J. A. Moretz and M. R. Morris, unpublished 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, our 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.

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REFERENCES

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

Proc. R. Soc. Lond. B

- 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. & Vehrencamp, S. L. 1998 *Principles of animal communication*. Sunderland, MA: Sinauer.
- Butlin, R. K. & 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.* **400**, 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. & 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 Ruzante'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*. *Am. 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. & Boag, P. T. 1987 Unrepeatable repeatabilities: a common mistake. *Auk* **106**, 116–121.
- McKinnon, J. S. & McPhail, J. D. 1996 Male aggression and colour divergent populations of the threespine stickleback: experiments with animations. *Can. J. Zool.* **74**, 1727–1733.
- Maynard Smith, J. 1982 *Evolution and theory of games*. Cambridge University Press.
- Maynard Smith, J. & Price, G. R. 1973 The logic of animal conflicts. *Nature* **46**, 15–18.
- Maynard Smith, J. & Parker, G. A. 1976 The logic of asymmetric contests. *Anim. Behav.* **24**, 159–175.
- Meyer, A., Morrissey, J. & Shartl, M. 1994 Recurrent evolution of a sexually selected trait in *Xiphophorus* fishes inferred from a molecular phylogeny. *Nature* **368**, 539–542. ●●1●●.
- Molles, L. E. & Vehrencamp, S. L. 2001 Songbird cheaters pay a retaliation cost: evidence for auditory conventional signals. *Proc. R. Soc. Lond. B* **268**, 2013–2019. (DOI 10.1098/rspb ●●2●●.)
- 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. *Behav. Ecol. Sociobiol.* (Submitted●●3●●.)
- Moretz, J. A. & Morris, M. R. 2003 Variation in aggression across three species of swordtail fishes: assessing the benefits of mirror tests. *Behav. Ecol. Sociobiol.* (Submitted●●3●●.)
- Morris, M. R. & Ryan, M. J. 1996 Sexual differences in signal–receiver coevolution. *Anim. Behav.* **52**, 1017–1024.
- Morris, M. R., Mussel, M. & Ryan, M. J. 1995 Vertical bars on male *Xiphophorus multilineatus*: a signal that deters rival males and attracts females. *Behav. Ecol.* **6**, 274–279.
- Morris, M. R., Elias, J. A. & Moretz, J. A. 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. & Morizot, D. 2001b Phylogenetic relationships among the Northern Swordtails (*Xiphophorus*) as inferred from allozyme data. *Copeia* **2001**, 65–81.

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- Morris, M. R., Hesselman, L. & Nicoletto, P. 2003 A polymorphism in female preference for a polymorphic male trait in the swordtail *Xiphophorus cortezi*. *Anim. Behav.* **65**, 45–52.
- Parker, G. A. 1974 Assessment strategy and the evolution of fighting behaviour. *J. Theor. Biol.* **47**, 223–243.
- Quinn, V. S. & Hews, D. K. 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. (DOI 10.1098/rspb ●●2●●.)
- Raleigh, R. F., McLearen, J. B. & 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. Am. Fish. Soc.* **102**, 637–641.
- Rauchenberger, M., Kallman, K. D. & 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. *Environ. 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. Aquat. Sci.* **49**, 1966–1968.
- Ryan, M. J. & Rand, A. S. 1998 Evoked vocal response in male tungara frogs: pre-existing biases in male responses? *Anim. Behav.* **56**, 1509–1516.
- Searcy, W. A. & Brenowitz, E. A. 1988 Sexual differences in species recognition of avian song. *Nature* **332**, 152–154.
- Zimmerer, E. J. & Kallman, K. D. 1988 The inheritance of vertical barring (aggression and appeasement signals) in pygmy swordtail *Xiphophorus nigrensis* (Poeciliidae, Teleostei). *Copeia* **1988**, 299–307.
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