Vertical bars on male *Xiphophorus multilineatus*: a signal that deters rival males and attracts females

Molly R. Morris, Michelle Mussel, and Michael J. Ryan
Department of Zoology, University of Texas, Austin, TX 78712, USA

We examined the function of the vertical bar pattern on male swordtails (*Xiphophorus multilineatus*) as a signal in both male–male competition and female choice. This pattern had previously been described as an aggressive signal because males intensified the bars during male–male encounters in the laboratory. Our field observations supported this observation and also showed that bars intensified when males courted females. The intensity of bars was correlated with access to females in the field. Within the size range of males that have bars, however, neither bar number nor male size appeared to influence access to females. We used freeze-branding to remove the bars from males in the laboratory so that we could control for characters correlated with bar intensity, and tested males and females separately so that we could separate the influence of these two components of sexual selection. We compared the responses of males and females to males that had their bars removed and control males freeze-branded between the bars. Test males responded more aggressively to males without bars as compared to control males. In addition, females showed a preference for control males over males that had their bars removed. These results suggest that the bars may function as a signal that deters rival males and attracts females. Key words: aggressive signal, courtship, dichromatism, female choice, male–male competition, Poeciliidae, sexual selection, vertical bars, *Xiphophorus multilineatus*. [Behav Ecol 6:274–279 (1995)]

The significance of intrapopulation variation in color patterns in fish has been examined extensively (Barlow, 1983; deMartini, 1985; Endler, 1983; Long and Houde, 1989; McLennan and McPhail, 1989; Semler, 1971; Stepien et al., 1988). Many species of teleost fish can rapidly modify dichromatistics and often do so during social interactions (deMartini, 1985). Several studies have examined changes in coloration and pigment patterns as signals of aggression, and it has also been suggested that some of these signals play a role in female choice (Heiligenberg, 1976; Kingston, 1980; Martin and Hengstebek 1981; Neil, 1984; Thresher, 1984). One of the difficulties in determining whether these color and pigment changes function as signals that deter rival males and attract females is that they are often correlated with other male traits. Few studies have examined the function of these color or pigment patterns through experimental manipulations that control for correlated characters (although see Kodric-Brown 1989; Semler, 1971).

Through field observations and laboratory manipulations, we examined the function of a pigment pattern that can be facultatively expressed on males in the swordtail fish *Xiphophorus multilineatus*. *X. multilineatus* is a member of the northern swordtail clade (Rauchenberger et al., 1991). The vertical bars found on *X. multilineatus* and several other species in the genus *Xiphophorus*, as well as in other genera of poeciliid fishes (e.g., Poecilidae, Phallocthys), are composed of hundreds of closely associated melanophores (Gordon, 1931). The intensity of the bars increases dramatically during male–male interactions in several species of *Xiphophorus* (Franck, 1964; Zimmerer and Kallman, 1988). Male size is highly variable in *X. multilineatus* due to four alleles at the *P* locus on the Y chromosome, which control the age at which males reach sexual maturity and cease growth (Kallman, 1984, 1989). The three largest size classes of males have vertical bars, while females and males from the smallest size class do not. Zimmerer and Kallman (1988) demonstrated that the bars have a polygenic basis with one sex-linked factor having a major effect. The bar genes appear to influence both bar number and bar intensity. Bar number and male size are positively correlated in the larger males, and the lack of bars in small males is due to a suppressor gene (Zimmerer and Kallman, 1988).

The goals of this study were to determine (1) whether changes in the intensity of the bars in the field occurred in the same situations as changes in the laboratory, (2) whether variation in the intensity of the bars or in the number of bars influenced access to females, and (3) whether variation in the intensity of the bars or in the number of bars influenced access to females and attracted males.

METHODS

Field observations

We made field observations with snorkel and mask in March 1990 in the Rio Coy south of Ciudad Valles, San Luis Potosi, Mexico (18°N, 89°W). All observations were made between 0900 and 1400 h.

We used the same methods as described in Morris et al. (1992) with *X. nigrensis*. Before behavioral observations commenced, we counted the number of females and males within a 1-m² area. The river bottom dropped off within 3 m of the bank, and most of the fish stayed in this shallow portion of the river (approximately 1.5 m deep). A rope marked with tape every 1 m was tied parallel to the bank of the river to aid in locating quadrats that had been sampled. We made the assumption that when we moved to a new area we were observing different males. Results from a previous study with *X. nigrensis*, the sister species to *X. multilineatus*, in which
marked males returned to within 1 m of the same site in subsequent days, support this assumption (Morris et al., in press).

Males used in the focal observations were observed for several minutes prior to the beginning of the observation period to determine if they were likely to remain in the area of the quadrat. We made focal observations on 22 males. Each male observed was classified as large (>32 mm) or intermediate (>25 mm but <32 mm) by holding a small ruler, clearly marked at 25 mm and 32 mm, up close to the fish. We did not catch the fish to measure them, and therefore these estimates are used only as relative measurements of male size and not indicators of genotype. We counted the number of bars on 13 of the 22 focal males. When bar number varied from side to side, an average number of bars for the two sides was recorded.

We made focal observations for 10 min or until the focal male was lost from view. When observers were in position (approximately 1 m from the quadrat), their presence did not appear to disturb the behavior of the fish. Males courted females and chased males within 10 cm of the observers. Not all behaviors recorded occurred within the original 1-m² quadrat, but all did occur within a 0.5-m range of the quadrat. Focal periods averaged 7.98 min and ranged from 3 to 10 min. The difference between the duration of the focal observations for the large (x = 8.55 min, SD = 2.22) and intermediate size classes (x = 7.54 min; SD = 2.63) was not statistically significant (t = 0.98; df = 21; p = .34). During focal observations, we recorded the number of times each male displayed to females (glided back and forth either to the side or in front of a female; Ryan and Causey, 1989). We also recorded the number of times a male was chased by another male (flies), the number of times he chased other males (chases), and the relative size of the other male involved in a chase when possible. We noted changes in bar intensity in many cases. Based on observations in the field and the laboratory, these changes were translated into a qualitative index of bar intensity with four categories: completely faded (not visible), faded, intermediate, and dark.

We determined whether male size influenced the rate of interactions with other males (chases/min or flies/min) or interactions with females (court/min) with one-way analyses of variance. We also examined the relationships between bar number and interactions with males and females with linear regressions to determine whether bar number influenced these interactions in a manner similar to male size.

To determine whether bar number or bar intensity might influence a male’s mating success, we examined the relationships between both of these component of the bars and access to females. We measured access to females as the number of females on the 1-m² quadrat prior to the focal observations divided by the number of males on the quadrat. The maximum intensity of the bars does not vary among individuals (Zimmerer and Kallman, 1988). However, bar intensity changed depending on whether males were chasing males, courting females (bars intensified), or fleeing (bars faded, see Results below). The duration of a chase was very similar to the duration of a court (male glides back and forth in front of a female). We subtracted the number of times a male fled (bars fade) from the total number of times he chased another male or courted a female (bars intensified) to get an index of the relative time the bars were intensified. We tested relationships for significance with linear regressions.

Laboratory experiments
We determined the function of the bars in X. multilineatus by testing the responses of both males and females to control males with bars as compared to males with bars removed. We removed bars from males by freeze-branding (Hert, 1986; Raleigh et al., 1973). Twelve pairs of males were matched for size (within 0.5 mm) and anesthetized with MS222. One male from each pair was freeze-branded on the bars (bars removed) and one between the bars (control). Given a difference in size in some cases (within 0.5 mm), the larger of the two males was chosen to be the bars removed male. Pigmentation in the braded areas faded after 2–3 days, and the behavior of the males did not appear to be affected by the manipulation. Freeze-branding in this manner produced no other visible marks. During contests, the intensity of the bars on control males was either intermediate or dark. The bars removed males simulated males with a bar intensity of completely faded. Freeze-branded bars (bars removed and controls) are referred to as the experimental males.

The fight intensity of test males in contests with control males compared to their fight intensity in contests with bars removed males the same size as the control males was used as an indicator of male response to bars. Test males were 0.5–4.5 mm smaller than the experimental males, and only one test male was tested with each pair of experimental males. Experimental and test males were kept isolated in individual 2.5 l tanks throughout the testing period. Tests were conducted in a 45 × 60 × 41 cm tank with gravel on the bottom and black plastic covering the ends and back side. We placed one test male and one experimental male on either side of an opaque partition that divided the test tank into two equal parts. After 24 h, we removed the partition and recorded the number of bites delivered by each male, the length of the contest, and the winner of the contest. Contest length was measured as time from when the first male approached the time when one male became dominant. A male was considered dominant when the other male lowered his dorsal fin and retreated when approached. We used bites/min as a measure of fight intensity. The following day, the same test male was tested against the other experimental male from the pair. We tested half of the test males against the bars removed males first and half against the control males first to control for any influence of prior fighting experience (Franck and Ribowski, 1987). We compared the fight intensity of test males in contests with control males to their fight intensity in contests with bars removed males using a Wilcoxon matched-pairs signed-rank test. We also examined relationships between fight intensity and size difference between males to determine whether the relative size of the opponents influenced how the males responded to the bars.

We tested the responses of females with the same 12 pairs of experimental males used to test the responses of males. Prior to testing, males and females were separated for at least 1 week. The test aquarium measured 45 × 90 × 41 cm and was divided into five equal sections. Plexiglass separated the sections at each end from the three central sections. One male from each pair was placed on either end of the test tank, and a female was placed in an opaque cylinder in the center of the test tank. All three fish were allowed to acclimate for 10 min. After removing the opaque cylinder, we recorded for 20 min the time the female spent in each of the sections near the males. The males were then switched end to end and the entire test repeated to control for side-bias. We compared the total time females spent with control males and with bars removed males using a Wilcoxon matched-pairs signed-rank test.

RESULTS

Field observations
The average number of females per m² quadrat was 8.0 (SD = 5.29, N = 22), while the average number of males was 3.18
The intensity of bar interactions for the males was positively correlated with the number of males per quadrant (r = 0.5, p = 0.01). Number of females was positively correlated with the number of males per quadrant (r = 0.5, p = 0.01). The number of bars varied between the males and females (Table 1). In the males, the intensity of bar interactions was highest for the males with the highest number of bars (r = 0.5, p = 0.01). The intensity of bar interactions was highest for the females with the highest number of bars (r = 0.5, p = 0.01). The intensity of bar interactions was highest for the males with the highest number of bars (r = 0.5, p = 0.01). The intensity of bar interactions was highest for the females with the highest number of bars (r = 0.5, p = 0.01).

Table 1: Relationships between the number of bars and the intensity of bar interactions.

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<thead>
<tr>
<th>Behavior</th>
<th>Large males</th>
<th>Intermediate males</th>
<th>Small males</th>
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<td>x</td>
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<td>Large males</td>
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<td>Intermediate males</td>
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Figure 1: Relations between two components of the vertical bars on males and females. A. Relationship between the number of bars and the intensity of bar interactions. B. Relationship between the number of bars and the intensity of bar interactions in males. C. Relationship between the number of bars and the intensity of bar interactions in females. D. Relationship between the number of bars and the intensity of bar interactions in males and females.
DISCUSSION

Field observations supported previous laboratory observations (Franck, 1964; Zimmerer and Kallman, 1988) that the bars on X. multilineatus males intensified in aggressive interactions and faded when a male was submissive. The range of male–male interactions and intensity of the bars, however, was greater in the laboratory than in the field. Aggressive interactions in the field did not include biting but consisted almost exclusively of chasing and fleeing, and the bars never faded completely on males in the field as they did in the laboratory. In addition, we observed that the bars on males intensified during courtship, suggesting that the bars may also function in female choice. We used the observation that the intensity of the bars faded when a male was fleeing and intensified when males were courting and chasing to arrive at a relative index of the time a male’s bars were intensified. Since this index of bar intensity was positively correlated with access to females and bar number was not correlated with access to females, we suggest that among the males that have bars, bar intensity may play a more important role than bar number in gaining access to females.

Correlations between aspects of the bars and access to females could result from factors correlated with the bars (e.g., male size, condition, behavior) rather than the bars themselves. In addition, since access to females in the field was measured as the number of females per male per quadrat, access could result from females preferring to associate with certain males or from certain males being better at excluding rival males. We controlled for male size and other characters that might be correlated with the bars by manipulating the bars in the laboratory. We then tested males and females separately so that we could distinguish the influences of female choice and male–male competition. Our laboratory results demonstrated that control males with bars elicited less aggressive behavior from the smaller test males than did males of the same size as control males with bars removed. In addition, females preferred to associate with the control males with bars compared to those with no bars. Therefore, when the bars are expressed at some level of intensity (or not completely faded), they may function to deter rival males and attract females in X. multilineatus.

Our results suggest that the presence of the bars should reduce the aggressive behavior of rival males and attract females and that increasing the intensity of the bars should increase access to females. Therefore, why don’t all of the males have bars expressed at their highest intensity all of the time?
The facultative nature of the bars in the larger males, and the complete suppression of the bars in the small males, strongly suggest a cost to the bars in *X. multilineatus*. We have shown that having dark bars should enhance rather than reduce a male's mating success. Costs could result from predation pressures or social interactions. If vertical bars increase conspicuousness, they could increase risk of predation. In addition, there may be a cost to the bars in aggressive interactions due to injury or unnecessary loss of energy for individuals that continue and/or escalate a contest in which they cannot win (Maynard Smith and Harper, 1988; Rohwer and Ewald, 1981). The presence of the suppressor gene that reduces bar expression in small *X. multilineatus* males strongly suggests that the cost/benefit ratio for the bars is size dependent.

In a previous study of unmanipulated *X. multilineatus* males, size was a significant determinant of fight outcome, and fight intensity decreased as size difference between contestants increased, indicating that males assessed the size of their opponents (Morris et al., in press). As expected, the relationship between fight intensity and difference in size in contests with control males was also negative, although not statistically significant. The lack of significance could result from the smaller range of size differences examined in the present study compared to the study of unmanipulated males. What was unexpected, however, was the significant positive relationship between fight intensity and difference in size in contests where the larger male had his bars removed. When we remove variation in fight intensity due to differences between test males by examining the difference in fight intensity between contests with bars removed and contests with control males, we get an even clearer indication that fight intensity in relation to the difference in size between opponents was changed by removing the bars. Further tests are needed to determine whether this change results from the behavior of the smaller test males, the larger experimental males, or both. One could compare the behavior of test males in contests with males without bars of various sizes, or the behavior of one opponent could be held constant in some manner. While it is difficult to imagine why a smaller test male would fight more intensely with a larger male without bars than with a smaller male without bars, it seems likely that larger males might increase their fight intensity as their opponent's size decreases in relation to their own.

Because the number of vertical body bars on *X. multilineatus* males is correlated with size (Zimmerer and Kallman, 1988) and size determines fight outcome, male *X. multilineatus* could use the number of bars to assess male size. In our field study, number of bars was significantly correlated with the number of times a male fled. However, this relationship could result from assessment of body size and not assessment of bar number. The male we observed with the greatest number of bars is the only male observed chasing males larger than himself, suggesting that males may sometimes rely on bar number over actual size in their assessment of an opponent. Interactions with females, on the other hand, were not greater for males with more bars or larger size. Females may not differentiate between males as long as they use courtship behavior (only the smallest size class of males do not court). This is apparently the case in the closely related species *X. nigrensis*. In *X. nigrensis*, male–male competition was sensitive to differences in intermediate and large males (Morris et al., 1992), while female preference tests indicated that females preferred large courting males over small noncourting males, but showed no preference for large courting males over intermediate courting males (Ryan et al., 1990). Another possibility is that females use bar number to assess males, but only within the context of another variable that we could not control in the field (e.g., male size, bar intensity, or bar symme-

try). Experiments currently underway examine the relationships between number of bars, male size, and female preference by manipulating the number of bars on males in the laboratory.

In summary, within the size classes of males that have bars in *X. multilineatus*, the component of the bars an individual can rapidly modify (bar intensity) appeared to influence access to females in the field to a greater extent than the component correlated with body size (bar number). Laboratory results only pertain to extreme differences in bar intensity, but they also suggest that expressing the bars at some intensity (not completely faded) should increase a male’s access to females due to both deterring rival males and attracting females. This system provides an interesting opportunity to examine the evolution of a signal that can be rapidly modified and yet functions as a signal in both components of sexual selection.

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