Growth and male ornamentation in *Xiphophorus montezumae*

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Introduction

Swordtails (Teleostei: Poeciliidae, genus *Xiphophorus*) are an important model to elucidate how male adult body size and ornamentation influences fitness. Females of several swordtail species prefer males with larger body size (Ryan and Wagner 1987; Rosenthal and Evans 1998; Fisher and Rosenthal 2007); males with larger body size are better fighters in intrasexual encounters (Benson and Basolo 2006), and also exhibit higher swimming endurance and velocity (Ryan 1988; Kruesi and Alcaraz 2007). In this sense, the greater the body size of an individual the higher its competitive ability. One important life-history trait that determines adult size is early growth; adult body size is a result of cumulative growth during an individual’s lifetime. Male differences in growth may affect population structure by producing different dominance status between individuals due to variation in size (Aubin-Horth and Dodson 2004; Alcaraz 2005).

Male ornamentation also plays an important role in sexual selection. Female swordtails prefer to mate with males that display elaborate secondary sexual traits,
for example, ultraviolet coloration (Cummings et al. 2003), stereotyped courtship displays (Rosenthal et al. 1996), and melanophore pigment patterns (Morris et al. 1995; Fernandez and Morris 2008). Perhaps the most distinctive feature of swordtails (Darwin 1871) is the eponymous ornament: the sword, which is present in the males of some Xiphophorus species. The sword comprises an array of nine traits organized in four components: elongation of a specific set of caudal fin rays, a lower melanophore stripe, an upper melanophore stripe, and coloration between the upper and lower melanophore stripes which can be orange, yellow, and/or green (Basolo 1996). In some species, females prefer to mate with males bearing longer swords (Basolo 1990a); this appears to reflect a pre-existing perceptual bias, since the preference appears in species that diverged from a swordless common ancestor (Basolo 1990b). Nevertheless, the sword has been secondarily lost several times in the history of the swordtail lineage (Marcus and McCune 1999), and varies considerably in size and elaboration among species (Rauchenberger et al. 1990). Both predation risk (Rosenthal et al. 2001; Cummings et al. 2003) and changes in female preference (Rosenthal et al. 2002; Wong and Rosenthal 2006) are likely to drive the secondary loss of elaborate swords.

Little is known, however, about the mechanisms underlying variation in sword elongation among individuals of the same species. Interspecific crosses by Zander and Dzwillo (1969) estimated that the trait is largely associated with four genetic loci. Variation in sword length has also been attributed to environmental factors, such as food availability (Basolo 1998), predation risk (Basolo and Wagner 2004), and the population level of male ornamentation (Walling et al. 2007). A comparative analysis between species showed that differences in growth trajectories of both sword and body size were correlated with adult body size; sword growth is faster in swordtail species with larger adult size than in smaller species (Marcus and McCune 1999). An important question, then, is whether the pattern observed among species is also exhibited among individuals of the same population.

Sexual maturation in males activates the elongation of the sword, reduces body growth rate, and ultimately induces the cessation of body growth (Marcus and McCune 1999). The sword in the Montezuma swordtail (Xiphophorus montezumae) is the most exaggerated when comparing all swordtail species, it grows longer than the body of the bearer (Rauchenberger et al. 1990). Little is known about the intrinsic differences that determine sword elongation among individual males. The goal of this study was to test whether juvenile body growth rate and the age and size at sexual maturation explain the differences in sword growth rate among individuals, as well as variation in sword and body size among mature X. montezumae males.

Materials and methods

Maintenance and breeding

Juvenile X. montezumae were obtained from six breeding pairs originally collected from Capuchinas Spring, in San Luis Potosi, Mexico. Each breeding pair was housed in a 40-L glass tank with a 16:8 hour light:dark schedule. Water conditions were held constant at a pH value of 7.0, salt content of 0.4 g L⁻¹, and temperature of 25 ± 1°C. Fish were fed twice a day; their diet was alternated between live germ-free Tubifex worms and Tetramin flakes (36% protein), ad libitum. A third of the water content in all tanks was changed every 10 days.
The juveniles used for this study were born in the laboratory. Broods from each breeding pair were placed in individual 60-L communal tanks with water conditions set as described above. Seven days after birth, five fish were randomly selected from each communal tank and set apart in isolation, until the onset of the development of secondary sexual traits. Each was assigned randomly to one of two compartments of a 25-L tank divided by an opaque plastic screen. In this way, fish were maintained in physical and visual isolation. Olfactory communication might have occurred between the two fish that shared a tank; however, its effect on individual growth rate was minimized by randomly rotating the fish between the tanks each time they were measured (approximately every 30 days).

We checked for the initial stages of external secondary sexual traits, which are highly recognizable in males of Montezuma swordtails and other poeciliids (Marcus and McCune 1999; Dries et al. 2001), and consist primarily of the fusion of anal fin rays into a gonopodium, the appearance of dorsal fin pigmentation and the development of a sword primordium. The onset of gonopodium differentiation is an indicator of male maturation (Snelson 1989). As soon as any incipient male traits were observed, males were housed individually in a new 25-L glass tank for the rest of the experiment. We reared a total of 28 juveniles (17 males, 11 females) but gathered data only on the males.

**Morphometrics**

Standard length (SL; snout to hypural plate; ±0.5 mm) was measured for all fish approximately every 30 days (measurement date was registered), starting the seventh day after birth, and until growth rates plateaued to near zero (approximately after 20 months from birth). To minimize handling stress, we used a photo tank (150 mm length; 100 mm width; and 15 mm deep) marked with a scale in millimeters. We took a picture of one fish at a time with a CANON® Ultra video recorder (set in picture mode), and used image analysis software (NIH Image; Rasband 1997) for morphometrics. We measured sword length (distance from the inferior proximal margin of the caudal fin to the distal farthest limit of the sword) in all males from the onset of the sword primordium until the end of the experimental period. Body mass ($M$; ±0.01 g) was measured twice in the experiment using a plate balance: at the onset of sword elongation and at the end of the experiment. This measurement was not taken periodically or during the juvenile stages to avoid stress produced by handling without the use of anesthesia or by the use of it; previous studies report long-term effects of anesthesia in body growth in teleost fish (Fabrizio and Pessutti 2007).

**Growth parameters**

We modeled body growth using the standard von Bertalanffy (1968) equation:

$$L(t) = L_\infty (1 - e^{-kt} \exp(t + t_0))$$  \hspace{1cm} (1)

where $L(t)$ is the SL at time ($t$); $L_\infty$ the asymptotic maximum SL; $t_0$ the hypothetical time when SL equals zero; $t$ the time; and $k$ the growth rate constant.

Growth in sword length was fitted to a logistic model:

$$\text{Sword length} = \ln[(1/x_i) - (1/u)]$$  \hspace{1cm} (2)
where \( u \) is the upper bound parameter for the sword length (\( u = \text{mean sword length at the end of the experiment [final sword length]} \)).

We also calculated key parameters describing male body and sword growth at different life-history stages. Juvenile growth rate was estimated considering size data during the juvenile stage (using measurements of SL from the age of 7 days to the onset of gonopodium differentiation). As a measure of male condition at the onset of sword elongation, the Fulton condition factor was estimated for each individual male according to Chellappa et al. (1995), using the following equation:

\[
\text{Condition factor} = \left(100 \times M\right)\text{SL}^{-3}
\]  

The age at the onset of sword elongation was identified as the earliest recognizable stage of extension of the inferior fin rays that constitute the sword (primordium shorter than 4 mm in length).

The maximum growth rate of the sword was identified for each individual male as the linear slope of the logarithmic phase of the logistic growth curve when the data were ln-transformed; that is, excluding the lag phase of the curve and the steady state or decreasing growth phase (Wareing and Philips 1981; Sibley and Calow 1987). Finally, sword index (SI; Rauchenberger et al. 1990) was calculated for males at the end of the experiment.

\section*{Statistical analysis}

The von Bertalanffy and logistic growth functions describing male body and sword growth, respectively, were fitted to the observed data (mean values of all males used for this study) to nonlinear regression analysis using SPSS version 13.0 for Windows (SPSS Inc. 2004).

To estimate if growth during the juvenile stage in \( X. \) montezumae has an effect on adult size and sword length, we conducted two multiple regressions: the first using juvenile growth rate, male condition, and age at the onset of sword elongation as independent variables against the standard length at the end of the experiment (dependent variable). The second multiple regression was conducted to test the relationship between the same set of independent variables describing male juvenile growth and the sword length at the end of the experiment as the dependent variable. To test for the effects of juvenile growth on the development of the sword, a third multiple regression was used to estimate whether juvenile growth rate and male age and/or size (SL) achieved at the onset of sword elongation can explain the maximal growth rate of the sword. Finally, a simple linear regression was conducted to estimate the relationship between male body size and sword length at the end of the experiment.

\section*{Results}

\section*{Male growth trajectories}

The von Bertalanffy growth function described growth trajectory of the male \( X. \) montezumae (nonlinear regression; \( r^2 = 0.98; F_{(1, 0.05)} = 734.14; P < 0.01 \) in the following equation: \( \text{SL}(t) = 50.9 \left(1 - e^{-0.2 \exp[3.4]}\right) \)). Mean sword growth of all males
showed a sigmoid trajectory (nonlinear regression; \( r^2 = 0.95; \ F(1, \ 0.05) = 179.78; \ P < 0.01 \)), described in the following logistic equation:

\[
L(t) = \ln \left( \frac{1}{x_t} \right) / C0 \cdot \left[ 1 - \frac{1}{64} \right].
\]

Both models are shown in Figure 1.

Mean body size (SL) of male 7-day-old juveniles was 12.1 ± 3.1 mm (SD). During the period of juvenile growth, male size increased at a rate of 0.09 ± 0.04 mm day\(^{-1}\) (SD). Gonopodium differentiation was observed at a mean age of 164 ± 52 days (SD), which coincided with the onset of sword elongation. Male size at sword onset showed a mean SL of 40.0 ± 3.5 mm (SD). The maximum sword growth rate of all males was 0.20 ± 0.04 mm day\(^{-1}\); this fast-increasing period of sword length lasted a mean time of 207 ± 47 days. Males reached a sword length at the end of the experiment (after 20 months since birth) of 64.4 ± 7.2 mm, and body size (SL) of 50.8 ± 2.6 mm (Figure 1); mean SI was 1.3 ± 0.1.

**Relationship between body and sword growth parameters**

Both juvenile growth rate and the age at the onset of sword elongation predicted male size at the end of the experiment (Panel A of Table 1). The largest males at the end of the experiment had higher juvenile growth rates and developed the sword at later age (Figure 2).

Neither juvenile growth rate, nor age or condition at sword onset predicted sword length at the end of the experiment (Panel B of Table 1). The maximum sword growth rate, however, was positively related to male SL at the onset of sword elongation, but not to juvenile growth rate or the age at sword onset (Panel C of Table 1). Thus, males that were larger at the onset of sword growth were the ones developing a sword at higher rates (Panel C of Table 1). However, male size at the end of our experiment was negatively related to final sword length (linear regression; \( r^2 = 0.36; \ P = 0.03 \)).
Table 1. Multiple regression results comparing the effect of juvenile growth rate, age, and male condition at sword onset on male size (SL; Panel A); and on sword length at the end of the experiment (Panel B). A third multiple regression (Panel C) shows the effect of male juvenile growth rate, and the age and male size (SL) at sword onset on sword growth rate (ranged left above table).

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variables</th>
<th>$\beta$</th>
<th>$B$</th>
<th>$t$-Value</th>
<th>$P$-Value</th>
<th>$r^2$ (model)</th>
<th>$P$-Value (model)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Panel A</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Body size (SL at the end of the experiment)</td>
<td>Intercept</td>
<td>-68.85</td>
<td>-1.61</td>
<td>0.14</td>
<td>0.66</td>
<td>0.01</td>
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<tr>
<td></td>
<td>Juvenile growth rate</td>
<td>3.34</td>
<td>390.34</td>
<td>2.87</td>
<td>0.02</td>
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<td></td>
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<tr>
<td></td>
<td>Sword onset</td>
<td>3.71</td>
<td>0.35</td>
<td>3.18</td>
<td>0.01</td>
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<td></td>
<td>Condition at sword onset</td>
<td>0.12</td>
<td>976.37</td>
<td>0.42</td>
<td>0.68</td>
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<tr>
<td><strong>Panel B</strong></td>
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<tr>
<td>Sword length (at the end of the experiment)</td>
<td>Intercept</td>
<td>-153.04</td>
<td>-0.80</td>
<td>0.44</td>
<td>0.28</td>
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<td>Juvenile growth rate</td>
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<td>679.44</td>
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<td>Sword onset</td>
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<td>Condition at sword onset</td>
<td>0.64</td>
<td>16,650</td>
<td>1.61</td>
<td>0.14</td>
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<tr>
<td><strong>Panel C</strong></td>
<td></td>
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<tr>
<td>Sword growth rate</td>
<td>Intercept</td>
<td>-0.09</td>
<td>-1.07</td>
<td>0.31</td>
<td>0.70</td>
<td>0.01</td>
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<td>Juvenile growth rate</td>
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<td>Sword onset</td>
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<td>Size at sword onset</td>
<td>0.83</td>
<td>0.01</td>
<td>4.12</td>
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</table>

Note: $\beta$, error probability and $B$, regression coefficient (ranged left under table).
Discussion

As with reports on other *Xiphophorus* species (Marcus and McCune 1999), males grew rapidly until the onset of sexual maturity; growth then slowed, coinciding with the start of sword elongation. The decrease in male body growth rate when approaching sexual maturity can be explained by the change in energy allocation from somatic growth to the development of reproductive traits (Sibley and Calow 1987; Alexander 1999). The diversion of energy allocated from somatic growth to sexual maturation in male *X. montezumae* may involve testicular maturation, gonopodial differentiation, increase of the dorsal fin area, and/or the elongation of the lower caudal fin rays (Kallman and Schreibman 1973; Kallman 1983; Marcus and McCune 1999). The development of the sword is also accompanied by the increasing energetic costs of routine swimming activities (Basolo and Alcaraz 2003).

Both growth rate and the size at which males initiate sexual maturation varied considerably among individuals in this study. The juvenile males that displayed higher growth rates developed a sword later than those males which grew at a lower rate. Early sexual maturation can be an advantage since it may reduce a male’s risk of dying without reproducing (Stearns 1992). However, maturation in this species, as in other swordtails (*Xiphophorus nigrensis*, Ryan et al. 1992; *Xiphophorus helleri*, Basolo 2008), may be costly in terms of the decrease in both growth rate and size at maturity. Additionally, in swordtails, maturation is also accompanied by increasing fitness costs associated with the sword: energetic costs of swimming (Basolo and Alcaraz 2003), reduced swimming velocity (Kruesi and Alcaraz 2007), and increased predation risk (Rosenthal et al. 2001). Several lines of evidence suggest that sexual selection favoring sword elongation is counterbalanced by natural selection. The loss of the sword and the preference for the sword in several lineages of *Xiphophorus* (Rosenthal et al. 2002; Wong and Rosenthal 2006) may reflect direct selection against the expression of a sword.
Juvenile males who grew at a higher rate and started maturation later also reached a larger adult size. Later-maturing males could have a long-term advantage by reaching a greater adult size. Lagarde et al. (2001) found a similar result in the steppe tortoise (*Testudo horsfieldi*) in which both age at maturity and the elaboration of sexually selected ornaments were correlated with growth rate. Although juvenile growth and the onset of sexual maturation explained adult size in male swordtails, neither juvenile growth rate, condition nor age at maturity explained maximum sword elongation. However, at the end of the experiment, sword length was negatively correlated with SL. The smaller size of the males bearing longer swords suggests resource partitioning between body size and sword elongation. Increases in both body size and sword elongation are energetically demanding in terms of somatic growth; but sword elongation comes also with an increasing energetic cost associated with swimming activity (Basolo and Alcaraz 2003). Basolo (1998) proposed that, under restricted food conditions, *X. helleri* divert energy from body growth to sword elongation, as seems to occur in Montezuma swordtails during sword elongation.

Our results contrast with the data reported by Basolo and Wagner (2004) in *X. helleri*, in which SL and sword length were positively correlated. However, the results reported were taken from a wild population in which the abiotic and social environment, not to mention differential survival and sublethal predation on swords, could play an important role structuring the covariation between body size and sword length. Further, swords are substantially smaller in *X. helleri* (SI of 0.6–0.8) than in *X. montezumae* (0.7–1.3; Rauchenberger et al. 1990). Moreover, our analysis focused on males that had reached their maximum body size under controlled conditions, while Basolo and Wagner (2004) were likely to have collected males of different ages in the wild.

Additionally, males in this experiment reached 51 mm SL and 64 mm sword length, corresponding to a mean SI of 1.3. Field-caught males (Capuchinas spring, San Luis Potosi) had similar size (SL = 50.5 ± 8.6 mm; n = 97) but had considerably shorter swords (54.9 ± 18.6 mm) and consecutively a lower SI (1.1; Kruesi and Alcaraz unpublished data) than the males reared in this study. Thus, it seems that under laboratory conditions the sword can reach a larger size than under natural conditions. The relatively shorter sword of males captured in the field may suggest that the wild population is composed mainly of younger males than the ones reared in this study. Our results show that the sword continues growing with age, which is accompanied by the increasing energetic cost of swimming (Basolo and Alcaraz 2003), and possibly higher predation risk (Rosenthal et al. 2001). Thus, the increase in sword length through life may play an important role in male survival, making it costly for males in the wild to have long swords.

Variation in growth can be explained through differences in foraging efficiency, food assimilation efficiency, energetic food conversion and its allocation to different activities, and overall metabolism (Peck et al. 2004). The data reported here do not account for the particular individual functional responses playing a role in growth differences; instead, this study, conducted under controlled conditions, reveals intrinsic inter-individual differences in life-history traits and their relation with male ornamentation. Our data support the notion that early life-history traits may provide valuable information about an individual’s role in a population. Our results show that variation among individuals in life-history traits, such as growth rate, adult size, and the timing of maturation, plays an important role in adult size and male
ornamentation that might strongly affect performance in subsequent stages and ultimately individual reproductive success and survival.

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References


