

# Population divergence of private and non-private signals in wild guppies

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**Abstract** Private signalling, where conspecifics use mating signals that are difficult to detect by predators, can reduce the compromise between opposing natural and sexual selection. We investigated whether guppies, *Poecilia reticulata*, use an hypothesized private signal. In some areas, guppies, who can detect ultraviolet (UV) light, coexist with dangerous predators, but at least some of these cannot detect UV. In these populations of guppies, UV might be used as a private signal. We tested this hypothesis by quantifying the UV and non-UV colouration of male guppies from paired high- and low-predation sites in five rivers. We found evidence in support of the use of UV as a private signal: male guppies living with the predator generally had greater UV reflectance than male guppies living without the predator. Closer inspection revealed differences in this trend among rivers and thus suggested the influence of modifying factors. We also found that the non-UV colour of male guppies does not differ consistently between high- and low-predation environments. This

result suggests that a number of factors in addition to predation act on male colour in guppies.

**Keywords** Private signalling · Secondary sexual traits · Colour · Predation · *Poecilia reticulata* · UV

## Introduction

Secondary sexual traits evolve in response to both natural and sexual selection. These traits are often conspicuous and are used during mating to attract the opposite sex or to interact with members of the same sex (Andersson 1994; Maynard-Smith and Harper 2003). By virtue of their conspicuousness, secondary sexual traits may also increase the susceptibility of the bearer to predation (Endler 1980). Populations subject to high levels of predation should evolve, through natural selection, a reduced trait expression to decrease conspicuousness. Thus many secondary sexual traits evolve as a compromise between opposing natural and sexual selection (Endler 1980). Given this apparent compromise between obtaining mates and avoiding predators, selection might favour mating signals that break the above constraint, for example, by facilitating signalling between conspecifics while reducing predation risk. One way to escape this constraint is through the evolution of “private signals”: signals that can be perceived by conspecifics but not, or to a lesser degree, by predators (Endler 1978, 1983; Cummings et al.

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2003). Our goal is to examine the evidence for a private signal in a fish species whose secondary sexual traits are a classic example of the compromise between effective signalling to potential mates and avoiding detection by predators.

Guppies (*Poecilia reticulata*) are a small freshwater fish native to Trinidad and north eastern South America. Natural guppy populations can be classified into two general types according to whether they live with dangerous fish predators (high predation) or without them (low predation). By ‘dangerous’, we mean that predation from these fish causes significant mortality and selection in guppies. Although this dichotomous contrast between high and low predation is crude, it is associated with strong differences in mortality rate (Reznick et al. 1996; Bryant and Reznick 2004; Gordon et al. 2009; Weese et al. 2010) and consistent differences in guppy life history, morphology, and behaviour (reviews: Endler 1995; Houde 1997; Magurran 2005). The high predation versus low predation contrast is therefore standard, useful, and predictive—although future work would certainly benefit from more quantitative measures of predation intensity.

We are here interested in male guppy colour patterns (colouration): a complex and highly variable arrangement of spots of different sizes, shapes, and colours (Endler 1978; Kemp et al. 2008). Despite high variability among individuals at a given sampling site, guppies in low-predation environments are considered to be more colourful than are those in high-predation environments (Haskins et al. 1961; Endler 1978; Houde 1997; Millar et al. 2006). This difference is thought to reflect the above-described balance between opposing natural and sexual selection. That is, females usually prefer to mate with more colourful males (Endler 1983; Kodric-Brown 1985; Houde 1987; Brooks and Caithness 1995; Endler and Houde 1995) but these same males are expected to be more likely to be seen by predators (Endler 1978, 1980). This rationale has been elegantly supported by introduction experiments in the wild, where guppies rapidly evolved greater colour in the absence of dangerous fish predators and evolve reduced colour in their presence (Endler 1980), although more recent work has not always found similar results (Karim et al. 2007; Kemp et al. 2008).

Guppies may not always be held hostage to this classic constraint, and instead might evolve private signals in visual “channels” outside the spectral (visual)

sensitivity of local predators. For example, some evidence has been found for one such private channel. In a portion of their range, guppies live with the potential predator *Macrobrachium spp.*, a freshwater prawn. *Macrobrachium*, as a decapod crustacean, is insensitive to long wavelengths of light, such as orange and red, but are sensitive to short wavelengths, including UV (Endler 1991; Kemp et al. 2008). Consequently, orange colour could be used by guppies as a private signal where prawn densities are high and dangerous fish predators are absent (Millar et al. 2006). This hypothesis is supported by two observations. First, male guppies living with a high abundance of prawns have *more* orange colour (Endler 1978, 1983, 1991; Millar et al. 2006). Second, females from at least some of these sites (e.g., Paria River) demonstrate a higher preference for orange than do females from other populations (Houde and Endler 1990).

In the present study, we look for evidence of a second private signal in guppies: a UV channel. Most of the work on natural variation in guppy colour has thus far been based on the part of the spectrum that is visible to humans (400–700 nm). Guppies, however, are also sensitive to UV light (Archer et al. 1987; Douglas and McGuigan 1989; Archer and Lythgoe 1990; Kemp et al. 2008), and the UV component of male colour may be important for female mate choice (Kodric-Brown and Johnson 2002; Smith et al. 2002; *but see* White et al. 2003). At the same time, predatory fishes found in different environments vary in their sensitivity to UV light. For example, the main predator in low-predation sites, Hart’s rivulus (*Rivulus hartii*), is sensitive to UV light, whereas at least one of the most important predators in high-predation sites, the millet (*Crenicichla sp.*), is not (Endler 1991; Kemp et al. 2008). Here then is the opportunity for a private UV signal to evolve in an environment where alternative visual signals are demonstrably costly to survival.

To test the above hypothesis, we quantified the non-UV and UV colour components of wild male guppies from paired sites with (high predation) and without (low predation) *Crenicichla* in five separate rivers. If signals visible to dangerous predators evolve as a compromise between natural and sexual selection, then males from low-predation populations should show a greater expression of non-UV colour than should males from high-predation populations, as has been previously demonstrated (Endler 1978). If

signals invisible to dangerous predators have allowed an escape from the constraint imposed by natural selection, then males from high-predation populations should show a greater expression of UV than should males from low-predation populations.

## Materials and methods

### Sampling sites

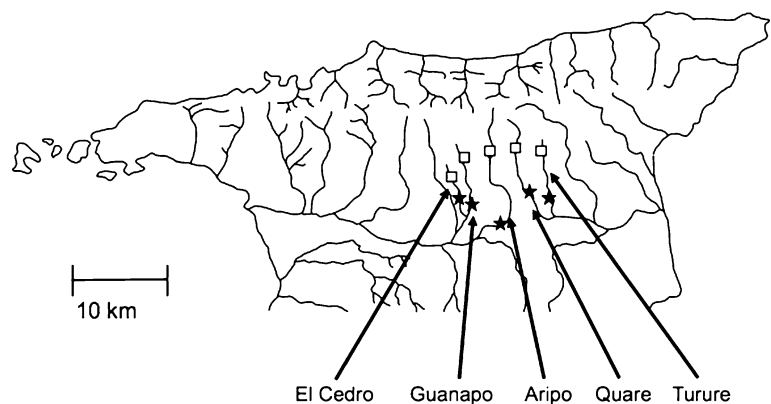
In March and April of 2004, we visited ten sites on the south slope of Trinidad's Northern Range Mountains. These were paired high- and low-predation sites in each of five rivers: Aripo, El Cedro, Guanapo, Quare, and Ture, (see Fig. 1, Table 1). Three of the rivers (Guanapo, El Cedro, and Aripo) are in the westward flowing Caroni drainage, whereas two (Quare and Ture) are in the eastward flowing Oropuche drainage. These two major drainages contain different ancestral lineages of guppies that have been separated for about 500 000 years and significant genetic differences exist between populations within these drainages (Fajen and Breden 1992; Suk and Neff 2009). In the present study, we treat the five rivers (as opposed to the two drainages) as separate replicates because (1) genetic differences are substantial between rivers even within a drainage, and (2) upstream low-predation populations within a given river are generally most closely related to the downstream high-predation populations within the same river (e.g., Alexander et al. 2006; Suk and Neff 2009; Willing et al. 2010). It is widely considered that each low predation site has been independently colonized from the immediate downstream high

predation population such that each high- and low-predation pair is an independent evolutionary pair. Thus our study design tests for parallel evolution among the guppy populations in our five study rivers. Specific collection sites were chosen based on accessibility and background knowledge about predation regimes (Endler 1978; Reznick et al. 1996).

At each site, we recorded potential predators during qualitative visual surveys, each lasting at least an hour. Minnow traps were then used to assay the abundance of *Rivulus* and *Macrobrachium*. These traps were baited with a standard amount of dried dog food and were left in the stream for an average of 45 min. We then calculated catch per unit effort (CPUE) as the number of *Rivulus* or *Macrobrachium* caught in an average trap over a standardized period (in this case, we used 1 h). We also recorded any other predators captured in the traps. We did not calculate CPUE for the other predators because the visual survey and traps would not provide an unbiased estimate. Instead, these methods were merely used to verify previous inferences for these sites about whether or not dangerous predators were present.

Physical habitat features can influence the evolution of guppy colour (Endler 1978, 1983; Grether et al. 2001; Reznick et al. 2001; Millar et al. 2006). We therefore quantified potentially relevant habitat features at each site. Note that we could not quantify all potentially relevant variables (e.g., the size and color of the substrate) and so our inferences are restricted to the specific habitat features that we could measure. First, we measured the wetted width of the stream at each of ten locations per site. Second, also at these ten locations at each site, we measured water depth at each of three equidistant points across the stream.

**Fig. 1** Map of northern Trinidad showing locations of the sampling sites. We sampled 25 male guppies from paired high-predation (filled stars) and low-predation (open squares) sites in five rivers on the south slope of the Northern Mountain Range



**Table 1** The locations of sampling sites on the south slope of Trinidad's Northern Range Mountains

River	Predation	Latitude	Longitude	Location
Aripo	High	20 P 0695829	1177496	PS 940 781
	Low	20 P 0693325	1181913	PS 931 817
El Cedro	High	No reading could be taken		PS 896 788
	Low	20 P 0689788	1178724	PS 895 797
Guanapo	High	20 P 0691156	1178883	PS 911 788
	Low	20 P 0689526	1184619	PS 893 844
Quare	High	20 P 0697672	1179461	PS 975 792
	Low	20 P 0697317	1181153	PS 969 810
Turure	High	20 P 0700344	1178573	QS 703 783
	Low	20 P 0699964	1181969	PS 999 819

Latitude and Longitude are from GPS readings. "Location" is based on co-ordinates from UTM grid locations read from 1:25,000 maps (Lands and Surveys Division, Port of Spain, Trinidad)

Third, at five locations per site, we quantified canopy openness with a concave spherical densiometer (Lemmon 1957). At each of these locations, densiometer readings were taken facing each cardinal direction while standing in the middle of the channel. Measurements for stream width, water depth, and percent canopy openness were log ten transformed to improve normality.

Spectral properties of the water have the potential to influence the evolution of fish colouration (Endler 1991; Boughman 2001; Reimchen 1989). To assess this possibility in our system, we took water samples from each collection site, and held them in the dark until all could be processed on the same day. All of the rivers were very clear at the time of sampling and visual differences in water colour were still visible

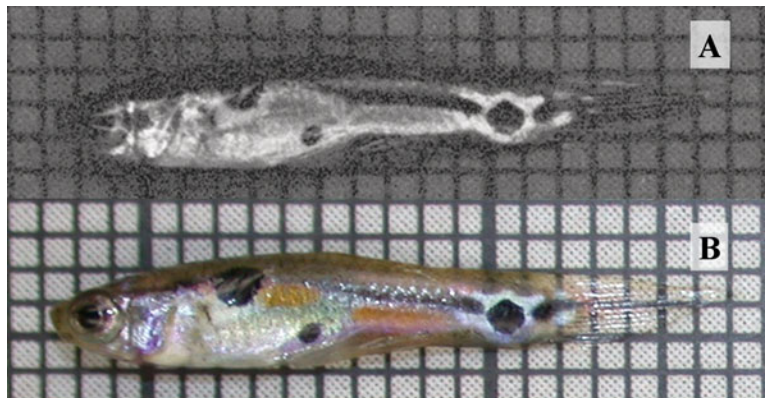
when the samples were run. Water samples were loaded into a blackened PVC tube (path length: 48.6 cm). Light from an Ocean Optics DH-2000 light source was directed through a collimating lens into the tube, and a bare fibre optic cable collected light at the far end of the tube and transmitted it to an Ocean Optics SD2000 spectrometer. We recorded transmission spectra (300–700 nm) as percent transmission relative to a standard sample of filtered water. To summarize the relevant information on transmission of ultraviolet light, we calculated a UV attenuation index ( $I_{UV}$ ). This index, calculated as [mean transmission 300 to 400 nm] / [mean transmission 300 to 700 nm], reflects the shape of the transmission spectrum regardless of its height (light intensity). Relative to the standard water sample, increased attenuation of UV decreases  $I_{UV}$ ; higher  $I_{UV}$  values are associated with relatively more UV transmission.

#### Fish collection and photography

Twenty-five male guppies were collected from each of the ten sites and killed with an overdose of tricaine methanesulfonate (MS-222). Each fish was placed, with its left side facing up, on a grid-ruled background and illuminated with two full spectrum fluorescent lights (Vitalite, Durotest Canada). These lights mimic the light spectrum of full sunlight. Colour photographs were taken with a Nikon CoolPix 995 or a Nikon D100, the latter equipped with a Sigma 105 mm macro lens. Each fish was photographed twice, once with and once without the camera's flash.

Immediately after the colour photographs of each fish, we took a UV photograph (Fig. 2). For this we used Kodak T-MAX 400 ISO black and white film

**Fig. 2** UV (a) and colour (b) photographs of a male guppy from the low-predation sampling site in the Aripo River



(sensitivity: 200–700 nm) in a Nikon F65 camera equipped with the above Sigma lens. To exclude non-UV light from the image, we used a filter (Kodak Wratten 18A) that allowed light transmission only from 300 to 400 nm. In combination, the lens and filter allowed the transmission of light from 360 to 400 nm. All rolls of film were processed as a single batch to ensure standardization.

#### Photo analysis – non-UV

All images were analyzed “blind” with respect to the site of origin, and in random order (across and within sites), by a single person (NPM). Colour photographs were analyzed using *Scion Image* (version Beta 4.02, <http://www.scioncorp.com/>) following the methods of Millar et al. (2006). These methods have been used for decades and have proven sensitive enough to observe differences in colour pattern that are the basis of the most convincing observational and empirical proof about selection in the wild (Endler 1980). Briefly, we measured body length (tip of the jaw to the end of the caudal peduncle), body area (entire side of the fish, excluding fins and tail), and the area of each colour spot on the left side of the body (excluding the fins and tail). Each colour spot was assigned to one of eight colour categories (after Endler 1978, 1991; Millar et al. 2006): orange (includes red), black (includes fuzzy black), yellow, blue (includes purple), green, violet-blue, bronze-green, and silver. The flash and non-flash photographs were viewed simultaneously when the spots were measured and the colours assigned. This facilitated appropriate categorization and measurement because some spots look different under different lighting conditions. In particular, the iridescent spots are highly reflective and hence easier to define using the flash photographs.

Colour spots that, on average, made up less than 10% of the total area of colour spots (yellow, bronze-green, blue, and silver) were not analyzed individually. They were, however, included in composite measures of colour such as “structural colour” and “total colour.” Our analyses here focused on two general measures of colour: the total number of spots of a given colour (“number of spots”) and the total area of a given colour divided by body area (“relative area”). To improve normality, relative areas were arcsine square-root transformed.

#### Photo analysis – UV

The UV image negatives were scanned and the resulting digital images were analyzed in *Adobe Photoshop* (Version 6.0.1, Adobe Systems Inc., California). With the lasso tool, we outlined the entire fish and then each colour spot. Outlining colour spots while viewing the UV image alone was difficult because some colours differ little in UV reflectance (Fig. 2). To ensure that our analysis based on UV images defined spots in the same way as the analysis based on colour images, we also viewed the colour image to facilitate the outlining of spots on the UV images. Glare from the fluorescent lights sometimes covered part of a spot, and these areas were excluded. For each lassoed area, we recorded the mean luminosity (using the Histogram tool), which ranged from 0 (black) to 255 (white). Mean luminosity is our measure of the average amount of UV reflectance of the selected area.

Spot luminosity could be affected by changes in lighting conditions, and so we also measured a reference luminosity in each UV photograph (e.g., Villafuerte and Negro 1998). We did so at two location types on the background: on grid lines and between grid lines. For each location type, we took four repeat measures: one measure per quadrat of the photograph.

From mean UV reflectance (luminosity) measurements of the spots, we calculated a metric of UV reflectance for each fish. For each colour spot, we multiplied the mean UV reflectance by the total spot area. We then summed the resulting values for all colour spots on the fish. This “total UV reflectance of the colour pattern” is partly a function of the area of the colour pattern. To create a metric that is area-independent (to allow comparisons between populations of guppies that differ in size or area of colour pattern), we then divided the total UV reflectance of the colour pattern *by the area of the colour pattern*. This index, the “relative UV reflectance of the colour pattern”, represents the amount of UV reflectance for a given area of colour pattern and was used as our metric of UV reflectance.

#### Statistics

We used SPSS (Version 11.0.1) for all statistical analyses. Analysis of non-UV colour was based on a MANOVA that included river (fixed—because we will require river-specific inferences), predation (fixed), and

the river-by-predation interaction as predictor variables. All 12 elements of colour pattern (Table 2) were included as response variables: relative area and number of black, orange, violet-blue, green, structural, and total spots. We found a strong interaction between the effects of predation and river (see Results), which precluded a straightforward interpretation of main effects. Our main question was whether high-predation sites differ in colour from low-predation sites within each river. We therefore next used MANOVA to test for the effects of predation separately within each river. These MANOVAs were followed by colour-specific ANOVAs to yield insight into which particular colours drove the observed patterns.

Our analysis of UV first considered the correlation between UV reflectance of the colour pattern on a fish and UV reflectance of the background in the photograph. This correlation was not strong, but was significant ( $r = 0.265$ ,  $P < 0.001$ ), and so we included UV reflectance of the background as a covariate in subsequent analyses. Our analysis of UV reflectance was therefore based on ANCOVA that included river and predation as fixed effects (with an interaction), UV reflectance of the background as the covariate, and relative UV reflectance of colour pattern as the dependent variable. Similar to our analysis of non-UV colour, we then used ANCOVAs for each river

separately, and then did the equivalent river-specific analysis for the mean UV reflectance of *each type of colour spot* (i.e., orange, black, etc.).

Because physical habitat features may influence the expression of guppy colour, we examined Pearson's correlations across all 10 sites between habitat features (stream width, depth, canopy openness,  $I_{UV}$ ) and aspects of non-UV colour and UV reflectance. Finally, to assess which variables were the most responsible for the variation we observed in colouration, we used an information theoretic approach to model selection (Burnham and Anderson 1998). We compared four different regression models that explained variation in non-UV colour (river, predation, river & predation, river & predation & canopy openness) and five different regression models that explained variation in UV colour (river, predation, river & predation, river & predation & canopy, predation and  $I_{UV}$ , river and  $I_{UV}$ ). AIC<sub>c</sub> differences ( $\Delta_i$ ) were used to determine the likelihood that a given model is the best model from among the candidate models. The best model has a  $\Delta_i$  value of zero. Models with  $\Delta_i$  values up to two have substantial empirical support, models with  $\Delta_i$  values from 4 to 7 have considerably less empirical support, and models with  $\Delta_i$  values above ten have essentially no empirical support (Burnham and Anderson 1998).

**Table 2** Statistical results for comparisons of non-UV colour between high- and low-predation sites in each of five rivers

Colour pattern element	Aripo		El Cedro		Guanapo		Quare		Turure					
	F	P	F	P	F	P	F	P	F	P				
Relative area of black	5.255	0.026	L	0.186	0.668	0.393	0.534	27.039	<0.001	L	4.759	0.034	L	
Relative area of orange	4.696	0.035	L	3.094	0.085	12.496	0.001	H	0.934	0.339	4.946	0.031	H	
Relative area of violet-blue	0.659	0.421		0.072	0.790	22.765	<0.001	H	0.528	0.471	1.882	0.176		
Relative area of green	1.341	0.253		1.728	0.195	0.138	0.712	11.513	0.001	H	0.236	0.629		
Relative area of structural	0.009	0.927		3.531	0.066	10.709	0.002	H	13.635	0.001	H	7.738	0.008	H
Relative total area	10.486	0.002	L	1.407	0.241	13.429	0.001	H	1.183	0.282	3.179	0.081		
No. of black spots	5.621	0.022	L	0.171	0.681	0.600	0.442	9.404	0.004	L	0.439	0.511		
No. of orange spots	16.835	<0.001	L	0.022	0.884	2.477	0.122	5.091	0.029	L	1.367	0.248		
No. of violet-blue spots	0.955	0.333		0.328	0.569	7.188	0.010	H	0.074	0.787	5.762	0.020	H	
No. of green spots	0.388	0.536		1.087	0.302	0.170	0.682	11.362	0.001	H	0.768	0.385		
No. of structural spots	6.517	0.014	L	0.000	1.000	0.514	0.477	1.046	0.311		3.308	0.075		
Total number of spots	24.248	<0.001	L	0.304	0.584	5.169	0.028	H	2.185	0.146	0.000	1.000		

Shown are  $F$  values for the predation effect in an ANOVA for each colour pattern element and associated  $P$  values. Significant differences are followed by an H (high predation) or an L (low predation) to indicate the population with the greater amount of that colour element. For all comparisons,  $df = 1$  between groups and  $df = 48$  within groups

## Results

### Predators

*Crenicichla* was seen at all of our high-predation sites. Other predators seen at these sites, although not always, included another cichlid (*Aequidens pulcher*) and predatory characins (*Astyanax bimaculatus* and *Hemibrycon dentatum*). None of these predators were seen at our low-predation sites. *Rivulus* was not caught at any of the high-predation sites, but its abundance at low-predation sites ranged from low (Guanapo, CPUE = 3.94 individuals per trap hour) to intermediate (Aripo, CPUE = 9.60; Turure, CPUE = 10.45) to high (El Cedro, CPUE = 17.29). We were not able to assay *Rivulus* abundance at the Quare low predation site because of access difficulties. *Macrobrachium* were very rare, with only four captured during our entire sampling period: one in the Aripo low-predation site (CPUE = 0.08) and three in the Turure low-predation site (CPUE = 0.39). This rarity of *Macrobrachium* even in low-predation sites on the south slope of the Northern Mountain Range was in sharp contrast to the north slope, where CPUEs based on identical methods are very high (Range: 1.69–28.61; Mean = 11.89; Millar et al. 2006).

### Non-UV colour

Male guppy colour differed significantly among rivers (MANOVA; Wilks'  $\lambda = 0.371$ , partial  $\eta^2 = 0.240$ ,  $df = 48$ ,  $P < 0.001$ ) and between predation regimes (Wilks'  $\lambda = 0.694$ , partial  $\eta^2 = 0.172$ ,  $df = 12$ ,  $P < 0.001$ ), with a significant interaction (Wilks'  $\lambda = 0.578$ , partial  $\eta^2 = 0.168$ ,  $df = 48$ ,  $P < 0.001$ ). This interaction arose because predation did not have the same effect on colour divergence in all five rivers (Table 2, ANOVAs). In the El Cedro, no differences were evident between high- and low-predation males (Wilks'  $\lambda = 0.647$ ,  $df = 12$ ,  $P = 0.110$ ). In the Aripo, low-predation males were *more* colourful than high-predation males (Wilks'  $\lambda = 0.460$ ,  $df = 12$ ,  $P = 0.001$ ), whereas in the Guanapo, low-predation males were *less* colourful than high-predation males (Wilks'  $\lambda = 0.288$ ,  $df = 12$ ,  $P < 0.001$ ). In the Turure and Quare, males from high- and low-predation environments differed in colour (Wilks'  $\lambda = 0.454$ ,  $df = 12$ ,  $P = 0.001$ ;  $\lambda = 0.342$ ,  $df = 12$ ,  $P < 0.001$ ), but in neither river were males from a particular

predation regime clearly more colourful overall. Instead, guppies from low-predation sites in these two rivers had more of certain colours but less of other colours relative to their high-predation counterparts (see Table 2, and Fig. 3a, b).

### UV reflectance

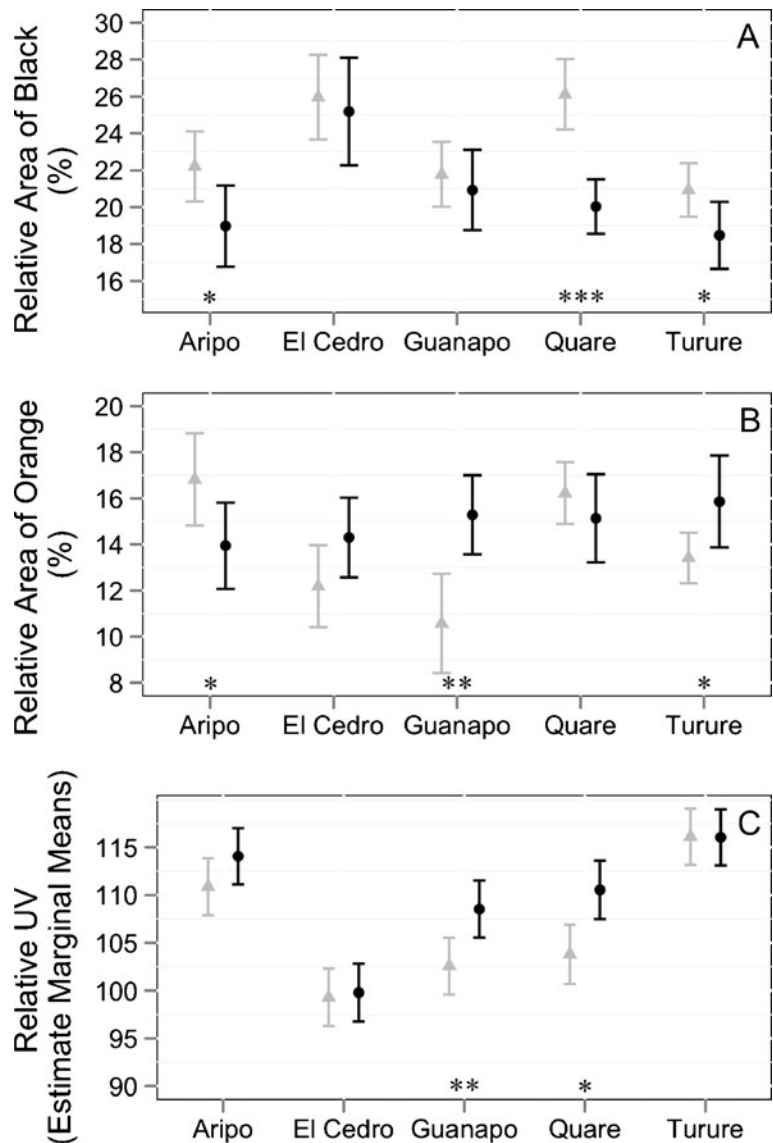
When all rivers were analyzed together, relative UV reflectance of the colour pattern was influenced by predation (more UV in high-predation sites,  $F = 11.600$ ,  $df = 1$ ,  $P = 0.001$ ), river ( $F = 36.237$ ,  $df = 4$ ,  $P < 0.001$ ), and background UV ( $F = 14.321$ ,  $df = 1$ ,  $P < 0.001$ ), without a significant interaction between river and predation ( $F = 1.941$ ,  $df = 4$ ,  $P = 0.104$ ). Visual inspection, however, suggest that an interaction might actually be present (Fig. 3c). We therefore also examined the influence of predation within each river. In two of the five rivers (Guanapo and Quare), high-predation males had significantly greater relative UV reflectance than did low-predation males. In the other three rivers, there was no significant difference (Table 3, Fig. 3c).

Despite these differences based on overall UV reflectance, we found very few differences between high- and low-predation males in the average UV reflectance of specific colours. No differences were found in the Guanapo, Quare, and El Cedro (all  $P > 0.05$ ). In the Turure, low-predation males had more UV reflectance from orange spots ( $P = 0.001$ ) and from all spots overall ( $P = 0.042$ ). In the Aripo, high-predation fish showed more UV reflectance from black spots ( $P < 0.001$ ) and less UV reflectance from structural colours ( $P = 0.048$ ). These results suggest that UV reflectance divergence between high- and low-predation environments is shared subtly across the UV component of multiple colour pattern elements, rather than being concentrated in one particular type of spot.

### Physical habitat features

Some aspects of non-UV colour were correlated with physical habitat features ( $I_{UV}$  index, canopy openness, and water depth, Table 4). Closer inspection, however, revealed that these correlations were driven by a single site. In particular, the Aripo low-predation site was very shallow, had a high  $I_{UV}$  index, and had very colourful males. When this site was removed from the analyses, no correlations were significant. We also

**Fig. 3** Variation in selected UV and non-UV components of male colour in low-predation (grey triangles) and high-predation (black circles) sites in five rivers. The specific non-UV colour components shown here were selected as those most commonly studied and reported in the literature. Points represent mean values ( $\pm$  95% confidence intervals) of the relative area of black (a), the relative area of orange (b), and the relative UV reflectance of the colour pattern while accounting for background UV: the Estimated Marginal Means are plotted (c). Relative area is the proportion (%) of the total body area covered by spots of that colour. Relative UV reflectance is the total UV reflectance divided by the area of the colour pattern (see *Methods*). Significant differences between high- and low-predation sites are indicated with asterisks: \*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ , \*  $P < 0.05$



failed to find any significant correlations between the abundance of *Rivulus* and aspects of male colour. No aspects of UV colour were correlated with physical habitat features or *Rivulus* CPUE (all  $P > 0.05$ ). In short, among-site variation in non-UV and UV colour was not correlated with physical habitat features or variation in *Rivulus* density (as proxied by CPUE).

In the model selection exercise, the models that included either predation or river as predictors by themselves had the best support (Table 5). The model with canopy openness included had no support, but the model that had depth as a predictor had considerable support for some aspects of colour.

Models that included  $I_{UV}$  had some limited support in explaining variation in UV colour.

## Discussion

### Non-private signals

It has been repeatedly stated that male guppies are more colourful in low-predation environments than in high-predation environments (Haskins et al. 1961; Endler 1978, 1980, 1983; Winemiller et al. 1990; Millar et al. 2006). Here we used methods that were



**Table 3** Statistical results for comparisons in relative UV reflectance of colour pattern between high- and low-predation males

Relative UV reflectance of colour pattern			
River	<i>F</i>	<i>P</i>	
Aripo	1.454	0.234	
El Cedro	1.531	0.222	
Guanapo	9.555	0.003	H
Quare	5.725	0.021	H
Turure	0.059	0.809	

Relative UV reflectance is the total UV reflectance divided by the area of the colour pattern (see *Methods*). Shown are *F* and *P* values for predation from ANCOVAs for each river analyzed separately. The model includes background UV as a covariate. Significant differences are followed by an H (high predation) or an L (low predation), which indicate the population with the greater relative UV reflectance. For all comparisons, *df* = 1

similar to the majority of previous work on guppy colour (measurements of the number and size of colour spots from photographs of wild-caught males) with a powerful “parallel evolution” sampling design (e.g., Langerhans and DeWitt 2004; Schluter 2000) that used paired high- and low-predation sites within separate drainages. We were therefore surprised to find that the effect of predation differed dramatically among

ivers. In comparison to high-predation males from the same river, low-predation males were more colourful in the Aripo, similarly colourful in the El Cedro, and less colourful in the Guanapo. Differences in male colouration within the Turure and Quare depended on the specific colour pattern elements under consideration (Table 2). We can see several reasons for the interesting discrepancy between our results and the conclusions reached in most previous studies.

One possibility is that variation in sexual selection (i.e., female preference) influences among-population variation in male colour. Female preferences certainly vary appreciably among populations (Houde 1988; Endler and Houde 1995; Brooks and Endler 2001; Rodd et al. 2002), and are sometimes correlated with aspects of male colour (Houde and Endler 1990). A second possibility is related to the fact that several of our low-predation populations were recently derived from introduced high-predation guppies (1981 for the El Cedro and 1967 for the Turure) and may not yet have had enough time to evolve the equilibrium phenotypes characteristic of low-predation environments. However, this constraint seems unlikely because guppies in other introductions have shown rapid adaptation of both life history (Reznick et al. 1997) and sometimes colour (Endler 1980; Kemp et al. 2008; Kemp et al. 2009).

**Table 4** Statistical results for correlations between physical habitat features and aspects of non-UV male colour

Colour pattern element	<i>I<sub>UV</sub></i>		Width		Depth		Canopy	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Relative area of black	-0.237	0.540	-0.579	0.102	-0.495	0.175	-0.677	0.031
Relative area of orange	0.576	0.104	-0.037	0.925	-0.444	0.232	0.330	0.352
Relative area of violet-blue	0.316	0.407	0.438	0.238	0.145	0.710	0.478	0.162
Relative area of green	-0.460	0.213	0.354	0.350	0.435	0.243	0.321	0.366
Relative area of structural	0.090	0.819	0.076	0.845	-0.137	0.725	0.627	0.052
Relative total area	0.174	0.655	-0.477	0.194	-0.716	0.030	0.060	0.868
No. of black spots	0.828	0.006	-0.533	0.139	-0.892	0.001	-0.243	0.499
No. of orange spots	0.777	0.014	-0.407	0.277	-0.803	0.009	-0.332	0.349
No. of violet-blue spots	0.383	0.309	0.248	0.520	0.069	0.860	0.381	0.277
No. of green spots	-0.349	0.357	0.415	0.266	0.336	0.377	0.328	0.355
No. of structural spots	0.661	0.052	-0.618	0.076	-0.795	0.010	0.139	0.701
Total number of spots	0.830	0.006	-0.527	0.145	-0.862	0.003	-0.085	0.815

Shown are Pearson’s correlation coefficients (*r*) and corresponding *P* values. Significant correlations are bolded. *I<sub>UV</sub>* is our index of the transmission of light of different wavelengths and is calculated as [mean transmission 300 to 400 nm] / [mean transmission 300 to 700 nm]. Relative to the standard water sample, higher *I<sub>UV</sub>* values are associated with greater transmission of short (UV) wavelengths

**Table 5** AIC<sub>c</sub> differences ( $\Delta_i$ ) of regression models explaining variation in aspects of male colour amongst the ten study sites

	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7
	River	Predation	River and predation	River, predation, and canopy	Predation and depth	Predation and I <sub>UV</sub>	River and I <sub>UV</sub>
No. of black spots	4.06	3.52	9.27	18.19	0.00		
No. of orange spots	1.47	<b>0.00</b>	5.96	14.96	0.29		
No. of violet-blue spots	0.24	<b>0.00</b>	5.79	14.42	5.25		
No. of green spots	0.69	<b>0.00</b>	5.86	17.82	3.28		
No. of structural spots	<b>0.00</b>	2.74	6.00	14.92	3.42		
Total number of spots	<b>0.00</b>	0.40	5.69	14.58	0.12		
Relative area of black	1.05	<b>0.00</b>	5.69	12.63	4.36		
Relative area of orange	0.36	<b>0.00</b>	5.97	14.82	6.72		
Relative area of violet-blue	2.58	2.38	7.79	16.02	<b>0.00</b>		
Relative area of green	0.59	<b>0.00</b>	5.90	14.90	6.67		
Relative area of structural	2.17	<b>0.00</b>	5.51	14.41	6.46		
Relative total area	<b>0.00</b>	0.51	5.74	14.24	0.27		
Total UV reflectance	0.06	<b>0.00</b>	5.13	13.16		3.12	4.05
Relative UV reflectance	<b>0.00</b>	0.21	5.56	13.67		5.00	3.16

The model with a  $\Delta_i$  value of zero is the best model, values of up to two have considerable support and models with values of ten and greater have little to no support

A third possibility is that colour evolution is influenced by more subtle aspects of the environment like physical habitat features (Endler 1980, 1991; Grether et al. 1999; Millar et al. 2006; Schwartz and Hendry 2009), and the extent to which these features differ between high- and low-predation environments is variable across rivers. Here we did not find robust correlations between physical habitat features and aspects of male colour, nor did we find that models that included physical habitat parameters performed better than models that were based on predation and river alone—but we did not, of course, measure all potentially relevant variables. A final possibility is that our analysis methods were not powerful enough to detect the real divergence in colour. For instance, our capacity to detect differences in certain colours is limited and biased by the specifics of the human visual system which differs from that of guppies and that of guppy predators. Other approaches, such as the use of spectrophotometry, alone or in combination with information about spectral sensitivity (i.e., visual modeling), measure colour differently and may therefore lead to different conclusions about colour divergence (e.g., Endler 1991; Kemp et al. 2008; Kemp et al. 2009). For example, recent work has shown that, while the magnitude of changes

in spot size and number can be minimal in some high-versus low-predation contrasts, other aspects of spot colour like spectral properties can change dramatically (Kemp et al. 2009). Counter to this argument that our colour analysis methods was not powerful enough is the fact that many studies have used similar or identical methodologies for colour analysis and have found consistent differences in colour between high- and low-predation sites.

In conclusion, our finding that some rivers do not show the classic high- vs. low-predation colour difference (see also Karim et al. 2007; Schwartz and Hendry 2007; Weese et al. 2010) suggests rich opportunities for the further use of male guppy colour in understanding how natural and sexual selection can drive evolution in contemporary time.

#### Private signals – UV

If predators favour the evolution of private signals in their prey, we would predict that (1) male guppies experiencing predation from *Crenicichla*, a UV-insensitive predator, would have a greater UV reflectance, and (2) female guppies in these high-predation sites would increasingly base their mating

decisions on male UV reflectance. Our study provides some support for the first of these two predictions: high-predation males typically have more relative UV reflectance when all rivers and sites are considered in the same analysis (Table 3, Fig. 3c). Despite this generalization, the extent of divergence between high- and low-predation sites in UV reflectance varied among rivers. In two of the five rivers, we found strong support for the hypothesis of a private UV signal. In the other three rivers, however, we found no difference in UV reflectance. Moreover, we found very few differences between high- and low-predation males in the UV reflectance of particular colour spots (e.g., of orange spots). Overall then, our study supported the prediction of private signalling in UV in some, but not all populations, again suggesting the importance of modifying factors.

One modifying factor may be the nature of selection on UV reflectance in low-predation sites. Upstream sites (low predation) are probably colonized from downstream (high predation) sites (Carvalho et al. 1996; Alexander et al. 2006; Crispo et al. 2006). When guppies from downstream sites, where we hypothesize UV is favoured as a signal, colonize upstream sites, selection against ultraviolet reflectance may be weak or absent. Although *Rivulus* likely sees well in the ultraviolet (Endler 1991), it preys only weakly on guppies (Endler 1978). Prawns, also likely to see well in the UV (Endler 1991; Kemp et al. 2008), are now largely absent from low-predation sites on the south slope. The loss of UV colour in low-predation environments might therefore be the result of mutation and drift, energetic costs, or trade-offs with other signalling traits. The strength of these forces, and how they vary among low-predation populations, would be an interesting avenue for future work. It will also be important to determine what information about male fitness, if any, females can glean from variation in UV reflectance. Finally, the role of avian predators is poorly understood in this system, yet has been found to be a significant source of predation on other poeciliids (Riesch et al. 2010).

In conclusion, our study provides one piece of evidence that some guppy populations use UV as a private signal when living with *Crenichla alta*—a dangerous, but UV-insensitive, predator. The next step might be to demonstrate that high-predation females have an elevated preference for increased UV reflectance of males (e.g., Cummings et al.

2003 for *Xiphophorus spp.*). It will be particularly interesting to determine the relative importance of UV reflectance and non-UV colour to mate choice decisions. For example, the observation that high-predation females often have weaker preferences for colour (Endler and Houde 1995; Schwartz and Hendry 2007, 2009) could be explained if high-predation females discriminate among males using different traits than their low-predation counterparts—traits that are not being measured, such as UV. Further work in this area will bring fruitful insights to UV-based mate choice and to population differentiation in guppies.

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