Covariation between predation risk, body size and fin elaboration in the green swordtail, *Xiphophorus helleri*

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Natural and sexual selection can have either opposing or synergistic effects on the evolution of traits. In the green swordtail *Xiphophorus helleri*, sexual selection arising from female choice is known to favour larger males and males with longer swords. We examined variation in male and female size and fin morphology among 15 populations that varied in their predation environments. Males and females from populations in which piscivorous fishes were present had longer and deeper bodies than did males and females from populations in which piscivorous fishes were absent. Controlling for a positive effect of body size on sword length, males from populations in which piscivores were present had relatively shorter swords than did males from populations in which piscivores were absent. The associations between morphology and predation environment may be due to direct effects of predation, indirect effects of predation, other sources of selection that covary with predator presence, or other environmental effects on trait expression. These results suggest that while sexual selection favours longer swords, natural selection may have an opposing effect on sword length in populations with predators. Natural selection on body size, however, may act synergistically with sexual selection in populations with predators; both may favour the evolution of larger body size. The body size results for *X. helleri* contrast with related taxa that have become model systems for the study of life history evolution. © 2004 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2004, **83**, 87–100.

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INTRODUCTION

The traits expressed in animals are often thought to reflect a balance between opposing sources of selection. Two sources of selection that are commonly observed to have opposing effects on trait evolution are natural selection due to predation and sexual selection due to female choice. Traits that make males more attractive to females often also increase the probability of being eaten by a predator (Mitchell & Mau, 1971; Moodie, 1972; Cade, 1975; Soper, Shewell & Tyrrell, 1976; Bell, 1979; Endler & Houde, 1995; Wagner, 1996). One well-documented example of the opposing effects of mate choice and predation involves morphological and life-history traits in the guppy *Poecilia reticulata*. In this poeciliid fish, female choice favours males with brighter coloration (Houde, 1987; Houde & Torio, 1992) and, in some populations, larger

males (Reynolds & Gross, 1992; Endler & Houde, 1995). Predation, in contrast, favours duller coloration and smaller size (Endler, 1980; Reznick & Endler, 1982; Gong, 1995). The result is that both coloration and size can vary among populations depending upon predation environment. In general, when natural and sexual selection have opposing effects on trait evolution, we expect to see a balance in which intermediate forms are favoured.

A synergistic effect of sexual selection and predation on trait evolution has been reported less frequently than have opposing effects. Males with preferred traits, however, may have a lower risk of predation for a number of reasons. First, males with preferred traits may be in better condition, and thus may be better at escaping from predators. In a meta-analysis of 69 studies, Jennions, Møller & Petrie (2001) found a positive relationship between ornament expression and male survival, an effect that they suggest is due to a positive effect of condition on both ornament expres-

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sion and survival. Whether this relationship is partially due to a greater ability of males with larger ornaments to avoid predators is not known. Second, males with preferred traits may show correlated behaviour that reduces their predation risk. In the field cricket *Gryllus integer*, males that produce more attractive song types are more cautious towards predators (Hedrick, 2000). Third, males with preferred traits may be more difficult for predators to capture and handle because of a direct effect of the trait on a predator's ability to capture and consume the male. Although few preferred traits have been shown to directly reduce the probability of being eaten by a predator, large body size may be different from many other types of traits because larger prey may be more difficult to capture (Fuiman & Magurran, 1994; Juanes & Conover, 1994; Juanes, 1994), more difficult to handle (Elner & Hughes, 1978), and could entail a higher risk of injury for a predator. Whether larger size reduces predation risk will depend, in part, upon predator size and the mechanism of prey capture.

For a trait to evolve in response to selection there must be genetic variation for the trait. In several poeciliid fish species, allelic variation at a single locus explains a large proportion of the variation in body size (Kallman, 1989; Trexler, 1989). This locus, the pituitary locus (P-locus), has been studied most thoroughly in the genus *Xiphophorus*, in which P-locus effects have been suggested in ten species (Kallman, 1989). In the southern platyfish *X. maculatus*, a suite of alleles called P-alleles that assort at the P-locus strongly influence the age and size at which males and females become sexually mature (Schreibman & Kallman, 1977; Bao & Kallman, 1982; Kallman, 1983, 1989). These effects are due to P-locus regulation of gonadotropin-releasing hormone, which regulates the initiation of sexual maturation (Bao & Kallman, 1982; Kallman, 1983, 1989; Halpern-Sebold, Schreibman & Margolis, 1986). While the P-locus has been found to affect sexual maturation, it does not appear to affect growth rate in either males or females (Kallman & Borkoski, 1978; Kallman, 1989). Environmental conditions can also affect age and size at sexual maturation. For example, early and late maturing genotypes of *X. maculatus* that were exposed to different diet conditions showed substantial overlap in their age at sexual maturation but much less overlap in their size at sexual maturation (McKenzie *et al.*, 1983). Like *X. maculatus*, the male green swordtail *X. helleri* can mature earlier at smaller sizes or later at larger sizes (Peters, 1964), and this difference is attributable to allelic variation at the P-locus (Kallman, 1989). In addition, Campton (1992) reported a heritability of 0.82 for the total length of males that were reared individually. While it appears that P-alleles have similar effects on sexual maturation in males and females, postmaturation growth varies between the sexes; after sexual maturation, body growth in male *X. helleri* ranges between 0 and 10% (Campton, 1992; A.L. Basolo, unpubl. data), while females grow 30–60% after sexual maturation (A. L. Basolo, unpubl. data). Because males grow little after sexual maturation, the P-alleles affect not only the age and size at sexual maturation, but also adult body size; earlier maturers are smaller as adults than are later maturers. For females, adult body size depends not only on the P-alleles, but also on the time since sexual maturation (thus the time for postmaturation growth) and on postmaturation environmental effects.

A characteristic feature of male *X. helleri* is the presence of an elaborate sword that consists of at least four components: elongation of a set of ventral caudal fin rays, an upper black stripe, a lower black stripe and internal coloration of orange, yellow or green (see Rauchenberger, Kallman & Morizot, 1990; Basolo, 1996). The sword of this species extends in a straight line from the body beyond the caudal fin margin and is displayed to females during courtship (Clark, Aronson & Gordon, 1954; Franck, 1964; Hemens, 1966). In the two populations that have been tested for a sword preference, females preferred males with longer swords (Basolo, 1990, 1998a). Tests using video stimuli also suggest that females prefer males with longer swords (Trainor & Basolo, 2000). The effect of sword length on the outcome of male–male competition, however, is not clear (Beaugrand, Caron & Comeau, 1984). Because of its length and coloration, the sword is potentially a conspicuous trait. It may be easily detectable not only by conspecific females, but also by predators, depending on water colour, ambient light level and turbidity (e.g. Franck, Dikomey & Schartl, 2001).

Larger male body size, like longer sword length, is favoured by sexual selection. In one population that has been tested, females preferred larger males to smaller males (Basolo, 1998b). In a second population, the strength of a female's mating response was positively correlated with male size (Basolo, 1998a). Tests using video stimuli also suggest that females prefer larger males (Rosenthal & Evans, 1998). Female preference for larger males has also been demonstrated for five related poeciliid fishes, *X. maculatus*, *X. nigrensis*, *Po. reticulata*, *Po. latipinna* and *Brachyrhaphis rhabdophora* (Zimmerer & Kallman, 1989; Ryan, Hews & Wagner, 1990; Reynolds, 1993; Ptacek & Travis, 1998; Basolo, 2004), although there is variation among populations in female size preferences for a sixth species, *X. pygmaeus* (Ryan & Wagner, 1987; Morris, Wagner Jr & Ryan, 1996). In addition to its effect on mate choice, male size has also been found to affect the outcome of male–male interactions in *X. helleri*; larger males tend to win in fights with smaller males (Ribowski & Franck, 1993). Thus,

both intrasexual and intersexual selection can favour larger male size.

X. helleri co-occur with piscivorous fishes at some localities, while at others these predators are absent. We were interested in investigating the relationship between predation environment and male and female morphology. Here we report the results of a study of geographical variation in male and female body and fin characters for 15 *X. helleri* populations, investigating how these characters vary between populations with and without predators.

METHODS

SAMPLING LOCATIONS

X. helleri are freshwater, livebearing poeciliid fishes occurring along the Atlantic slope and coastal plain of Central America from the Rio Nautla, Vera Cruz, Mexico to Belize, Central America (C.A.). These swordtails can be found in mountain streams and rivers as well as lowland bodies of water, including streams, ponds, rivers and lagoons.

Fifteen populations of *X. helleri* were sampled at field sites ranging from Vera Cruz, Mexico to Belize, C.A.; these were designated field sites 13, 53, 72, 74, 92, 93, 94, 95, 96, 104, 105, 106, 114, 119 and 121. A description of each site, including the location and a list of the sympatric fish species, is given in the Appendix. Field sites with at least one of four predators, *Cichlasoma friedrichsthali*, *C. octofasciatum*, *Petenia splendida*, or *Belonesox belizanus*, were designated predator-present environments and sites lacking all of these piscivorous fishes were designated predator-free environments. The first three predators are cichlid fishes, and the fourth is the largest of the poeciliid fishes. These four species are voracious predators of fish (Konings, 1989; Conkel, 1993; Greenfield & Thomerson, 1997; A. L. Basolo, pers. observ.), and will capture and consume adult *Xiphophorus* in the laboratory (A. L. Basolo, pers. observ.). Four of the sites (13, 53, 106, and 119) had at least one of the piscivorous predators present and hence were designated predator-present environments. The remaining 11 sites had no predaceous fishes capable of capturing adult swordtails and were designated predator-free environments (although birds, reptiles and mammals may prey on swordtails). The 15 sites were selected such that the effect of gene flow would be minimal; the populations were either located in different river drainages or separated by barrier waterfalls or distance.

FIELD METHODS

Fish were collected with a 1/4′ mesh 10 ft seine and scored alive. Sampling for all sites occurred during the dry seasons in 1992–95 and 1997–98. Fish that were sampled from Mexico were done so under a permit awarded by the Secretaria de Relaciones Exteriores and the Secretaria de Medio Ambiente Recursos Naturales y Pesca. Fish that were sampled from Belize were done so under a permit awarded by the Fisheries Department, Ministry of Agriculture and Fisheries. For each station, males were measured for: (1) standard length (the distance from the most anterior point of the body to the point just posterior to the caudal vein along the centre of the fish), (2) body depth (the largest distance from the dorsal to the ventral side), and (3) sword length (the distance from the insertion of ventral caudal fin rays 1–13 to the distal-most tip of these rays). Swords at all except one site were straight; at Site 104 they were curved upward. Due to the abnormal appearance of the swords at this site and the difficulty in measuring the length in the field due to the curvature, the data for sword length at this site were not included. At 12 sites, male caudal fin length (the distance from the insertion of the caudal rays to the distal tips along the centre of the caudal fin) was also measured (all sites except 13, 53, and 74). At each site, females were measured for: (1) standard length and (2) body depth. At 12 sites, female caudal fin length was also measured (all sites except 13, 53, and 74). Measurements were taken using digital calipers. We measured males with complete swords containing all four of the sword components. While males become sexually mature before all the sword components develop, their swords have not fully developed. The presence of the 'gravidity' spot was used to classify females as sexually mature.

STATISTICAL ANALYSES

We examined variation among populations in male and female morphology using one-way ANOVA with population as the independent variable. Planned comparisons were then used to compare male and female morphology between populations from predator-present and predator-free environments.

Body length was positively correlated with all of the other morphological traits. To examine variation among populations in these morphological traits after removing the effect of body length, we first tested for an interaction between standard length and population using ANCOVA. For those traits in which there was not a significant interaction between standard length and population, and thus no evidence of variation among populations in the relationship between standard length and the trait of interest, we obtained residuals from the regression of each trait on standard length. We then examined variation among populations in residual body depth, sword length and caudal fin length using one-way ANOVA with population as

the independent variable. Planned comparisons were then used to compare male and female morphology between populations from predator and predator-free environments.

We compared variation among populations in male and female morphology using two-way ANOVA with population and sex as the independent variables. In these analyses, a significant interaction between population and sex indicated that the degree of sexual dimorphism varied among populations. Planned comparisons were then used to compare the degree of sexual dimorphism between populations from predator and predator-free environments (i.e. whether the effect of sex on morphology varied between populations with and without piscivorous fishes).

For figures and summary statistics, we present relative trait values (e.g. relative sword lengths). These relative trait values are adjusted for the effect of stan-

dard length on the trait. We adjusted each trait to the mean standard length (SL) for all populations (37.5 mm for males and 33.5 mm for females) using the equation: $T_{adj} = T_{obs} + slope\ (SL_x - SL_{obs})$, where T_{adj} = the trait value after adjusting for standard length, T_{obs} = the observed trait value, slope = the slope of standard length regressed on the trait, SL_x = the mean standard length and SL_{obs} = the observed standard length.

RESULTS

VARIATION IN MALE MORPHOLOGY AMONG POPULATIONS

Male morphological measurements for each population are summarized in Table 1. There was significant variation among populations in standard length

Table 1. Summary statistics by population for standard length (SL), body depth (BD), sword length (SWL), relative sword length (SWL$_R$) and caudal fin length (CFL) for males and females

Population	Sex	SL	BD	SWL	SWL$_R$	CFL
Site 13*	Male	42.5 ± 9.4 (13)	13.5 ± 3.2 (12)	30.7 ± 16.1 (13)	26.2 ± 8.9 (13)	
	Female	41.9 ± 6.0 (7)	13.9 ± 3.0 (7)			
Site 53*	Male	40.5 ± 3.9 (45)	14.2 ± 1.5 (14)	26.5 ± 8.7 (45)	23.8 ± 7.2 (45)	
	Female	41.3 ± 3.5 (21)	15.0 ± 2.4 (4)			
Site 72	Male	37.4 ± 3.7 (43)	10.0 ± 1.2 (39)	31.4 ± 4.7 (43)	31.5 ± 2.4 (43)	10.6 ± 1.3 (9)
	Female	35.5 ± 2.0 (24)	10.8 ± 0.7 (20)			
Site 74	Male	34.8 ± 5.2 (6)	9.5 ± 1.7 (6)	27.0 ± 8.6 (6)	29.4 ± 5.0 (6)	
	Female	34.7 ± 4.1 (20)	10.7 ± 1.3 (20)			
Site 92	Male	30.3 ± 3.9 (14)	8.4 ± 1.2 (14)	19.1 ± 3.7 (14)	25.5 ± 2.8 (14)	8.8 ± 1.2 (14)
	Female	28.1 ± 3.1 (20)	8.4 ± 1.0 (20)	7.4 ± 0.8 (20)		
Site 93	Male	30.3 ± 3.9 (13)	8.6 ± 1.1 (13)	23.3 ± 4.2 (13)	29.7 ± 2.2 (13)	8.8 ± 1.1 (13)
	Female	29.7 ± 4.2 (22)	9.4 ± 1.4 (22)	7.7 ± 1.0 (22)		
Site 94	Male	29.6 ± 2.2 (20)	8.0 ± 0.7 (20)	22.1 ± 4.4 (20)	29.1 ± 3.2 (20)	8.6 ± 0.7 (20)
	Female	24.8 ± 2.3 (23)	9.4 ± 1.4 (23)	6.4 ± 0.8 (23)		
Site 95	Male	34.8 ± 7.2 (11)	10.2 ± 2.3 (11)	30.4 ± 8.8 (11)	32.8 ± 3.5 (11)	11.4 ± 2.1 (11)
	Female	37.6 ± 7.1 (20)	12.8 ± 2.7 (20)	10.7 ± 1.8 (19)		
Site 96	Male	37.7 ± 2.5 (8)	11.2 ± 0.9 (8)	31.6 ± 3.7 (8)	31.4 ± 2.6 (8)	11.5 ± 0.8 (8)
	Female	38.1 ± 3.8 (20)	12.6 ± 1.1 (20)	10.4 ± 0.9 (19)		
Site 104	Male	31.4 ± 4.0 (14)	7.3 ± 0.7 (4)			
	Female	27.7 ± 4.0 (16)				7.6 ± 1.0 (16)
Site 105	Male	30.7 ± 3.6 (5)	9.2 ± 1.2 (5)	28.6 ± 6.3 (5)	34.6 ± 4.5 (5)	10.0 ± 1.2 (5)
	Female	25.5 ± 3.8 (12)	8.8 ± 1.5 (12)	7.9 ± 1.4 (12)		
Site 106*	Male	41.8 ± 5.6 (10)	12.1 ± 1.8 (10)	28.6 ± 4.3 (10)	24.7 ± 3.5 (10)	13.4 ± 2.2 (10)
	Female	44.6 ± 4.1 (5)	14.4 ± 1.4 (5)	13.5 ± 1.3 (5)		
Site 114	Male	41.8 ± 3.6 (12)	11.4 ± 1.1 (12)	39.0 ± 4.1 (12)	35.2 ± 1.5 (12)	12.4 ± 1.4 (12)
	Female	32.9 ± 2.6 (5)	9.8 ± 0.9 (5)	9.0 ± 1.0 (5)		
Site 119*	Male	48.5 ± 6.6 (14)	16.1 ± 2.1 (14)	32.7 ± 6.4 (14)	22.8 ± 3.8 (14)	15.2 ± 2.1 (14)
	Female					
Site 121	Male	42.5 ± 7.0 (12)	12.3 ± 2.1 (12)	39.2 ± 9.2 (12)	34.8 ± 4.9 (12)	12.8 ± 2.5 (12)
	Female	37.2 ± 6.9 (16)	12.1 ± 2.6 (16)	10.2 ± 2.1 (16)		

Values are presented as mean ± SD (sample size for each measurement). *Predation site.

($F_{14,225} = 20.45$, $P < 0.0001$), body depth ($F_{14,179} = 29.92$, $P < 0.0001$), sword length ($F_{13,212} = 8.79$, $P < 0.0001$) and caudal fin length ($F_{11,124} = 24.50$, $P < 0.0001$).

Regression was used to examine the relationship of standard length with the other morphological characters. Body depth ($F_{1,192} = 1011.86$, $P < 0.0001$), sword length ($F_{1,224} = 234.82$, $P < 0.0001$) and caudal fin length ($F_{1,134} = 1113.04$, $P < 0.0001$) all increased as standard length increased (Fig. 1). Because there was not significant heterogeneity of slopes between populations for any of these relationships, as revealed by an interaction between standard length and population by ANCOVA (body depth: $F_{14,164} = 0.37$, $P = 0.981$; sword length: $F_{13,198} = 1.76$, $P = 0.052$; caudal fin length: $F_{11,112} = 1.11$, $P = 0.363$), we used these regressions to generate residuals for body depth, sword length and caudal fin length. There was significant variation among populations in residual body depth ($F_{14,179} = 35.00$, $P < 0.0001$), residual sword length ($F_{13,212} = 12.48$, $P < 0.0001$) and residual caudal fin length ($F_{11,124} = 3.61$, $P < 0.0005$).

MALE MORPHOLOGY AND PREDATION ENVIRONMENT

Most aspects of male morphology differed between populations of *X. helleri* from predator and predator-free environments. There were significant differences in male standard length ($F_{1,225} = 30.62$, $P < 0.0001$), body depth ($F_{1,179} = 78.97$, $P < 0.0001$) and caudal fin length ($F_{1,124} = 22.63$, $P < 0.0001$) between populations in environments with and without piscivorous fishes. Males from environments with piscivorous fishes were 25.0% longer, had 44.9% deeper bodies and had 37.6% longer caudal fins than did males from environments without piscivorous fishes (Figs 2, 3). There was not, however, a significant difference in sword length between these two environments ($F_{1,212} = 0.01$, $P = 0.916$).

There was a significant difference in residual body depth between populations of *X. helleri* from predator and predator-free environments ($F_{1,179} = 53.03$, $P < 0.0001$); males from populations with piscivorous fishes had relative body depths that were 11.7% greater (Fig. 2). This pattern was similar to that found for absolute body depth. There was a significant difference in residual sword length between populations with and without piscivorous fishes ($F_{1,212} = 20.91$, $P < 0.0001$). Thus, while there was no difference in absolute sword length, males from populations with piscivorous fishes had relative sword lengths that were 22.6% shorter than did males from populations without piscivorous fishes (Fig. 3). There was not a significant difference in residual caudal fin length between populations with and without piscivorous
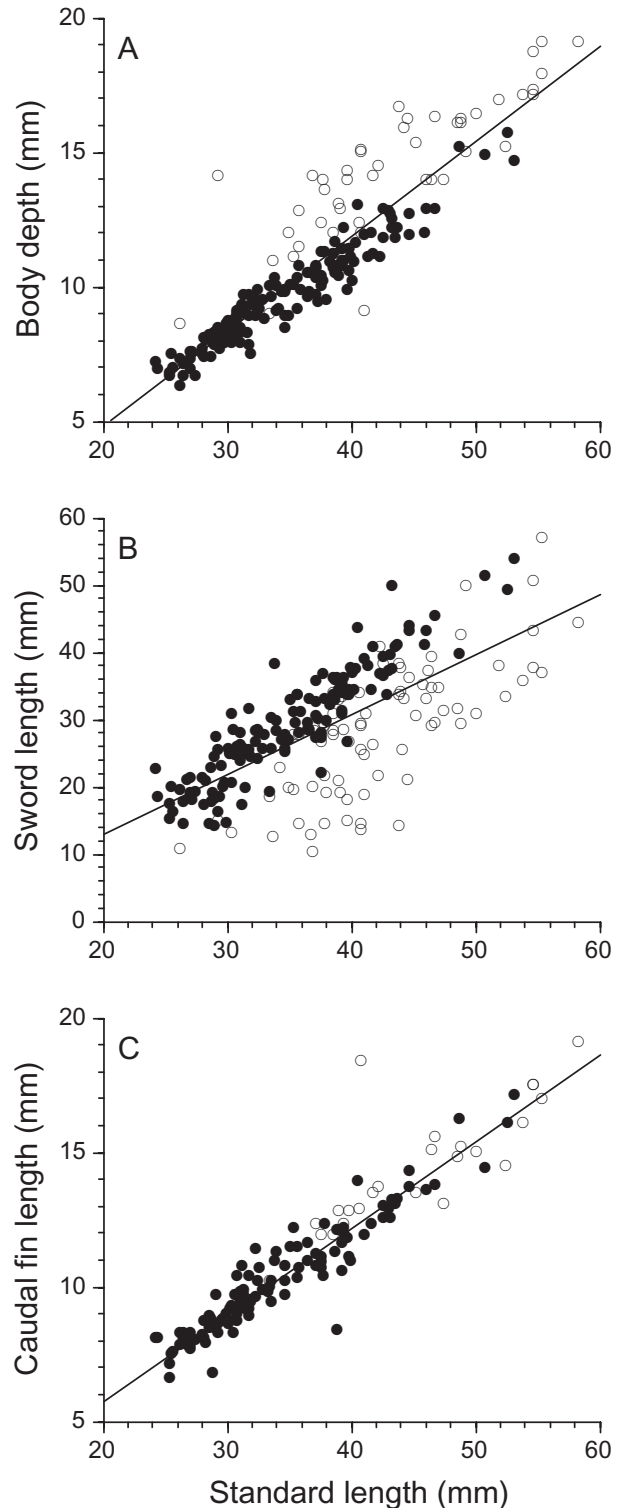

Figure 1. Regression of (A) body depth ($y = -2.28 + 0.35x$) (B) sword length ($y = -4.88 + 0.89x$) and (C) caudal fin length ($y = -0.64 + 0.32x$) on standard length for males from all of the populations sampled (predator-free environments = closed symbols, predator-present environments = open symbols).
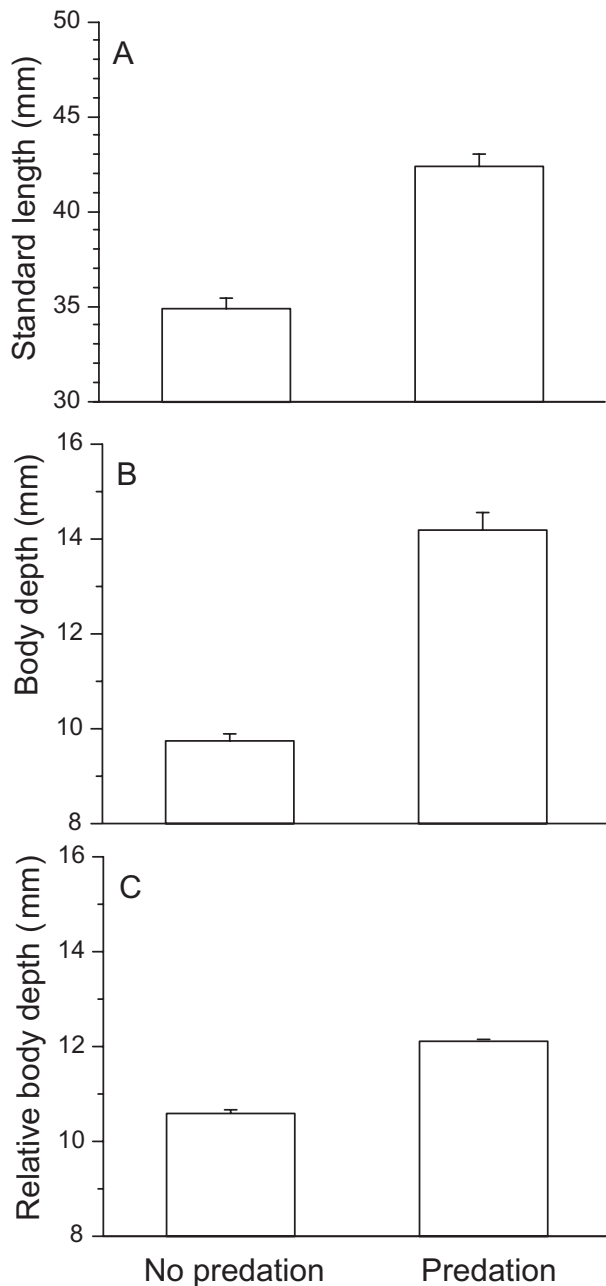
Figure 2. Comparison of male morphology between populations in predator-present and predator-free environments. The means (+ SE) of the population averages are presented for (A) standard length, (B) body depth and (C) relative body depth.

VARIATION IN FEMALE MORPHOLOGY AMONG POPULATIONS

Female morphological measurements for each population are summarized in Table 1. There was significant variation among populations in standard length ($F_{13,217} = 31.10$, $P < 0.0001$), body depth ($F_{12,181} = 26.74$, $P < 0.0001$) and caudal fin length ($F_{9,147} = 33.80$, $P < 0.0001$).

Regression was used to examine the relationship of standard length with body depth and caudal fin length. Body depth increased as standard length increased ($F_{1,192} = 2101.74$, $P < 0.0001$; Fig. 4). Because there was not significant heterogeneity of slopes between populations ($F_{12,168} = 1.61$, $P = 0.093$), we used this regression to generate residuals for body depth. There was significant variation among populations in residual body depth ($F_{12,181} = 9.17$, $P < 0.0001$). Thus, some of the variation among populations in female body depth is independent of body length variation. Caudal fin length also increased as standard length increased ($F_{1,155} = 1577.63$, $P < 0.0001$; Fig. 4). There was significant heterogeneity of slopes between populations for caudal fin length ($F_{9,137} = 2.89$, $P = 0.004$). Because the relationship between caudal fin length and standard length varied among populations, we were unable to compare residual caudal fin length among them.

FEMALE MORPHOLOGY AND PREDATION ENVIRONMENT

Some aspects of female morphology differed between populations of *X. helleri* from predator and predator-free environments. There was a significant difference in female standard length between populations with and without piscivorous fishes ($F_{1,217} = 45.84$, $P < 0.0001$); females from populations with piscivorous fishes were 33.1% longer (Fig. 5). There was also a significant difference in female body depth between populations with and without piscivorous fishes ($F_{1,181} = 39.43$, $P < 0.0001$); the bodies of females from populations with piscivorous fishes were 40.7% deeper (Fig. 5). Caudal fin measurements were available for only one population with piscivorous fishes. There was, however, a significant difference in female caudal fin length between this population and the populations without piscivorous fishes ($F_{1,147} = 4.82$, $P = 0.030$); females from the population with piscivorous fishes had caudal fins that were 57.2% longer (Fig. 5).

There was a significant difference in residual female body depth between populations with and without piscivorous fishes ($F_{1,181} = 16.92$, $P = 0.0001$); females from populations with piscivorous fishes had relative body depths that were 4.4% greater (Fig. 5). We
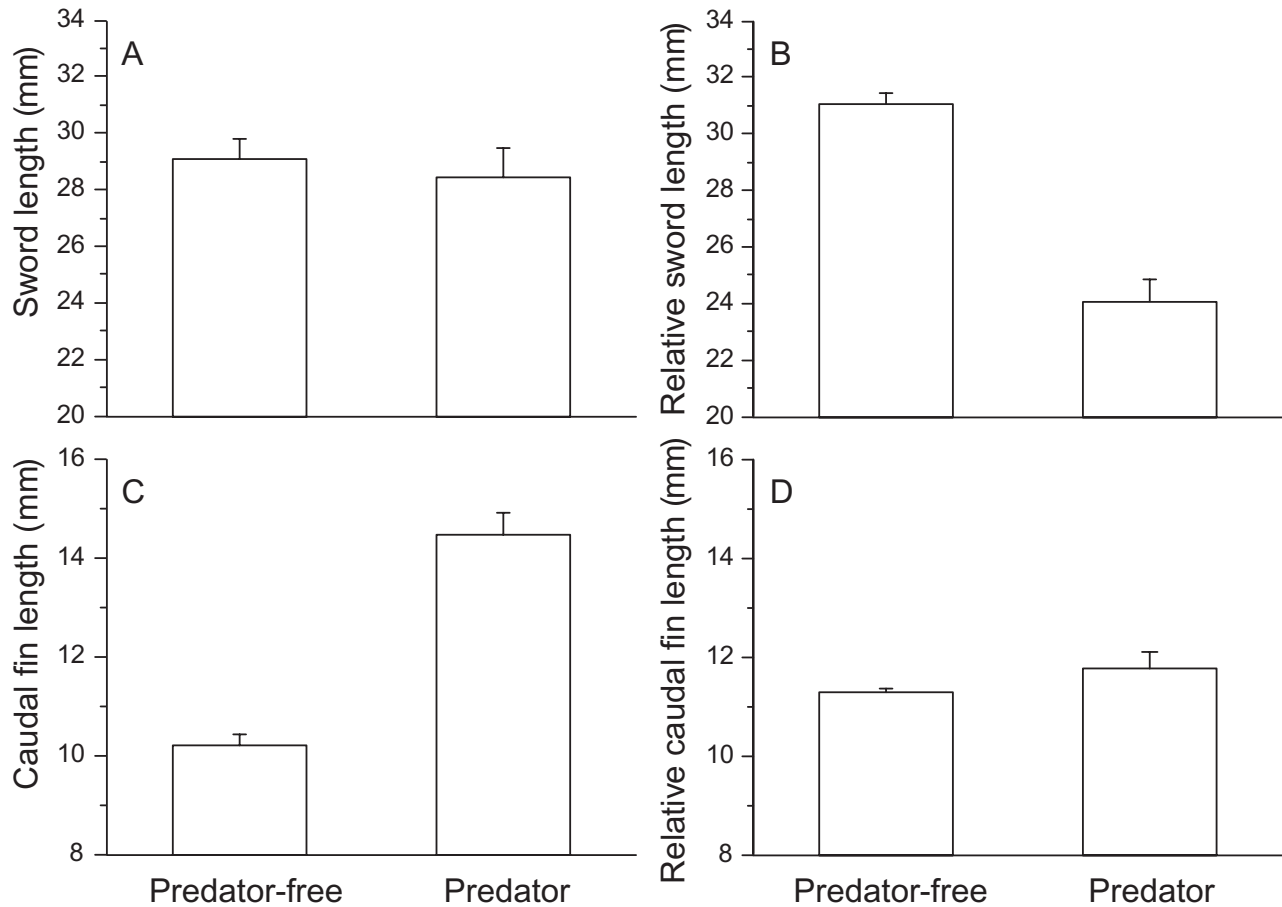
fishes ($F_{1,124} = 0.77$, $P = 0.383$). As a result, the observed difference in absolute caudal fin length between populations with and without piscivorous fishes can be accounted for by the difference in standard length between these populations.

Figure 3. Comparison of male morphology between populations in predator-present and predator-free environments. The means (+ SE) of the population averages are presented for (A) sword length, (B) relative sword length, (C) caudal fin length, and (D) relative caudal fin length.

were unable to compare relative caudal fin length because of variation among populations in the relationship between standard length and caudal fin length.

VARIATION IN SEXUAL DIMORPHISM

We compared the degree of sexual dimorphism in morphology using a two-way ANOVA with sex and population as the independent variables. In this analysis, the interaction between sex and population tests whether the effect of sex on morphology varies among populations. The interaction between sex and population had a significant effect on standard length ($F_{13,429} = 3.02$, $P = 0.0003$), body depth ($F_{12,344} = 2.89$, $P = 0.0008$) and caudal fin length ($F_{8,228} = 2.71$, $P = 0.0072$). There was thus variation among populations in the degree of sexual dimorphism for all three

traits. We used planned comparisons to assess whether the degree of sexual dimorphism differed between populations of *X. helleri* from predator and predator-free environments. Sexual dimorphism in standard length varied between populations with and without piscivorous fishes ($F_{1,429} = 4.22$, $P = 0.041$). Males from populations without piscivorous fishes were longer than were females (males: $\bar{x} = 34.9$ mm, SE = 0.5; females: $\bar{x} = 32.1$ mm, SE = 0.5) whereas males from populations with piscivorous fishes were slightly shorter in length than were females (males: $\bar{x} = 41.1$ mm, SE = 0.7; females: $\bar{x} = 41.9$ mm, SE = 0.7). In contrast, the degree of sexual dimorphism in body depth ($F_{1,344} = 0.17$, $P = 0.682$) and caudal fin length ($F_{1,228} = 2.09$, $P = 0.150$) did not differ between populations with and without piscivorous fishes. The caudal fin analysis, however, included *X. helleri* from a single population with piscivorous fishes.
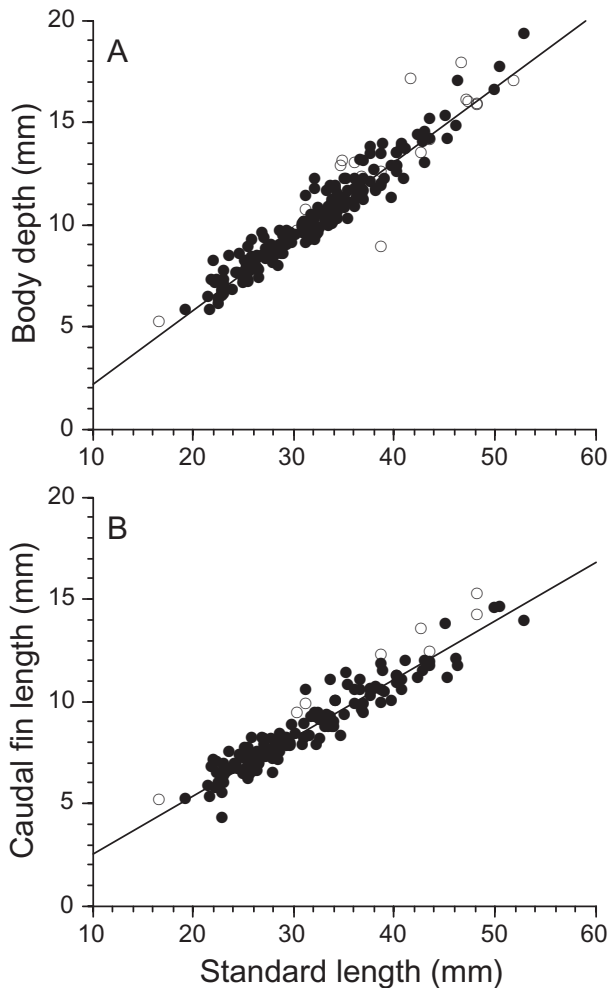
Figure 4. Regression of (A) body depth ($y = -1.45 + 0.36x$) and (B) caudal fin length ($y = -0.38 + 0.29x$) on standard length for females from all of the populations sampled (predator-free environments = closed symbols, predator-present environments = open symbols).

DISCUSSION

VARIATION IN MALE AND FEMALE BODY SIZE

Male and female *X. helleri* from populations with piscivorous fishes had longer and absolutely deeper bodies than did males and females from populations that lacked piscivorous fishes. The relationship between predator presence and body depth was evident even when controlling for the positive relationship between standard length and body depth. Although larger body size in populations with predators or with higher predator densities has also been reported for least killifish *Heterandria formosa* (Leips & Travis, 1999), crucian carp *Carassius carassius* (reviewed by Bronmark, Petersson & Nilsson, 1999), and Utah chub *Gila*

atraria (Johnson & Belk, 1999), the difference in size between *X. helleri* populations with and without predators contrasts with the effect of predation on body size in two related species of poeciliid fishes. In *Phalloceros caudimaculatus*, adults from populations with fish predators are smaller than are adults from populations without fish predators (Endler, 1983). In the guppy *Po. reticulata*, individuals from populations with a cichlid predator mature earlier, mature at smaller sizes and are smaller as adults compared with individuals from populations with only a less dangerous killifish predator of adults (Reznick & Endler, 1982; Reznick, Bryga & Endler, 1990; Strauss, 1990). The evolution of smaller body size in guppy populations with a higher risk of predation has been attributed to age-specific mortality, although numerous interacting factors are likely to affect the evolution of age and size at sexual maturation (see Abrams & Rowe, 1996; Reznick *et al.*, 1996). The age-specific mortality hypothesis (Gadgil & Bossert, 1970; Charlesworth, 1980) suggests that: (1) if adults have a higher risk of predation compared with juveniles, there will be a shift towards earlier maturation at a smaller size, or (2) if juveniles have a higher predation risk compared with adults, there will be a shift towards later maturation at a larger size.

Sexual selection in *X. helleri* appears to favour larger male size both because females prefer large males (Basolo, 1998a,b; Rosenthal & Evans, 1998) and because larger males are more successful in male–male competition (Ribowski & Franck, 1993). Our results are consistent with the hypothesis that natural selection due to predation likewise favours the evolution of larger size. This hypothesis requires: (1) that larger individuals incur a lower risk of predation, and (2) that some of the variation among populations in body size has a genetic basis. The effect of size on predation risk in *X. helleri* has not yet been examined. Larger individuals may be more invulnerable to predation by piscivorous fishes, however, because they exceed the gape width of many of these predators. As a result, predation in *X. helleri* populations with piscivorous fishes present may be directed primarily at juveniles and small adults, favouring the evolution of larger body size. In several species of poeciliid fishes, including *X. helleri*, variation among populations in adult body size is known to be at least partially due to heritable differences in size (Stearns, 1984; Kallman, 1989; Campton, 1992; Reynolds & Gross, 1992). Because male body size is also influenced by environmental factors (e.g. Campton, 1992), however, additional data are necessary to confirm that the reported differences in size are genetically based.

While our results are consistent with the hypothesis that predation by piscivorous fishes has directly favoured the evolution of longer and deeper bodies in
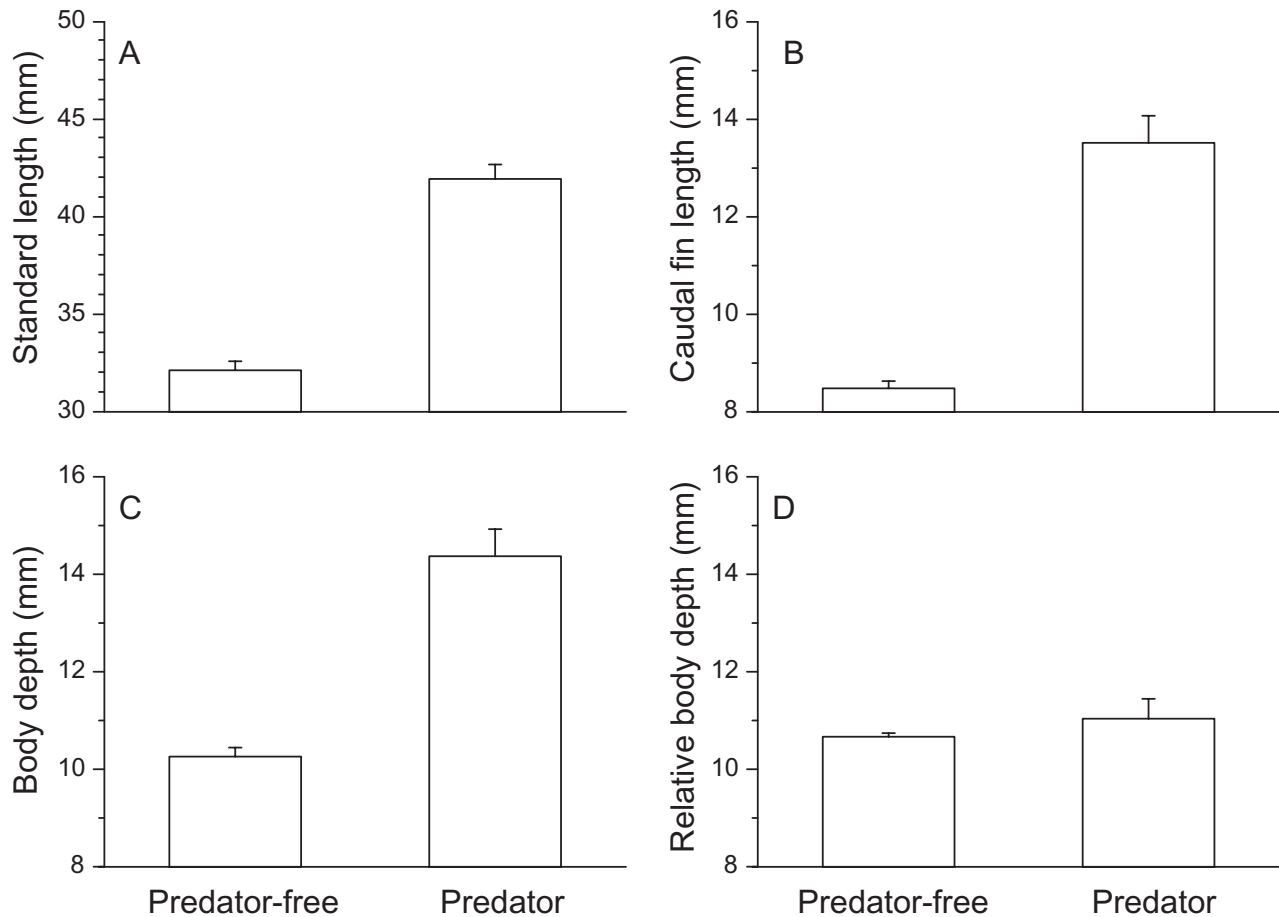
Figure 5. Comparison of female morphology between populations in predator-present and predator-free environments. The means (+ SE) of the population averages are presented for (A) standard length, (B) caudal fin length, (C) body depth and (D) relative body depth.

X. helleri, there are alternative hypotheses that could explain the observed variation among populations in male and female size. First, predation may indirectly favour the evolution of larger size. The presence of predators, for example, may reduce *X. helleri* density, and variation in density between populations with and without predators may affect adult size. The traditional expectation is that higher densities will favour larger, more competitive individuals (Stearns, 1992), but higher densities may also reduce food availability. While the effect of food availability on the evolution of body size is not clear (discussed by Reznick, Butler & Rodd, 2001), predation can, under some conditions, favour the evolution of larger size at sexual maturation due to an indirect effect of predation on food availability (Abrams & Rowe, 1996). Second, larger size in populations with predators may be due to an effect of predation on age structure. Predation, for example, may reduce average adult age, and thus average adult size, even if size has no effect on preda-

tion risk. This is an unlikely explanation for our results. Males grow very little after sexual maturation, thus size-independent predation should have little effect on the size distribution of adult males. While females do grow substantially after sexual maturation, size-independent predation should reduce the average female age, and thus the average female size. Our result that males and females are larger in populations with predators is inconsistent with this hypothesis. Third, the evolution of larger size may be favoured by environmental factors that are independent of the risk of predation by piscivorous fishes but that covary with this risk. This may include other sources of predation. For example, herons differentially prey on larger individuals in two species of poeciliids, mosquitofish *Gambusia affinis*, and sailfin mollies *Po. latipinna* (Britton & Moser, 1982; Trexler, Tempe & Travis, 1994). Fourth, larger size in populations with predators may be a purely phenotypic response to environmental factors. For example, if

food availability covaries with predator presence, individuals from populations with and without predators may differ in size without a difference in P-allele frequencies. Even if piscivorous fishes differentially attack smaller individuals, this source of predation is unlikely to be the sole cause of the observed variation among populations. Populations with and without predators likely differ in a variety of factors other than the presence or absence of predators, many of which may influence the age and/or size of individuals at sexual maturity (e.g. Trexler & Travis, 1990; Trexler *et al.*, 1994; Reznick *et al.*, 1996).

VARIATION IN SWORD AND CAUDAL FIN LENGTH

Male *X. helleri* from populations with and without piscivorous fishes did not differ in absolute sword length. Controlling for the positive association between standard length and sword length, however, males from populations without piscivorous fishes had relatively longer swords than did males from populations with such predators.

Females prefer males with longer swords in *X. helleri* (Basolo, 1990, 1998a; Trainor & Basolo, 2000). Our results are consistent with the hypothesis that natural selection due to predation has an opposing effect on the evolution of sword length, favouring males that have shorter swords. This hypothesis requires (1) that males with shorter swords incur a lower risk of predation and (2) that some of the variation among populations in sword length has a genetic basis. Swords are colourful, conspicuous traits that may make males more visible to predators, decrease their ability to escape from predators once detected, or make some types of swimming activities energetically costly (Basolo & Alcaraz, 2003). Although there is as yet no direct evidence that sword length affects predation risk, predators of a related swordtail preferentially attack males with sword extensions compared with males without sword extensions (Rosenthal *et al.*, 2001). The heritability of sword length is also not known. Environmental conditions, such as diet quality, do affect male sword length, but even a large difference in diet quality only has a small effect on sword growth (Basolo, 1998b).

While our results are consistent with the hypothesis that predation by piscivorous fishes has favoured the evolution of shorter swords, there are alternative hypotheses that could explain the observed variation among populations in male sword length. First, predation may indirectly favour the evolution of shorter swords. For example, predation might favour a shift in energy allocation from sword growth to body growth. Second, the evolution of shorter swords may be favoured by environmental factors that are independent of the risk of predation by piscivorous fishes but

that covary with this risk (see above). Third, shorter swords in populations with predators may be a purely phenotypic response to environmental factors, such as food availability, that covary with the risk of predation.

Males from populations with piscivorous fishes also had longer absolute caudal fins than did males from populations without such predators, but this difference disappeared when controlling for the positive association between standard length and caudal fin length. As a result, there is no evidence for an effect of predation environment on relative male caudal fin length. Comparison of female caudal fin lengths between populations with and without predators was hindered by the availability of female caudal fin data for populations with predators. Nonetheless, females from one population with predators had longer absolute caudal fins than did females from populations without piscivorous fishes. This result mirrors that for male caudal fin length. We were unable, however, to examine the association between predation environment and relative caudal fin length in females because of variation among populations in the relationship between standard length and caudal fin length.

COMPARISON OF MALE AND FEMALE MORPHOLOGY

Males from the populations without piscivorous fishes were relatively longer compared with females, whereas males from the populations with piscivorous fishes were not. This result suggests that the male and female optima for body length may be similar in populations with predators, but different in populations without predators. This could occur for a number of non-mutually exclusive reasons. First, the relative effect of natural selection on male and female length may differ between populations with and without piscivore predators. Natural selection, for example, may more strongly favour longer females in predator-present populations than it favours longer males, whereas natural selection may act more similarly on the sexes in predator-free populations. Alternatively, natural selection may more strongly favour smaller females than it favours smaller males in predator-free environments. Second, sexual selection on male length in predator-present populations may be weaker than it is in predator-free populations. In some animals, for example, an increase in the perceived risk of predation causes females to be less discriminating or to change the choice of male with which they mate (Houde & Endler, 1990; Hedrick & Dill, 1993; Endler & Houde, 1995; Gong, 1995), including in *X. helleri* (Johnson & Basolo, 2003).

While there was variation among populations in the degree of sexual dimorphism in body depth, the degree of sexual dimorphism did not vary with predation

environment. In most populations, females had deeper bodies compared with males. Similarly, while there was variation among populations in the degree of sexual dimorphism in caudal fin length, the degree of sexual dimorphism did not vary between populations with and without piscivorous fishes. In most populations, males had caudal fins that were substantially longer compared with those of females. It is not known whether female swordtails have preferences based on male caudal fin length, but female guppies have preferences for larger caudal fins (Bischoff, Gould & Rubenstein, 1985; Endler & Houde, 1995). Our observation that males have longer caudal fins compared with females suggests that caudal fin length could also be under sexual selection in *X. helleri*.

CONCLUSIONS

For *X. helleri*, there are consistent differences in body size between populations with and without piscivorous fishes. The mean length of males and females is greater in populations with predators than it is in populations without predators. Also, controlling for the effect of body length on sword length, males from populations without piscivorous fishes have relatively longer swords compared with males from sites with such predators. In other poeciliid fishes, it appears that adult size is smaller and coloration reduced in predation sites. Natural and sexual selection in these other species thus have opposing effects on the evolution of these traits. Although our results suggest that natural and sexual selection may have opposing effects on the evolution of sword length in *X. helleri*, our results also suggest that natural and sexual selection may have synergistic effects on the evolution of body length in *X. helleri*. While predation by piscivorous fishes may be one factor that affects the evolution of male and female morphology and life history in *X. helleri*, these traits are likely to be affected by multiple sources of selection (cf. Reznick *et al.*, 2001). Predation by piscivorous fishes is thus unlikely to be the sole cause of the reported differences between populations.

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APPENDIX 1

The following are descriptions of the 15 *X. helleri* sites that were sampled. Each description includes the site number, the site name, the location of the site, and the sympatric fish species present. C.A., Central America; MX, Mexico.

Site 13 (Palm Pond). Location: four miles west, southwest of Bermudian Landing on road to Rancho Dolores, Belize River drainage, Belize, C.A. Fish species present: *Astyanax aeneus*, *Cichlasoma friedrichsthali, C. octofasciatum*, *Gambusia* spp., *Heterandria bimaculata*, *Ophisternon aenigmaticum*, *Poecilia mexicana*, *Petenia splendida*, *Rivulus tenuis* and *X. maculatus*.

Site 53 (Savannah Stream). Location: at mile marker 28 along the Western Hwy, flows south towards Sibun River, Sibun River drainage, Belize, C.A. Fish species present*: A. aeneus, Belonesox belizanus, C. friedrichsthali, C. octofasciatum*, unidentified non-piscivorous *Cichlasoma*, *Gambusia* spp., *H. bimaculata*, *Po. mexicana*, *Pe. splendida*, *Rhambdia guatemalensis* and *X. maculatus*.

Site 72 (Rio On). Location: near bridge crossing river, 18.5 miles south of Georgeville on Chiquibul Road towards Augustine, Mountain Pine Ridge Forest Reserve, flows over waterfall to Macal River, Macal River drainage, Belize, C.A. Fish species present: *A. aeneus* and *H. bimaculata*.

Site 74 (Little Vaqueros Creek). Location: upstream from bridge crossing stream, 11.5 miles south of Georgeville on Chiquibul Road towards Augustine, Mountain Pine Ridge Forest Reserve, flows to Macal River, Macal River drainage, Belize, C.A. Fish species present: *H. bimaculata*.

Site 92 (Tiger Falls Stream). Location: just upstream from Tiger Falls, Mountain Pine Ridge Forest Reserve, flows over waterfall into Barton Creek, Belize River drainage, Belize, C.A. Fish species present: *H. bimaculata*.

Site 93 (King Vulture Falls Stream). Location: upstream from King Vulture Falls, Mountain Pine Ridge Forest Reserve, flows over waterfall into Barton Creek, Belize River drainage, Belize, C.A. Fish species present: *H. bimaculata*.

Site 94 (Upper Privassion Creek). Location: upstream from Thompson Fireline bridge, 1.64 m from Cooma Cairn Road, Mountain Pine Ridge Forest Reserve, flows into the Eastern Branch of Macal River, Macal River drainage, Belize, C.A. Fish species present: *H. bimaculata*.

Site 95 (Refugee Creek). Location: Valley of Peace, flows into Colorado Lagoon, Belize River drainage, Belize, C.A. Fish species present: *A. aeneus*, *H. bimaculata* and *R. guatemalensis*.

Site 96 (Three Flags Stream). Location: 11.5 miles NW of San Ignacio, flows into river behind Three Flags grocery on Western Hwy., Belize River drainage, Belize, C.A. Fish species present: *A. aeneus*, *H. bimaculata* and *Po. mexicana*.

Site 104 (Catemaco Hill). Location: 1.7 miles from Benito Juarez, flows downhill into Lake Catemaco, Vera Cruz, MX. Fish species present: *H. bimaculata*.

Site 105 (Pasture Channel). Location: north-west of Sontecomapan, Barra de Sontecomapan drainage, Vera Cruz, MX. Fish species present: *Po. mexicana*.

Site 106 (Goat Creek). Location: near Plan de San Luis on foot trail to Paquital, Isthmus of Tuentepec, flows to Rio Tolosito, Rio Coatzacoalcos drainage, Oaxaca, MX. Fish species present: *A. fasciatus*, *B. belizanus*, unidentified non-piscivorous *Cichlasoma*, *H. bimaculata*, *Poeciliopsis gracilis*, *Po. mexicana* and *X. clemenciae*.

Site 114 (Mahogany Creek). Location: 6 miles west of Macal River and four miles east of Augustine on Chiquibul Road, flows to upper branch of Macal River, Macal River drainage, Belize, C.A. Fish species present: *A. aeneus* and *H. bimaculata*.

Site 119 (Otter Stream). Location: upstream from bridge crossing stream, 1.2 miles south of Flowers Bank between Flowers Bank and Burrel Boom, joins Mussell Creek, Belize River drainage, Belize, C.A. Fish species present: *A. aeneus*, *B. belizanus*, *C. octofasciatum*, unidentified non-piscivorous *Cichlasoma*, *Gambusia* spp., *H. bimaculata*, *Pe. splendida*, *R. guatemalensis* and *X. maculatus*.

Site 121 (Dry Creek). Location: upstream and downstream from bridge crossing stream, 39.7 miles from Belmopan on Hummingbird Hwy, flows into Dry Creek, Sibun River drainage, Belize, C.A. Fish species present: *A. aeneus*, non-predatory *C. spilurum*, *H. bimaculata* and *Po. mexicana*.