A private ultraviolet channel in visual communication

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Although private communication is considered an important diversifying force in evolution, there is little direct behavioural evidence to support this notion. Here, we show that ultraviolet (UV) signalling in northern swordtails (*Xiphophorus*) affords a channel for communication that is not accessible to their major predator, *Astyanax mexicanus*, the Mexican tetra. Laboratory and field behavioural experiments with swordtails (*X. nigrensis*) and predators (*A. mexicanus*) demonstrate that male UV ornamentation significantly increases their attractiveness to females but not to this predator, which is less sensitive to UV. UV reflectance among swordtail species correlates positively with tetra densities across habitats, and visual contrast estimates suggest that UV signals are highly conspicuous to swordtails in their natural environment. Cross-species comparisons also support the hypothesis that natural selection drives the use of UV communication. We compared two species, one with high (*X. nigrensis*) and one with low (*X. malinche*) Mexican tetra densities. *Xiphophorus nigrensis* males reflect significantly more UV than *X. malinche*, exhibit significant UV sexual dimorphism, and UV is a salient component of the sexual communication system. In *X. malinche*, however, males reflect minimally in the UV, there is no UV sexual dimorphism, and UV does not play a part in its communication system.

Keywords: private communication; Poeciliidae; *Xiphophorus*; *Astyanax*; visual communication

1. INTRODUCTION

Courtship traits often evolve under the conflicting forces of sexual and natural selection. Sexual selection favours conspicuous traits, while natural selection generated by predation leads to decreased trait conspicuousness (Endler 1980; Andersson 1994; Zuk & Kolluru 1998). Private communication, wherein species use signals detectable by conspecifics but not predators, can release signals from conflicting selection pressures and lead to elaboration and diversification of traits. Private communication has figured prominently in behavioural–evolutionary theory (Silberglied 1979; Endler 1980; Stoddard 1999). Although selection experiments have shown that predators can influence evolution of colour patterns (Endler 1982, 1983), there is little direct behavioural evidence for private communication (Zuk & Kolluru 1998).

We investigate private communication in a well-known model system of fishes: northern swordtails, *Xiphophorus* (Ryan & Rosenthal 2001), and one of their major predators, the Mexican tetra, *Astyanax mexicanus* (Rosenthal *et al.* 2001). Swordtails are sexually dimorphic, and males of many species are characterized by large size, conspicuous pigmentation and swords (Rauchenberger *et al.* 1990)—traits that can be preferred by females (Basolo 1990; Rosenthal & Evans 1998). The Mexican tetra is known to eat fish (Koster 1957; Sublette *et al.* 1990), and our studies based on predator gut contents, predator densities in the wild and predation in large semi-natural enclosures, demonstrate that this species is the swordtail's major predator (Rosenthal *et al.* 2001). Furthermore, these predators are preferentially attracted to some of the same male display traits, size and sword, that female swordtails find so attractive (Rosenthal *et al.* 2001).

In this study, we examine swordtail ultraviolet (UV) ornamentation and its use as a private communication signal by examining the geographical relationship between densities of the UV-insensitive predator (the Mexican tetra) and swordtail UV ornamentation across five swordtail species, along with behavioural experiments examining the preference of females and tetras for male UV ornamentation in two species of northern swordtails. If UV ornamentation has evolved as a sexual signal for private communication then: (i) the signal should be more conspicuous in the signaller (males) than in the receiver (females); (ii) the degree of UV ornamentation and its efficacy as a courtship signal should vary with predator densities; and (iii) predators should not be attracted to swordtail UV ornamentation. In this study we demonstrate experimentally that swordtails have diverse signal traits within a signalling spectrum that is expanded relative to their major predator's visual sensitivity, which increases conspicuousness and attractiveness to females but not to these predators. This study thus offers strong empirical support for the notion that private communication can lead to phenotypic diversification.

2. MATERIAL AND METHODS

(a) *Spectral measurements*

UV photographs (figure 1) were taken with a Nikon FM2 camera, a Zeiss Jena f4, 60 mm Ultraviolet–Objektiv lens and a Wratten 18A filter. We used Fuji RTPII film (tungsten, 64 ASA, f-22). Illumination was with 215 W UV Blacklite lamps.

Reflectances were measured using an Ocean Optics PS1000 spectrophotometer and Oriel 150 W xenon lamp (model 66056) with fishes illuminated at 45° to normal. Reflectance values are relative to a 99% (300–700 nm) reflectance standard

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Figure 1. UV and visible spectrum imaging of male *Xiphophorus nigrensis*. UV and visible photographs were taken with a Nikon FM2 camera, Zeiss Jena f4, 60 mm Ultraviolet-Objektiv lens, and Fuji RTPII film (tungsten, 64 ASA, f-22). (*a*) Colour photograph in human visible (400–700 nm) range. Enlarged sword is shown for detail. (*b*) Photograph taken with UV bandpass filter (300–400 nm: Wratten 18A) and illuminated with 215 W UV Blacklite bulbs. (*c*) Composite of the above two photographs. Image (*a*) was partitioned into the four CMYK (cyan–magenta–yellow–black) channels used in photoreproduction. Image (*b*) was substituted for the magenta channel in (*a*).

(Spectralon). We calculated a 'UV index', the ratio of mean percentage reflectance in the UV bandwidth (300–400 nm) over the mean percentage reflectance across the entire UV visible spectrum (300–700 nm) from the base of the sword. We compared this variable among the following species (figure 2): *X. cortezi* ($n = 3$ (individuals), 9 (reflectances)); *X. malinche* ($n = 6$, 20); *X. multilineatus* ($n = 2$, 10); *X. nezahualcoytl* ($n = 5$, 16); *X. nigrensis* ($n = 3$, 10).

(b) Geographical distribution

Both microspectrophotometry (Kleinschmidt & Harosi 1992) and molecular sequencing (Yokoyama & Yokoyama 1993) studies have shown that *A. mexicanus* (which is synonymous to *A. fasciatus* (Koster 1957; Sublette *et al.* 1990)) has no UV cone. Our measurements of *A. mexicanus* lens transmittance (see below) show that the lens does not transmit much of the UV bandwidth, thereby reducing the effectiveness of other cone classes, such as the short-wavelength sensitive cone (SWS), at detecting this region of light. Thus we predicted the distribution and abundance of this predator should covary with swordtails' UV reflectance. We compared the relative amount of UV reflectance on swords ('UV index'), with the density of *A. mexicanus*. Rosenthal (2000) estimated mean *A. mexicanus* densities for each locality using visual census estimates from 8 to 16, 5 m × 1 m transects at each site. These predation densities were estimated at the same localities that males were collected for 'UV index' measurements. We computed the Spearman rank correlation coefficient between UV index and predation risk. To correct for the phylogenetic dependence across species, we also calculated a correlation coefficient of the independent contrasts using COMPARE v. 4.4 (Martins 2001).

(c) Female preference experiments

Twenty female *X. nigrensis* (from the nacimiento of the Río Choy, San Luis Potosí, Mexico) and twenty female *X. malinche* (from Tlatzintla, Hidalgo, Mexico) were tested in a 120 cm × 30 cm × 48 cm aquarium with two 500 W quartz–halogen lamps. Downwelling and sidewelling irradiance measurements from *X. nigrensis* habitats (see § 2f) were used to recreate the natural light environment within the testing aquarium. Each lamp was diffused (with Teflon, which diffuses both UV and visible light) and filtered (Rosco 365 + Cinegel 4360 + GAM
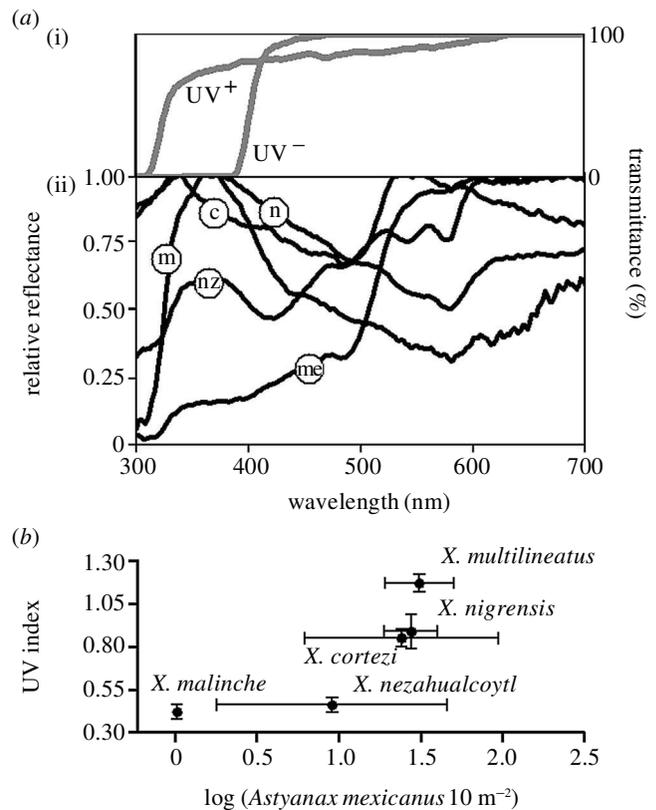

Figure 2. UV reflectance and predation intensity. (*a*)(i) Transmission spectra for the filter treatments used in the experiment: UV⁻ (GG-400 SCHOTT) and UV⁺ (Rhöm GS 2458 with GAM 10-40). (ii) Representative sword reflectance spectra normalized to the peak reflectance of male *Xiphophorus nigrensis* (n), *X. multilineatus* (m), *X. cortezi* (c), *X. nezahualcoytl* (nz), and *X. malinche* (me). (*b*) Means and ± 1 s.e.m. of log *Astyanax mexicanus* density and UV index (mean UV (300–400 nm) reflectance/mean full spectrum (300–700 nm) reflectance) of base of sword reflectance for five species of Río Pánuco basin swordtails. The phylogenetic relationships among these species are (*X. nezahualcoytl* ((*X. malinche*, *X. cortezi*) (*X. nigrensis*, *X. multilineatus*))) (Rauchenberger *et al.* 1990).

1532) to resemble a typical downwelling irradiance spectrum measured at 0.5 m depth in the nacimiento of the Río Choy. Horizontal irradiance was mimicked using filters (Lee 725 + Cinegel 4330) and a Teflon background. The testing aquarium was divided into five sections, with males confined to each of the end sections and females were allowed to roam across the three centre regions. The two 24 cm × 30 cm end regions of the aquarium were divided from the central region by either UV⁺ (UV-transmittant, Rhöm Plexiglas GS2458) or UV⁻ (UV-blocking filter, Schott GG-400) filters. The UV⁻ filter transmitted 95.8% of total flux within the experimental tank (300–700 nm), resulting in a total light flux (300–700 nm) of $2.30 \times 10^{-6} \mu\text{E cm}^{-2}\text{s}^{-1}$. Tank irradiance measurements were collected using an EPP2000C StellarNet Inc. spectrophotometer and a HOBI Laboratories 10 m underwater fibre with cosine collector. Total intensity between the two filters was equalized with a diffusion filter (GAM 10–40) above the UV⁺ side that resulted in 95.6% transmittance of experimental flux from 300 to 700 nm, resulting in a total light flux (300–700 nm) of $2.33 \times 10^{-6} \mu\text{E cm}^{-2}\text{s}^{-1}$ and a 1% difference in intensity between the two experimental treatments. Total intensity differ-

ences between treatments in the 400–700 nm range resulted in a 1% intensity difference but in the reverse direction: UV⁻ $(2.29 \times 10^{-6}\,\mu\text{E cm}^{-2}\,\text{s}^{-1})$ and UV⁺ $(2.27 \times 10^{-6}\,\mu\text{E cm}^{-2}\,\text{s}^{-1})$. Previous female choice studies using live males under laboratory conditions that prohibited UV reflection (Ryan *et al.* 1990), or video playbacks with monochromatic displays that also lacked UV (Rosenthal *et al.* 2002), have demonstrated strong female preferences. Thus, eliminating the UV bandwidth does not disrupt females' ability to recognize conspecifics.

We measured female association time with males (see Ryan & Wagner 1987; Basolo 1990; Rosenthal & Evans 1998; Rosenthal *et al.* 2002) as follows. Initially, females were placed in an opaque cylinder at the centre of the tank and allowed to acclimatize for 5 min, after which we lifted the cylinder and began recording the female's position. A female associated with a male when she was within the 24 cm 'goal area' adjacent to his flanking region, and no association response was recorded when she was in the neutral zone in the central region between the two 'goal areas'. Females were tested for four 10 min preference trials with a 3 min acclimatization period between each trial. Trials included: (i) pre-test control to evaluate preference for UV⁺ or UV⁻ conditions without males present; (ii) test with males behind filters; (iii) second test with males but with filters reversed; and (iv) post-test control with males removed. For each species, four pairs of males were matched for standard length, courtship display rate, and sword length (two sworded pairs, two non-sworded pairs). Each pair of males was tested with five females. Paired *t*-tests were computed for pooled treatment trials (males present), and pre- and post-test controls.

(d) Predator preference experiments

The same experimental procedure and *X. nigrensis* males used above to document mate preference were also used to study *A. mexicanus* prey preferences in the laboratory. Previous laboratory experiments (Rosenthal *et al.* 2001) have demonstrated robust predatory preferences of *A. mexicanus* for specific *X. nigrensis* male traits (sworded males over non-sworded males and large over small males). The tank environment and behavioural assay in Rosenthal *et al.* (2001) were similar to those in the present study, with the difference being that in the present study males were paired for size and presence of sword and varied systematically only in terms of the filters used. Twenty-one tetras ranging from 40 to 52 mm were used in this experiment. As in previous predator preference experiments, tetras were deprived of food for 24–48 h to ensure the predator's motivation. Tetras were collected at Del Rio, Texas. Trials were 3 min in length to prevent habituation (Rosenthal *et al.* 2001).

We repeated the prey preference experiment in the nacimiento of the Río Choy, San Luis Potosí, Mexico, using *A. mexicanus* co-occurring with *X. nigrensis*. A Plexiglas tank was positioned *in situ*, with the same UV⁺ and UV⁻ filters. Twenty Mexican tetras ranging in size from 41 to 90 mm were tested for filter preference with five pairs of sworded *X. nigrensis* males. Total light intensity differences were controlled as above.

(e) Intra- and interspecific variation in ultraviolet ornamentation

We collected reflectance measurements from live male and female *X. nigrensis* and *X. malinche* from corresponding body regions using the same methods described above. These species were chosen because *X. nigrensis* is among the species with the highest Mexican tetra density (Rosenthal 2000) and is the species whose reproductive behaviour has been best documented (Ryan & Rosenthal 2001). *Xiphophorus malinche*, however, is found without tetras. Reflectance measurements were collected using an EPP2000C StellarNet Inc. spectrophotometer and SpectraWiz software, an Oriel 150 W xenon lamp (model 66056) and water light guide (model 77800) with fishes illuminated from above to mimic the downwelling light environment at 30° off the zenith dorsal area. Reflectance values are relative to a 99% (300–700 nm) reflectance standard (Spectralon). We compared spectral reflectance measurements from eight female and nine male (five of which were subjects in the behavioural experiment detailed above) *X. nigrensis* from corresponding body regions that exhibited high UV reflectance (from figure 1). The mean spectral reflectance averaged numerous reflectance measurements from different body regions including caudal ($n_{\text{female}} = 8$, $n_{\text{male}} = 10$ spectra), ventral ($n_{\text{female}} = 16$, $n_{\text{male}} = 16$), and horizontal stripe ($n_{\text{female}} = 16$, $n_{\text{male}} = 16$) areas along with the log UV intensity ($\log(\Sigma_{\lambda=300}^{400} R(\lambda))$) for each spectrum. Mean spectral reflectance and log UV intensity from the same body regions were collected from eight female and six male (four of which were subjects in the behavioural experiment below) *X. malinche* from caudal ($n_{\text{female}} = 16$, $n_{\text{male}} = 10$ spectra); ventral ($n_{\text{female}} = 16$, $n_{\text{male}} = 16$); and horizontal stripe regions ($n_{\text{female}} = 14$, $n_{\text{male}} = 14$). Mean sword spectral reflectances were calculated from base and mid-sword regions from each species: five male *X. malinche* ($n = 11$ spectra), and four male *X. nigrensis* ($n = 8$). Wilcoxon signed-rank tests were computed to compare the amount of UV reflectance (log(UV)) between species (males of *X. nigrensis* and *X. malinche*) and within species (males and females of each species).

(f) Visual contrast estimates

Estimates of luminance contrast from different photoreceptor classes were used as a simple measure of visual contrast, C_c, of the *X. nigrensis* sword against background light measured in its habitat as:

$$C_c = (Q_{c,s} - Q_{c,b})/Q_{c,b}, \tag{2.1}$$

where $Q_{c,s}$ is the estimate of photoreceptor quantum catch of the diffusely reflecting sword radiance and $Q_{c,b}$ is quantum capture of background radiance:

$$Q_{c,s} = \sum_{\lambda=300}^{700} \frac{I_D(\lambda) R_s(\lambda)}{\pi} T(\lambda) A_c(\lambda), \tag{2.2}$$

$$Q_{c,b} = \sum_{\lambda=300}^{700} \frac{I_H(\lambda)}{\pi} T(\lambda) A_c(\lambda), \tag{2.3}$$

where $I_D(\lambda)$ is the downwelling irradiance measurement at 0.5 m depth in the native environment of *X. nigrensis*, and represents the dominant illuminant spectrum for the top layer of the sword (the UV-reflecting portion, see figure 1) in this shallow environment; $R_s(\lambda)$ represents the mean sword spectral reflectance of *X. nigrensis* measured in the position that mimics the reflected downwelling irradiance towards a viewer positioned in the same horizontal plane as the displaying male *X. nigrensis*; $T(\lambda)$ represents whole lens transmittance; $A_c(\lambda)$ is spectral absorptance of photoreceptor cone class, *c*; and $I_H(\lambda)$ is horizontal irradiance measured in tandem with each downwelling irradiance spectrum. Both target ($I_D(\lambda) R_s(\lambda)/\pi$) and background ($I_H(\lambda)/\pi$) radiance approximations are calculated using the simplifying assumptions that swords reflect diffusely and that the viewer's field of view is nearly hemispherical.

Paired measures of downwelling ($I_D(\lambda)$) and horizontal ($I_H(\lambda)$) irradiance spectra were collected at various locations with *X. nigrensis* present in the nacimiento of the Río Choy in March 1999 with an Ocean Optics PS1000 spectrophotometer with cosine collector modified to collect light underwater (Cummings & Partridge 2001). Absorptance spectra $A_c(\lambda)$ were estimated using reported photoreceptor peak sensitivities, λ_{max} and chromophore mixtures for *Xiphophorus* (UV-sensitive cone $\lambda_{max} = 360$ nm, and SWS cone $\lambda_{max} = 407$ nm (Rush 1996)) and *Astyanax* (SWS cone $\lambda_{max} = 453$ nm (Kleinschmidt & Harosi 1992)), and cone photoreceptor length and specific absorbance measurements of *Poecilia reticulata* (J. C. Partridge, personal communication). Lens transmittance spectra were measured on 10 *A. mexicanus* and 12 *X. nigrensis* using a Shimadzu UV201 spectrophotometer. All transmission spectra were normalized to the measured transmittance at 700 nm, and $T(\lambda)_{50}$ and $T(\lambda)_{90}$ wavelengths at 50% and 90% transmittance, respectively, were calculated from these normalized transmittance curves.

3. RESULTS

(a) Ultraviolet imaging and sword reflectance

Northern swordtails tend to lack colours conspicuous to humans (figure 1a), but exhibit substantial diversity in coloration outside of the human visible spectrum and into the UV bandwidth. The UV ornamentation of swordtails is concentrated on the mid- and ventral body sections, with minimal reflectance on the dorsal regions (figure 1). Four of the five northern swordtail species we measured reflect substantially in the UV (figures 1b and 2a).

(b) Geographical distribution

Swordtail UV ornamentation covaries with densities of *A. mexicanus*. We quantified the expression of UV on the most prominent male secondary sexual trait, the sword. Species in areas of high predator density reflect proportionately more UV (Spearman rank correlation coefficient = 1.00, $p < 0.05$; figure 2b). Analysis using phylogenetically independent contrasts also showed a strong correlation between these two variables, but given the small sample size the relationship was no longer significant (independent contrast correlation = 0.81, $p < 0.20$). This pattern suggests an opportunity for private communication of sexual advertisement signals because *A. mexicanus* should be less sensitive to signalling by their prey in these wavelengths.

(c) Ultraviolet-mediated preferences in swordtails and tetras

Females of a northern swordtail species, *X. nigrensis*, which co-occur with tetras at high densities (figure 2b), significantly preferred males with UV ornamentation (figure 3a). In an experiment that controlled for total light intensity across treatments (figure 2a top panel), females spent almost twice the time associating with conspecific males behind filters that passed (UV$^+$) rather than blocked (UV$^-$) UV (figure 3a; mean time (s) with males present: UV$^+$ = 576.6, UV$^-$ = 302.25, $t_{(2\ tail,19)} = 4.19$, $p = 0.000\ 49$; pre-test control: UV$^+$ = 215.6, UV$^-$ = 156.9, $t_{(2\ tail,19)} = 1.55$, $p = 0.14$; post-test control: UV$^+$ = 137.0, UV$^-$ = 168.2, $t_{(2\ tail,19)} = 0.62$, $p = 0.56$).

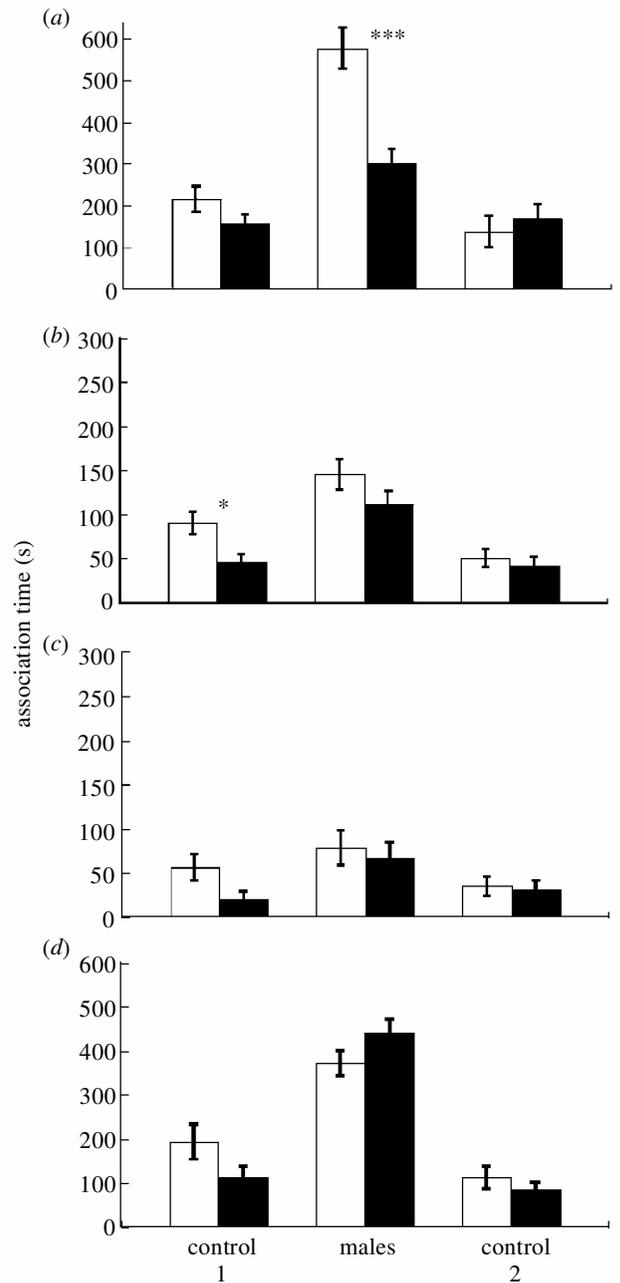*Astyanax mexicanus* did not prefer male swordtails with UV ornamentation. They exhibited no difference in

Figure 3. (a) Female *Xiphophorus nigrensis* ($n = 20$) mean association time, with ±1 s.e.m., viewing *X. nigrensis* males behind UV$^+$ (open bars) and UV$^-$ filters (filled bars). Pre-test controls (control 1) represent association time in front of filters before males were introduced into the tank. Post-test controls (control 2) represent association time after males are removed from tank. (b) *Astyanax mexicanus* from Del Rio, Texas ($n = 21$) mean association time, with ±1 s.e.m., viewing the same *X. nigrensis* males in the same laboratory setup as (a). (c) *Astyanax mexicanus* from the nacimiento of the Río Choy, Mexico ($n = 20$) measured *in situ*, using the same filters as laboratory setup with the tank situated in the river and illuminated with natural daylight. (d) Mean association time, with ±1 s.e.m., of female *X. malinche* ($n = 20$) viewing *X. malinche* males in the same laboratory setup as (a) and (b). Significance level is indicated by $^* p < 0.05$ and $^{***} p < 0.001$.

association time when tested with the same *X. nigrensis* males and filters used in the above experiment (UV$^+$ = 145.7, UV$^-$ = 111.8, $t_{(2\ tail,20)} = 1.30$, $p = 0.21$;

figure 3*b*). The predator's lack of UV response was also confirmed in the wild with *A. mexicanus* captured and tested in their native habitat where they co-occur with their prey *X. nigrensis* ($UV^+ = 78.25$, $UV^- = 66.4$, $t_{(2\ tail,19)} = 0.44$, $p = 0.66$; figure 3*c*). This lack of preference was evident despite a bias in the controls. With no swordtails present, *A. mexicanus* tended to prefer the UV^+ side of the tank (pre-test controls in the laboratory: $UV^+ = 90.8$, $UV^- = 46.5$; $t_{(2\ tail,19)} = 2.22$, $p = 0.038$; in the wild: $UV^+ = 56.65$; $UV^- = 19.95$; $t_{(2\ tail,19)} = 1.97$, $p = 0.063$). This bias may represent a preference for darker habitats. Because *A. mexicanus* lacks UV-sensitive cones the UV^+ side should appear darker ($UV^+ = 96.0\%$; $UV^- = 97.5\%$ of transmitted flux from 400 to 700 nm). Despite this bias for the UV^+ side of the tank in the control, the predators showed no bias in their attraction to UV-reflecting versus non-UV-reflecting swordtails, showing that the enhanced sexual attraction that UV reflectance affords to male swordtails does not incur a cost from this predator.

Although UV ornamentation is an important courtship signal in a swordtail species experiencing high Mexican tetra densities, it is not for a species that communicates in the absence of this predator (figures 2*b* and 3*d*). Female *X. malinche* exhibited no preference for conspecific male UV ornamentation (figure 3*d*; mean time (s) with males present: $UV^+ = 373.25$, $UV^- = 442.65$, $t_{(2\ tail,19)} = 1.50$, $p = 0.151$; pre-test control: $UV^+ = 194.0$, $UV^- = 114.3$, $t_{(2\ tail,19)} = 1.50$, $p = 0.149$; post-test control: $UV^+ = 113.85$, $UV^- = 85.1$, $t_{(2\ tail,19)} = 0.83$, $p = 0.417$).

(d) *Intra- and interspecific variation in ultraviolet ornamentation*

UV reflectance is sexually dimorphic in *X. nigrensis* and thus is likely to have evolved via sexual selection. Males reflect significantly more UV than females in all body regions measured, and the most conspicuous male secondary sexual trait, the sword, exhibits the most prominent reflectance peak in the UV bandwidth as one would expect of a sexual ornament (figure 4*a*). Male *X. nigrensis* exhibit significantly greater UV reflectance than females in corresponding body areas: mean ± 1 s.e.m. log(UV) for body caudal regions: male = 3.238 ± 0.099, female = 2.179 ± 0.012, $Z = -2.521$, $p = 0.012$; ventral regions: male = 3.335 ± 0.050, female = 2.936 ± 0.067, $p = 0.001$; horizontal stripe: male = 3.206 ± 0.061, female = 2.923 ± 0.051, $Z = -3.258$; $p = 0.001$. However, no such sexual dimorphism in UV ornamentation is observed in *X. malinche* (figure 4*b*): mean ± 1 s.e.m. caudal regions: male = 2.68 ± 0.102, female = 2.62 ± 0.261, $Z = -1.274$, $p = 0.203$; ventral regions: male = 3.059 ± 0.058, female = 3.103 ± 0.062, $Z = 0.827$, $p = 0.408$; horizontal stripe: male = 2.92 ± 0.062, female = 2.93 ± 0.063, $Z = 0.031$, $p = 0.975$. Interspecific variation in male UV reflectance shows that male *X. nigrensis* reflect substantially more UV than *X. malinche* males: caudal regions: $Z = -2.701$, $p = 0.007$; ventral regions: $Z = -3.309$, $p = 0.001$; horizontal stripe (mid body) regions: $Z = -3.107$, $p = 0.002$; sword: mean ± 1 s.e.m. UV(log): *X. nigrensis* = 3.344 ± 0.128, *X. malinche* = 2.759 ± 0.112, $Z = 1.960$, $p = 0.050$.
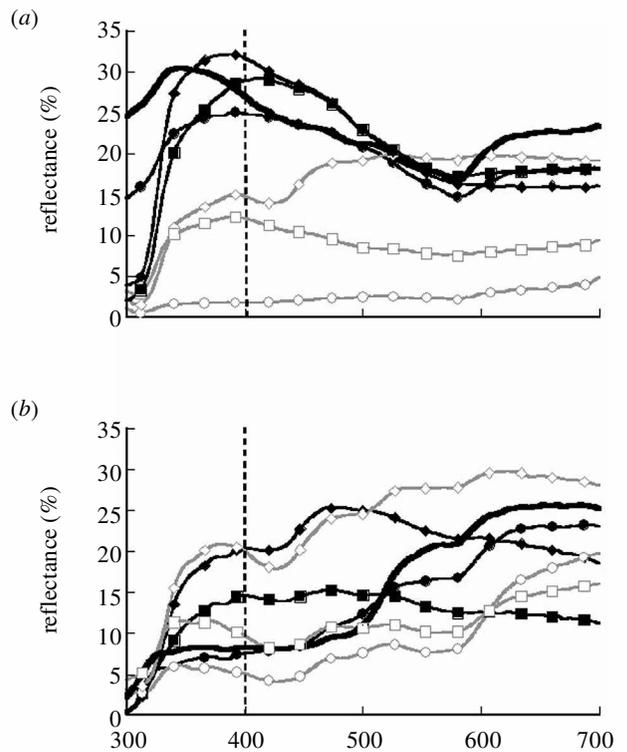
Figure 4. Mean spectral reflectance of *Xiphophorus nigrensis* (*a*), and *X. malinche* (*b*), males (black lines) and females (grey lines) for caudal (circles), ventral (diamonds), and horizontal stripe (squares) body areas and male sword (solid line). The dashed line at 400 nm represents the upper boundary for UV wavelengths.

(e) *Visual contrast estimates*

The mean values of $T(\lambda)_{50}$ and $T(\lambda)_{90}$ show that the *X. nigrensis* lenses transmit much of the UV bandwidth (mean ± 1 s.e.m. $T(\lambda)_{50} = 303.5 \pm 1.32$ nm, $T(\lambda)_{90} = 329.6 \pm 2.06$ nm). However, *A. mexicanus* have lenses that remove much of the UV bandwidth ($T(\lambda)_{50} = 342.1 \pm 4.14$ nm, $T(\lambda)_{90} = 375.4 \pm 9.38$ nm). We used a visual model to assess the degree to which UV ornamentation in *X. nigrensis* contributes to increased conspicuousness to their own species and to Mexican tetras. Visual contrast estimates of the *X. nigrensis* sword against background radiances in the wild suggest that UV sensitivity and signalling offer *X. nigrensis* four times the level of conspicuousness perceived by their own species compared with *A. mexicanus* (*Xiphophorus* C_{UV} / *Astyanax* C_{SWS} = 3.27 ± 0.67, *Xiphophorus* C_{SWS} / *Astyanax* C_{SWS} = 2.17 ± 0.36).

4. DISCUSSION

Most mate attraction signals have evolved under sexual selection favouring male conspicuousness (Andersson 1994). In many cases, however, the degree of signal elaboration and diversification is checked by predation. As males become more conspicuous to females they usually become more conspicuous to predators (Andersson 1994; Zuk & Kolluru 1998). Predation typically causes either decreased signalling or a shift to another sensory modality. Rarely does predation promote the diversification of a signal (Zuk & Kolluru 1998). Private communication is an exception, however, in that it allows signal diversification

to proceed unchecked by predation (Endler 1982; Zuk & Kolluru 1998). Although it has been suggested that private communication has been an important component of signal evolution, there is little or no behavioural evidence supporting this theory (Zuk & Kolluru 1998).

In this study, we show that UV ornamentation in *X. nigrensis* has all the components of a trait evolving by sexual selection: UV ornamentation is highly sexually dimorphic, conspicuous to conspecifics in their natural environment, and preferred by females. Males reflect substantially more UV than females across all body parts, and have the most prominent UV peaks on male secondary sexual traits (e.g. the sword). The UV ornamentation increases male conspicuousness to females by reflecting light that is offset from the dominant bandwidth of the visual background. This contrast of signal to background (conspicuousness) will be more easily perceived by species with photoreceptor sensitivity in this short-UV bandwidth than species lacking such photoreceptors. Hence, given the difference in UV sensitivity between predator and prey, visual contrast estimates of the UV-reflecting sword against natural background radiances suggest that the sword is at least three times more conspicuous to *X. nigrensis* than it is to the tetra. Most importantly, this conspicuous ornamentation influences male attractiveness to females, as *X. nigrensis* females significantly prefer males with UV reflectance. Here, as in many sexual communication systems, it appears that females prefer more conspicuous males (Andersson 1994). Our results suggest that in *X. nigrensis*, the UV may be necessary to elicit female attraction to the sword, as other studies have shown that females do not show a preference for swords under laboratory conditions where UV cues are absent (Rosenthal *et al.* 2002).

Predation pressures usually constrain the evolution of sexually dimorphic, conspicuous traits that are preferred by females (Endler 1978, 1983, 1991; Tuttle & Ryan 1981; Ryan *et al.* 1982; Alcock 1984; Andersson 1994; Promislow *et al.* 1994). Traits that are often most attractive and conspicuous to females are also detectable by the predator, and this usually results in a decrease in male display rate (Morris 1980), decrease in the expression of the conspicuous trait (Endler 1978, 1983), a switch to an alternative display behaviour (Cade 1975; Burk 1982; Lloyd & Wing 1983) or a different advertisement modality (Spangler 1984; Henry 1994). Here we have a system that represents an exception to this rule. In *X. nigrensis*, male UV ornamentation increases attractiveness to females by twofold, while eliciting no significant increase in the predator's attention (figure 3). This increase in attractiveness or attention may be largely confined to aquatic viewers, as there is relatively little UV reflectance on the dorsal regions of the body (figure 1), thereby reducing the likelihood of detection from above (e.g. birds).

The expression of UV ornamentation in the swordtails does not exhibit the compromise between conspecific attractiveness and predator avoidance that is commonly associated with secondary sexual traits. Other swordtail traits, such as male size and presence of sword, do exhibit such a compromise. Large males are more attractive to females in *X. nigrensis* (Rosenthal & Evans 1998), and the presence of a sword is attractive to females of other swordtail species (Basolo 1990). These two traits, larger size and

swords, are also more attractive to predators (Rosenthal *et al.* 2001). UV ornamentation, having escaped the constraints of predation pressure, allows for a potential increase in reproductive success without any increased risk in predation. This escape from predation costs may account for the expression of UV ornamentation on males of all sizes and both sworded and non-sworded males. Hence, males that lack swords and preferred size may still garner some female attention by the expression of UV ornamentation.

It has been theorized that the 'arms race' between signallers and predatory eavesdroppers can lead to rapid divergence between signalling species or populations, particularly if there is geographical variation in the degree of predation pressure (Verrell 1991; Zuk & Kolluru 1998). The evolution of a private communication channel, as an escape from predators, can lead to reproductive isolation and signal diversity (Lande 1981; West-Eberhard 1983; Verrell 1991). The covariation between swordtail UV ornamentation and densities of a major UV-insensitive predator, along with the differences in female preference for UV ornamentation between *X. nigrensis* and *X. malinche*, suggests that spatial variation in intensity of selection may have produced this signal diversity in northern swordtails. In these fishes, the diversity of male spectral ornamentation ranges from long-wave reflectance in *X. malinche* (figure 4*b*) to UV and short-wave reflectance in *X. nigrensis* (figure 4*a*). The degree to which these traits are expressed among species correlates with the distribution and abundance of the UV-insensitive predator. *Xiphophorus malinche* lives in a predator-poor environment and does not exhibit substantial UV reflectance. Furthermore, our behavioural experiments show that the reflectance they do exhibit has no influence on their female's perception of male attractiveness (figure 3*d*).

Evolution of private communication in the visual modality may be more rare than in other sensory modalities (Endler 1991; Zuk & Kolluru 1998). Visual signals tend to be continually produced and photoreceptors tend to have broad and overlapping sensitivity functions, so light in the 'visible' waveband (400–700 nm) can be detected to varying degrees by animals with different photoreceptor sensitivities within that range. Thus, a common response to predator-generated selection is for the prey species signalling within this 'visible' range to reduce the production of the conspicuous and female-preferred traits in high predation areas (Endler 1978, 1983). The use of UV signals in communication presents a possible exception to this generality, as many animals have lenses and corneas that prevent this bandwidth from reaching the retina (Douglas & Marshall 1999). The presence of these short-wave filters in the ocular media has yet to be fully understood, but may serve to offset costs associated with the UV waveband reaching the retina, such as the physiological costs of UV-induced damage on retinal cells (Zigman & Bagley 1971; Ham *et al.* 1976, 1979) or target detection costs in environments where UV is highly scattered and results in image degradation (Walls & Judd 1933; Lythgoe 1979; Douglas & McGuigan 1989).

UV signalling occurs in a variety of other animals (Silberglied 1979; Bennett *et al.* 1996; Kodric-Brown & Johnson 2002; Smith *et al.* 2002), and has the potential to serve as a private communication channel in aquatic

organisms to a greater degree than terrestrial organisms because of the great variation in UV sensitivity among aquatic predators (see Losey *et al.* 1999). Thorpe *et al.* (1993) conducted an exhaustive survey of lens transmittance from 120 fish species (from 49 families), finding that nearly 75% of those species exhibited UV-absorbing lenses. Many of these species are piscivores, and having UV-absorbing lenses may aid in prey detection in the aquatic environment by decreasing the amount of 'veiling light' (light scattered into the line of sight between target and viewer from particles in the medium) that can occlude an image when viewed at a distance (Lythgoe 1979). Light is scattered by water molecules, as well as small particles suspended within the water column, with shorter wavelengths scattering much more frequently than longer wavelengths. Hence, sensitivity to the shortest wavelengths (UV) can lead to a great deal of visual noise when trying to detect distant targets in aquatic environments. In these environments, UV signalling may work for animals that communicate at short distances (e.g. courtship events), but be particularly ill-suited as a prey detection cue owing to the high cost of image degradation. By contrast, other communication channels might not be protected from predator invasion by such foraging or physiological costs, as evidenced by the evolution of extended auditory sensitivity that allows some flies (see Daniel *et al.* 1992) and bats (see Bruns *et al.* 1989) to localize the acoustic signals of their prey. Thus the evolution of UV reflectance might be an especially effective means to maximize the communication advantages derived from conspicuous signals, while decreasing the disadvantages of increased mortality often associated with such signals.

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