OIKOS 113: 1–12, 2006

Disentangling the selective factors that act on male colour in wild guppies

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Millar, N. P., Reznick, D. N., Kinnison, M. T. and Hendry, A. P. 2006. Disentangling the selective factors that act on male colour in wild guppies. – Oikos 113: 1–12.

The colour pattern of male guppies (*Poecilia reticulata*) is thought to evolve as a compromise between sexual selection (favouring conspicuousness) and natural selection (favouring crypsis). Underpinning this classic explanation is the observation that guppies living with dangerous fish predators are less colourful than guppies living without these predators. However, high fish-predation sites are generally farther downstream than low fish-predation sites, and so may also differ in physical habitat features related to stream size, as well as in the abundance of predatory prawns (*Macrobrachium crenulatum*). The goal of our study was to disentangle the effects of fish predation on colour evolution from the potential effects of physical habitat features and predation by prawns. We collected 20 male guppies from each of 29 sites in two Trinidadian rivers. We then quantified the colour pattern of these fish; each spot was measured for size and assigned to a colour category. For each site, we determined the fish predation regime and quantified stream size, water colour, canopy openness, and prawn abundance. We then used regressions to assess the relative importance of these factors in explaining variation in guppy colour. Supporting previous work, the presence of predatory fishes was the most important explanatory variable for many components of colour pattern. Physical habitat features explained some of the remaining variation, but in inconsistent ways between the two rivers. The abundance of predatory prawns also explained variation in male colour. Our results suggest that predatory fishes impose the strongest selection on the colour pattern of male guppies but that other factors are also important.

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Secondary sexual traits are molded by the interaction between sexual and natural selection (Andersson 1994). Populations experiencing different strengths of either type of selection should therefore differ in these traits. With respect to natural selection, the role of predation has been considered in the most detail (McPhail 1969, Endler 1978): populations experiencing higher predation typically have reduced values of sexually selected traits. However, features of the physical habitat, such as transmission properties of the environment (Reimchen 1989, Boughman 2001), reflective properties of the substrate (Endler 1980, Cummings and Partridge 2001), and the availability of pigments (Hill 1993, Grether et al. 1999), may also be important aspects of natural selection. Just as these aspects of natural selection may differ among populations, so too may aspects of sexual selection. For example, geographic and male-male variation in mate choice have been invoked as explanations for population differences in sexually competition selected traits (Houde 1988, Houde and

Accepted 31 August 2005

ISSN 0030-1299

Endler 1990, Hamon and Foote 2005). In short, a host of interacting selective factors can influence the divergence of secondary sexual traits among populations. Here we use a correlative approach to quantify the contribution of different selective factors to the evolution of colour in male guppies, *Poecilia reticulata*.

Male colour pattern in guppies is a complex arrangement of spots that vary in colour, size, shape and position. The colours fall into three basic categories: carotenoid pigments (orange, red and yellow), melanic pigments (black) and structural colours (blue and iridescent). Although some spectral properties of some colour spots are phenotypically plastic, such as the saturation and brightness of carotenoid pigments (Grether et al. 2001a), the basic colour, size, and position of spots are thought to be genetically determined (Winge and Ditlevsen 1947, Endler 1983, Kodric-Brown 1989). The specific colour pattern of an individual is determined by many X- and Y-linked genes (Haskins et al. 1961) and is highly heritable (Winge and Ditlevsen 1947, Haskins et al. 1961, Houde 1992).

The colour of male guppies has become a model system for examining natural and sexual selection (Haskins et al. 1961, Endler 1978, Houde 1997, Brooks 2002). Male colour varies greatly within and among populations and appears to evolve as a compromise between natural and sexual selection. Sexual selection, acting largely through female choice, generally favours large and numerous colour spots, particularly those based on carotenoids (Endler 1983, Kodric-Brown 1985, Houde 1987, Brooks and Caithness 1995). Natural selection, owing to predation by fishes, strongly disfavours these same colour patterns (Endler 1978, Endler 1980). Thus, the classic interpretation of differences in male colour among guppy populations is that sexual selection increases colour in the absence of dangerous fish predators and natural selection reduces colour in the presence of these predators. Although this explanation is elegant and well supported, it has largely ignored the potential roles of physical habitat features and non-fish predators.

Physical habitat features vary dramatically among guppy populations and may correlate to varying degrees with fish predation. In general, fish predation varies along the upstream–downstream axis, with sharp changes occurring at waterfalls that prevent upstream colonization by predacious fishes (Haskins et al. 1961, Seghers 1973, Liley and Seghers 1975, Endler 1978). The headwaters and tributaries of rivers are therefore characterized by low fish-predation (only the weak predatory fish, *Rivulus hartii*, is present), whereas the downstream sections are generally characterized by high fish-predation (several dangerous fish predators are present). Many physical habitat features vary along this same upstream–downstream axis (Hynes 1971, Endler 1978, 1983, Grether et al. 2001b, Reznick et al. 2001, thereby potentially confounding interpretations based solely on fish predation. This co-variation between fish predation and physical habitat features is not perfect, however, bec ause (1) predation regime often shifts in a stepwise manner (across waterfalls) whereas habitat features may vary at smaller or larger scales, and (2) some rivers lack dangerous fish predators even in downstream sections. This partial decoupling of fish predation from physical habitat features provides an opportunity to disentangle the effects of multiple selective factors acting on guppy colour.

Three physical habitat features are of particular interest. First, the openness of the forest canopy influences primary productivity and therefore the availability of carotenoids and other resources (Grether et al. 1999, 2001b). Carotenoid availability then limits the brightness and saturation of red and orange spots (Kodric-Brown 1989). Because females prefer males with brighter and more saturated colours (Kodric-Brown 1989), canopy openness might influence the evolution of carotenoid-based colours (Hill 1993). Second, spectral transmission properties of the water determine the extent to which different colours are conspicuous (Reimchen 1989, Endler 1991, Boughman 2001, Scott 2001). Variation in transmission properties should thus cause evolutionary divergence in signals (i.e. male colour) and signal reception (i.e. female preference for male colour; Endler 1992, Scott 2001). Third, substrate characteristics, such as the size and colour of background particles, determine how closely a male colour pattern matches the background and thus its level of conspicuousness to both females and predators (Endler 1980). Based on these expectations, we concentrated on the potential role of these three habitat features, as well as overall stream size.

In addition to fishes, guppies are preyed upon by birds and invertebrates. Bird predators (e.g. kingfishers) are present in Trinidad, but we discount their influence on colour because (1) they are rarely seen in the streams we study (Haskins et al. 1961, Endler 1978, all authors pers. obs.) and (2) they view fish from above, a perspective from which most colour patterns are not visible. In contrast, two lines of evidence suggest that invertebrate predators, such as the freshwater prawn *Macrobrachium crenulatum*, may be very important in the evolution of male colour. First, guppies familiar with prawns exhibit greater caution when inspecting *Macrobrachium* than do guppies with no such experience (Magurran and Seghers 1990). Second, males at sites with both *Rivulus hartii* and *Macrobrachium* differ in colour from males at sites with *Rivulus* alone (Endler 1978, 1983). Of additional interest is the potential for prawns to play a different role than fish in the evolution of guppy colour. First, *Macrobrachium* are less abundant at sites with predatory fishes – because these fishes likely also eat prawns (Phillip 1993, Winemiller and Ponwith 1998). As a result, selection by prawns is expected to be stronger at sites where fish predation is weaker. Second, the visual system of *Macrobrachium* is thought to be sensitive to short wavelengths (i.e. blue light), but insensitive to the long wavelengths (i.e. orange and red light; Endler 1978, 1991), whereas fish predators are sensitive to both blue and orange/red light.

No studies of any fish species have examined how physical habitat features, fish predation, and invertebrate predation interact to influence the evolution of colour pattern. Our goal is to disentangle the relative roles of these different selective factors. To do so, we quantified these potential selective factors and sampled guppies from multiple sites in two Trinidadian rivers, one with and one without variation in the presence of predatory fishes.

Methods

We studied the Marianne and Paria drainages on the north slope of Trinidad's northern range mountains. Although not as intensively studied as drainages on the south slope of these mountains, these rivers contain an analogous high vs low fish-predation gradient (Endler 1983, Reznick et al. 1996). They also have the benefit of being less impacted by humans. The Marianne is characterized by spatial variation in predatory fishes owing to barrier waterfalls on its tributaries (Fig. 1). The Paria, in contrast, contains no strong predatory fishes because of a large barrier waterfall close to the ocean (Reznick et al. 1996; Fig. 1). Otherwise, the two rivers show similar gradients in size, slope, and other environmental factors. Our study design thus allowed analyses conducted both with (Marianne, 15 sites) and without (Paria, 14 sites) variation in the presence of predatory fishes. Sites were selected to maximize spatial variation within each watershed, and therefore the potential for variation in predation, habitat, and guppy colour. The distance of each site from the ocean, as the guppy swims, was measured on 1:25 000 scale maps (Edition 2, Government of Trinidad and Tobago 1978: Sheets 5, 14, 15).

Colour analysis

Near the end of the dry season in March 2002, we collected 20 mature male guppies from each of 29 sites (Fig. 1). The fish were killed with an overdose of tricaine methanesulfonate (MS-222) and immediately photographed with a digital camera set at a standard height above a grid-ruled background. MS-222 treatment increases the number and size of black spots but does not affect these properties for any other colour spots (N. Millar, unpubl.). Two photographs were taken of each fish in the shade, one with a flash and one without. Using Scion Image (version Beta 4.02, http://www.scioncorp.com/), 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), length, height and area of each colour spot on the left side of the body (excluding the fins and tail). The images were analyzed "blind" with respect to site and in random order by a single person (NPM).

Each colour spot was assigned to one of nine colour categories (after Endler 1978, 1991): orange (includes red), black, fuzzy black, yellow, blue (includes purple), green, violet-blue, bronze-green and silver. The last three of these colours are considered iridescent (Endler 1978,

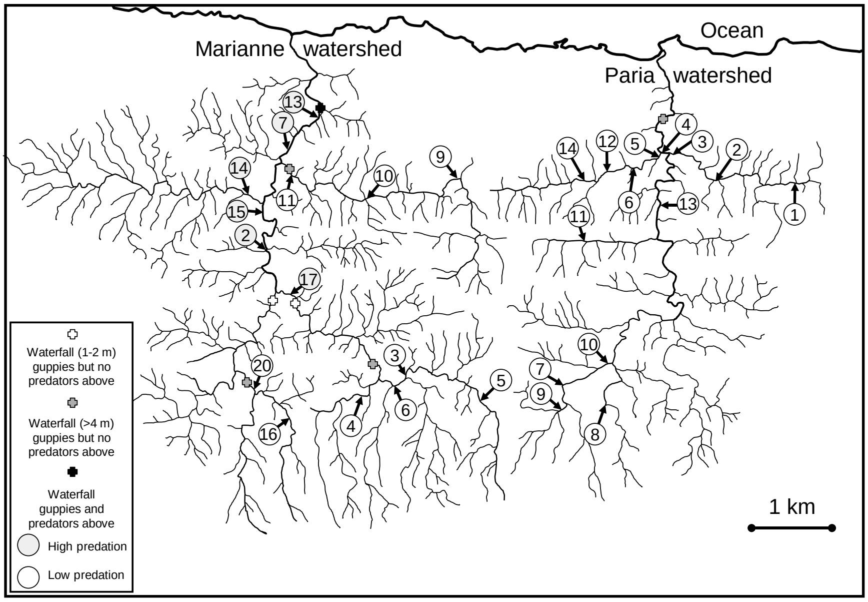

Fig. 1. Distribution of sampling sites on the Marianne and Paria rivers on the north slope of Trinidad's northern mountain range. Site numbers are not sequential in the Marianne because we collected guppies from additional locations but were unable to obtain habitat data.

1991). The flash and non-flash photographs were viewed simultaneously when the spots were measured and the colours assigned. This comparison 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.

We focused on several complementary measures of colour pattern: the total number of spots of a given colour ("number of spots"), the total area of the body covered by spots of a given colour ("total area"), the total area of a given colour divided by body area ("relative area"), and the average length of spots of a given colour divided by body length ("relative spot length"). Mean values were calculated for each colour measure at each site. Our analyses were thus based on some absolute measures of colour (i.e. not relative to body size) and some relative measures of colour (i.e. relative to body size). To achieve normality, relative sizes were arcsine square-root transformed.

Predators

We categorized each collection site as either "high" or "low" fish-predation. We based this assignment on our own (2002–2005) and previous (Reznick et al. 1996) observations of predatory fishes in the sites, as well as the size of downstream barrier waterfalls (Fig. 1). Low fish-predation sites contained the killifish *Rivulus hartii* (Endler 1983) and high fish-predation sites contained up to three species of gobies (*Eleotris pisonis*, *Gobiomorus dormitor* and *Dormitator maculatus*) and the mountain mullet (*Agonostomus monticola*) (Endler 1983, Reznick et al. 1996). Categorizing fish predation as a binary variable (high vs low) is a simplification. However, it is extremely difficult to quantify the intensity of fish predation, and so this dichotomy is the customary approach and makes our results comparable to previous studies (Endler 1978, Reznick et al. 2001).

We assayed the abundance of *Macrobrachium* in a subset ($n = 21$) of our sites from both drainages in March 2004 (13 sites in the Marianne, 5 sites in the Paria) and 2005 (12 sites in the Marianne, 7 sites in the Paria). Standard silver-coloured minnow traps were baited with six pellets of dry dog food and placed in slow to medium current where the water was at least 25 cm deep. Traps were separated by at least 4 m and were set for 40 min, after which the number of *Macrobrachium* was counted. For each site, we then calculated catch per unit effort (CPUE), whereby one trap-hour is one unit of effort. Sixteen sites were sampled at least twice (mean number of samples per site $= 2.95$) and repeat measurements for a given site were averaged. Passive trapping is a combined measure of abundance and activity (Collins et al. 1983, Dorn et al. 2005). As such, CPUE is a good indicator of predation pressure, but not necessarily of predator density.

Physical habitat features

In March 2003, we quantified physical habitat features. At each site, we first established 5–10 transects, evenly spaced every 5–20 m along the stream. The number of transects and their spacing varied among sites in order to match the area from which guppies were collected. For each transect, we measured the wetted width of the stream and established three equidistant points across the channel. At each of these points, we measured stream flow (Swoffer model 2100 flow meter with the impeller positioned 60% of the distance from the substrate to the surface), water depth and substrate type (rock, mud, roots, wood, sand, leaf or moss). When the substrate was a rock, we measured its median diameter. We excluded rocks > 200 mm because this improved normality and because rocks of this size would have little effect on the evolution of guppy spot size. At each site, canopy openness was quantified with a concave spherical densiometer (Lemmon 1957), which generates openness estimates comparable to those obtained by hemispherical photography (Englund et al. 2000). At each of five equidistant points between the most upstream and the most downstream canopy openness was measured facing each of the four cardinal directions while standing in the middle of the channel. Measurements for stream width, water depth, flow, substrate size and percent canopy openness were log 10 transformed and site means were calculated.

We measured spectral properties of the water at each site based on water samples collected from the field and stored in the dark until all could be processed on the same day. Light from an Ocean Optics PX-2 light source was directed through a collimating lens into a blackened PVC tube (path length: 48.6 cm) that contained the water sample. A bare fibre optic cable collected transmitted light at the far end of the tube and directed it to an Ocean Optics SD2000 spectrometer. Transmission spectra (300–700 nm) were recorded as percent transmission relative to a standard sample of filtered water. Transmission spectra were consistent across multiple runs from the same sample and across samples collected at a given site on different days.

To summarize the relevant information contained in each transmission spectra, we calculated a spectral attenuation index (SI). This index, calculated as [mean transmission 600 to 650 nm] – [mean transmission 400 to 450 nm], reflects the shape of the transmission spectrum regardless of its height. Increased relative attenuation of red wavelengths decreases SI whereas increased relative attenuation of blue wavelengths increases SI. In relation to our standard water sample SI was less than 25 for clear water and greater than 25 for tannic water, the latter having proportionally greater attenuation of short wavelengths and hence appearing red.

Statistics

We used SPSS (Version 11.0.1) for all statistical analyses, and all analyses were based on site means, except where noted. First, we used single-factor ANOVAs to compare physical habitat features and colour pattern elements among three river/predation categories: Marianne high fish-predation, Marianne low fish-predation, and Paria (all low fish-predation). Second, we used stepwise linear regressions to determine which candidate explanatory factors contributed significantly to explaining variation in each colour pattern element ($p = 0.050$ as the entrance criterion, $p = 0.100$ as the exit criterion). This was done within the Marianne alone and within the Paria alone because ANCOVAs revealed substantial drainage by habitat interactions for many colour pattern elements. ANCOVAs were used to test for interactions between drainage and physical habitat features for each physical habitat feature that significantly predicted variation (in either river) for the set of colour pattern elements in Table 3. Third, we used simple linear regressions across both drainages to ask how much of the variation in each colour pattern element was explained by fish predation alone. We then used partial regression coefficients from multiple regressions that included all factors (regardless of their significance) to examine the direct effects of predation. This last analysis controls for correlations between predation and other factors that affect colour. We then ran the multiple regressions again, this time using all individuals instead of site means. This analysis was not used to determine statistical significance but rather to partition the total variation among potential causal factors. Fourth, we used simple linear regressions to determine the relationship between each environmental factor and distance from the ocean. Three sites from the Marianne and three sites from the Paria were omitted from the canopy openness regression (and only this regression) because these sites were deforested and did not represent natural headwater sites.

The *Macrobrachium* data were based on a subset of the total sites, and the smaller samples sizes necessitated a less parameterized comparison of models. We therefore compared only four different regressions models: 1) log *Macrobrachium* CPUE and fish predation, 2) log *Macrobrachium* CPUE only, 3) log *Macrobrachium* CPUE and log depth, and 4) fish predation only. Depth was included in one model because *Macrobrachium* spend most of their time on the substrate, while guppies most often stay in the water column. *Macrobrachium* in deep water may therefore be less able to prey upon guppies than those in shallow water. We then used an information theoretic approach to model selection (Burnham and Anderson 1998) to determine whether models including *Macrobrachium* CPUE were as good as or better than models with fish predation alone. Specifically, AIC_c differences (Δ_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 Δ_i value of zero. Models with Δ_i values up to 2 have substantial empirical support, models with Δ_i values from 4–7 have considerably less empirical support, and models with Δ_i values above 10 have essentially no empirical support (Burnham and Anderson 1998). We also used simple linear regressions to examine relationships between *Macrobrachium* abundance and aspects of guppy colour pattern.

Results

Fish predation

Within the Marianne, guppies from high fish-predation sites had more and larger blue spots but fewer and smaller orange spots than did guppies from low fish-predation sites (Table 1, 2). Similarly, guppies from high fish-predation sites had a greater proportion of their body covered with blue and a smaller proportion of their body covered with orange (Table 1, 2). Guppies from high fish-predation sites had shorter spots (all spots combined) than low fish-predation guppies (Table 1, 2). No differences were evident for the other colour pattern elements. Paria guppies differed substantially in colour from Marianne guppies, even those at low fish-predation sites. In particular, guppies in the Marianne had more spots (all colours) that were shorter, whereas guppies in the Paria had a greater proportion of their body covered with orange (Table 1).

Fish predation alone explained large amounts of the variation in colour pattern elements when analyses were based on site means. For example, fish predation explained 49–64% of the variation in blue, 23–61% of the variation in orange, and 4–41% of the variation in total colour (Table 3). When analyses were based on all individual fish, however, the proportion of variation explained was much lower. For example, predation explained only 5–11% of the variation in blue, 3–24% of the variation in orange, and 1–11% of the variation in total colour (Table 3).

Physical habitat features and distance from the ocean

High fish-predation sites were wider and deeper than low fish-predation sites, but did not differ significantly from

Table 1. Summary of physical habitat features and selected colour pattern elements. Shown are the means ± 1 SD calculated across the range of site means. The P value is for an overall ANOVA comparing the three groups. Greek letter superscripts indicate homogeneous subsets of sites based on Tukey tests.

	Marianne (high)	Marianne (low)	Paria (low)	P
N (sites)	6	9	14	
Stream width^2 (cm)	$2.82 \pm 0.20^{\alpha}$	$2.32 \pm 0.28^{\beta}$	$2.50 \pm 0.22^{\beta}$	0.002
Water depth^3 (cm)	$1.51 \pm 0.17^{\alpha}$	$1.06 \pm 0.21^{\beta}$	$1.13 \pm 0.18^{\beta}$	<0.001
Water flow^3 (m s^{-1})	0.05 ± 0.02	0.05 ± 0.03	0.04 ± 0.03	0.506
Canopy openness^2 (%)	1.42 ± 0.09	1.33 ± 0.31	1.22 ± 0.23	0.224
Substrate diameter^2 (mm)	1.22 ± 0.10	1.18 ± 0.26	1.32 ± 0.13	0.185
Spectral index^1	20.78 ± 9.57	17.05 ± 5.10	22.05 ± 7.98	0.313
Distance (km)	$3.32 \pm 1.39^{\alpha}$	$6.63 \pm 2.18^{\beta}$	$4.18 \pm 2.46^{\alpha}$	0.015
Guppy length^2 (mm)	1.19 ± 0.03	1.22 ± 0.03	1.21 ± 0.03	0.222
Guppy body area^2 (mm^2)	1.68 ± 0.05	1.73 ± 0.08	1.70 ± 0.06	0.221
Number of spots				
Blue	$1.44 \pm 0.26^{\alpha}$	$0.84 \pm 0.27^{\beta}$	$0.67 \pm 0.19^{\beta}$	<0.001
Orange	$1.86 \pm 0.24^{\alpha}$	$2.44 \pm 0.42^{\beta}$	$2.17 \pm 0.21^{\alpha,\beta}$	0.003
Black	2.69 ± 0.43	2.84 ± 0.74	2.68 ± 0.31	0.743
Total (all colours)	$8.98 \pm 0.74^{\alpha,\beta}$	$9.18 \pm 1.00^{\alpha}$	$8.21 \pm 0.50^{\beta}$	0.011
Relative area (%)				
Blue	$11.79 \pm 2.12^{\alpha}$	$7.00 \pm 1.96^{\beta}$	$5.85 \pm 1.25^{\beta}$	<0.001
Orange	15.14 ± 1.68	20.08 ± 2.44	23.22 ± 1.40	<0.001
Black	18.64 ± 2.75	20.33 ± 3.99	21.13 ± 2.37	0.259
Total (all colours)	$37.72 \pm 1.50^{\alpha}$	$39.97 \pm 2.29^{\alpha,\beta}$	$41.28 \pm 1.51^{\beta}$	0.002
Relative spot length (%)				
Blue	$18.70 \pm 2.32^{\alpha}$	$12.86 \pm 3.50^{\beta}$	$11.98 \pm 2.36^{\beta}$	<0.001
Orange	21.06 ± 2.40	25.24 ± 2.14	29.43 ± 2.04	<0.001
Black	$23.34 \pm 2.25^{\alpha}$	$26.07 \pm 3.71^{\alpha,\beta}$	$27.98 \pm 2.87^{\beta}$	0.015
Total (all colours)	24.43 ± 0.62	26.11 ± 1.60	27.85 ± 1.01	<0.001

[1] [mean transmission 600 to 650 nm] – [mean transmission 400 to 450 nm].

[2] $\log_{10}$ (x) transformed values.

[3] $\log_{10}$ (x+1) transformed values.

[4] arcsine $\sqrt{(x)}$ transformed values.

Table 2. Factors explaining variation in male guppy colour across all sites in the Marianne (n = 15). Shown are the results of stepwise linear regressions testing for effects of predation regime (low: 0, or high: 1), stream width, water depth, flow, canopy openness, substrate size, spectral index and distance from the ocean. The r^2 value is for the overall model and the standardized regression coefficients (β) are for the significant predictor variables at the final step.

Dependent variable		Significant predictor variables			
	r^2		β		β
Blue colour					
Relative area	0.789^{d}	predation	$+1.166^{d}$	distance	$+0.574^{c}$
Total area	n.s.				
Relative spot length	0.495^{c}	predation	$+0.704^{c}$		
Number of spots	0.757^{d}	predation	$+1.141^{d}$	distance	$+0.557^{b}$
Orange colour					
Relative area	0.588^{d}	predation	-0.766^{d}		
Total area	0.610^{d}	predation	-0.781^{d}		
Relative spot length	0.490^{c}	predation	-0.700^{c}		
Number of spots	0.620^{c}	predation	-0.569^{c}	canopy	-0.453^{b}
Black colour					
Relative area	0.582^{d}	canopy	-0.763^{d}		
Total area	0.684^{c}	canopy	-0.654^{c}	depth	-1.111^{c}
		distance	-0.711^{b}		
Relative spot length	n.s.				
Number of spots	0.321^{b}	canopy	-0.567^{b}		
Total colour					
Relative area	0.393^{b}	canopy	-0.627^{b}		
Total area	0.472^{c}	depth	-0.687^{c}		
Relative spot length	0.322^{b}	predation	-0.568^{b}		
Number of spots	n.s.				
Bronze-green colour					
Relative area	0.588^{c}	width	-0.554^{b}	canopy	$+0.421^{b}$
Total area	0.791^{d}	substrate	-0.621^{d}	distance	$+0.481^{c}$
Relative spot length	0.408^{c}	width	-0.639^{c}		
Number of spots	0.562^{c}	width	-0.519^{b}	canopy	$+0.438^{b}$

[a] $P \leq 0.100$; [b] $P \leq 0.050$; [c] $P \leq 0.010$; [d] $P \leq 0.001$; n.s. – no predictor variables were significant.

Table 3. How much colour variation is explained by fish predation? Coefficients of determination (r^2) are for simple linear regressions (simple) based on site means (between sites: $n = 29$) and all individual fish (overall: $n = 575$). Corresponding letter superscripts indicate P values for the overall significance of the regression. Also shown are squared coefficients of partial correlation $(r')^2$ for fish predation in a multiple linear regression (multiple) that included all habitat features. Here, P values indicate the significance of the partial correlations.

	Between sites		Overall	
	Simple r^2	Multiple $(r')^2$	Simple r^2	Multiple $(r')^2$
Number of spots				
Black	(−) 0.002	(−) 0.001	(−) 0.000	(−) 0.003
Blue	(+) 0.606^{d}	(+) 0.497^{d}	(+) 0.101^{d}	(+) 0.038^{d}
Orange	(−) 0.232^{c}	(−) 0.412^{c}	(−) 0.033^{d}	(−) 0.032^{c}
Bronze green	(−) 0.079	(−) 0.048	(−) 0.018^{c}	(−) 0.006
Total (all colours)	(+) 0.036	(−) 0.001	(+) 0.005	(−) 0.017^{c}
Relative area				
Black	(−) 0.085	(−) 0.155	(−) 0.013^{c}	(−) 0.008^{b}
Blue	(+) 0.640^{d}	(+) 0.516^{d}	(+) 0.108^{d}	(+) 0.042^{d}
Orange	(−) 0.613^{d}	(−) 0.425^{c}	(−) 0.235^{d}	(−) 0.074^{d}
Bronze green	(−) 0.098	(−) 0.060	(−) 0.028^{d}	(−) 0.009^{b}
Total (all colours)	(−) 0.323^{c}	(−) 0.261^{b}	(−) 0.060^{d}	(−) 0.028^{d}
Relative spot length				
Black	(−) 0.218^{b}	(−) 0.266^{b}	(−) 0.024^{d}	(−) 0.018^{c}
Blue	(+) 0.488^{d}	(+) 0.355^{c}	(+) 0.052^{d}	(+) 0.021^{c}
Orange	(−) 0.501^{d}	(−) 0.263^{b}	(−) 0.156^{d}	(−) 0.034^{d}
Bronze green	(−) 0.113	(−) 0.066	(−) 0.031^{d}	(−) 0.009^{b}
Total (all colours)	(−) 0.412^{d}	(−) 0.195^{b}	(−) 0.112^{d}	(−) 0.026^{d}

[a] $P \leq 0.100$; [b] $P \leq 0.050$; [c] $P \leq 0.010$; [d] $P \leq 0.001$.

low fish-predation sites in flow, canopy openness, substrate diameter or spectral index (Table 1). Sites farther away from the ocean were narrower (significant for both drainages), shallower (significant for the Marianne, almost significant for the Paria), and contained less tannic water (significant in both drainages; Table 4). Canopy openness did not change with distance in the Paria but decreased with distance in the Marianne (marginally non-significant; Table 4).

In the Marianne, after accounting for the effects of fish predation, several habitat features were found to influence colour. First, guppies at sites farther from the ocean had more blue and bronze-green colour but less black colour (Table 2). Second, guppies at sites with more open canopies had less orange, black, and total colour but more bronze-green colour (Table 2). Third, guppies at sites with shallower water and smaller substrates had less black and total colour but more bronze-green colour (Table 2). The other colours did not correlate with any physical habitat features (Table 2). In contrast, the habitat effects in the Marianne were not evident in the Paria. For example, distance from the ocean did not explain variation in any colour and orange and total colour increased with increasing canopy openness. In fact, no physical habitat feature significantly predicted variation in the same direction for a colour pattern element in both the Marianne and the Paria (Fig. 2). This difference was reflected in significant interactions between habitat features and drainage in ANCOVAs; 11 of 28 interactions were significant at $P < 0.05$. These results point to considerable variation among streams in how environmental factors influence the evolution of colour.

Table 4. Coefficients of determination (r^2) and unstandardized regression coefficients (β) from simple linear regressions of physical habitat features (site means of transformed values) versus distance from the ocean (km) for each drainage.

	Marianne ($n = 15$)		Paria ($n = 14$)	
	r^2	β	r^2	β
Width (cm)	0.639^{d}	−0.112	0.362^{b}	−0.054
Depth (cm)	0.688^{d}	−0.099	0.266^{a}	−0.038
Flow (m s^{-1})	0.024	−0.002	0.158	+0.004
Canopy openness (%)[1]	0.311^{a}	−0.044	0.002	+0.005
Substrate diameter (mm)	0.086	−0.025	0.015	+0.006
Spectral index[2]	0.295^{b}	−1.557	0.452^{c}	−2.177

[1] Sites 4, 5 and 6 on the Marianne (i.e. $n = 12$) and sites 7, 8 and 9 on the Paria (i.e. $n = 11$) were excluded because of human impacts on forest canopies.
[2] [mean transmission 600 to 650 nm] − [mean transmission 400 to 450 nm].
[a] $P \leq 0.100$; [b] $P \leq 0.050$; [c] $P \leq 0.010$; [d] $P \leq 0.001$.

Incorporation of physical habitat features did not eliminate the apparent importance of fish predation in the evolution of male guppy colour. The effects of fish predation were seemingly modified by physical habitat features because r^2 values from simple linear regressions with fish predation alone differed from $(r')^2$ values for fish predation in multiple regression models by up to 0.238 (Table 3); most of these changes were decreases in the variation explained by predation when habitat factors were included. Despite this apparent influence, habitat factors generally did not interact significantly with predation as only 3 of 140 habitat-predation interactions were significant in ANCOVAs (data not shown).

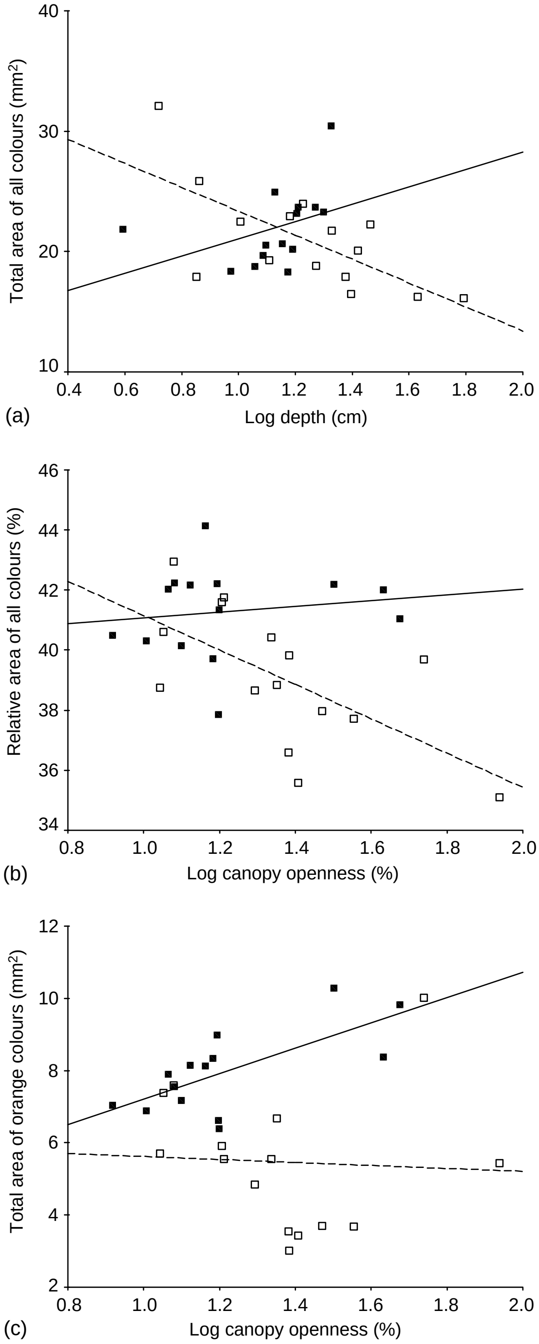

Fig. 2. Relationships between colour pattern elements and physical habitat features for the Marianne (open squares) and Paria (solid black squares). Within the Marianne, guppies in deeper sites have a smaller area of total colour (a), $r^2 = 0.472$, $P = 0.005$), but this relationship does not hold within the Paria ($P = 0.158$) indicating an interaction between drainage and the effect of depth (interaction term, $P = 0.006$). Within the Marianne, guppies in sites with a more open canopy have a smaller relative area of total colour (total area of colour divided by body size (b), $r^2 = 0.393$, $P = 0.012$), but this relationship does not hold within the Paria ($P = 0.624$), indicating an interaction between drainage and the effect of canopy (interaction term $P = 0.024$). (c) Within the Paria, guppies in sites with a more open canopy have a greater area of orange colour ($r^2 = 0.470$, $P = 0.007$), but this relationship does not hold within the Marianne ($P = 0.851$, interaction term $P = 0.135$).

Macrobrachium predation

We found a strong negative correlation between prawn catch per unit effort (CPUE) and the relative area of blue colour on male guppies (Fig. 3a, $n = 20$, $r^2 = 0.381$, $P = 0.004$). We also found a positive correlation between prawn CPUE and the mean relative area of orange colour (Fig. 3b, $n = 20$, $r^2 = 0.288$, $P = 0.015$). In the model selection exercise, models including *Macrobrachium* abundance could not be omitted from consideration. In many cases, the model with fish predation alone was best, but the model that included both fish predation and prawn abundance had considerable empirical support (AIC_c differences <3) and, in one case, was the best model (Table 5). These results suggest that *Macrobrachium* does substantially contribute to variation in some aspects of male guppy colour. One outlier (Marianne site 14) was removed from these analyses because it lies in a side channel to which predacious fishes have only occasional access. This was also the site with the most tannic water. Inclusion of this site in the regression analysis did not change the trends but did decrease statistical significance (e.g. arcsine relative area of blue, $n = 21$, $r^2 = 0.312$, $P = 0.009$; arcsine relative area of orange, $n = 21$, $r^2 = 0.167$, $P = 0.066$). In the model selection exercise, inclusion of this site did not change the results (Δ_i for the model of *Macrobrachium* abundance and fish predation were still all less than 3).

Discussion

Guppy colour patterns have long been thought to evolve as a compromise between sexual selection favouring conspicuousness and natural selection favouring crypsis (Haskins et al. 1961, Endler 1978). This basic premise remains unquestioned, but has been qualified by the results of recent studies. First, sexual selection is now known to vary geographically, with female guppies in different locations choosing mates based on different criteria (Endler and Houde 1995, Brooks and Endler 2001). Second, the action of natural selection on colour appears to be more complicated than a simple "high fish-predation" versus "low fish-predation" contrast. For example, the effect of predation will depend on the type of predator and its visual system, the background against which a guppy is viewed, encounter dynamics (distances and frequencies), the ambient light spectrum, and the transmission properties of the forest canopy and the water (Endler 1978). Environmental factors and the nature of the predator will therefore influence signal

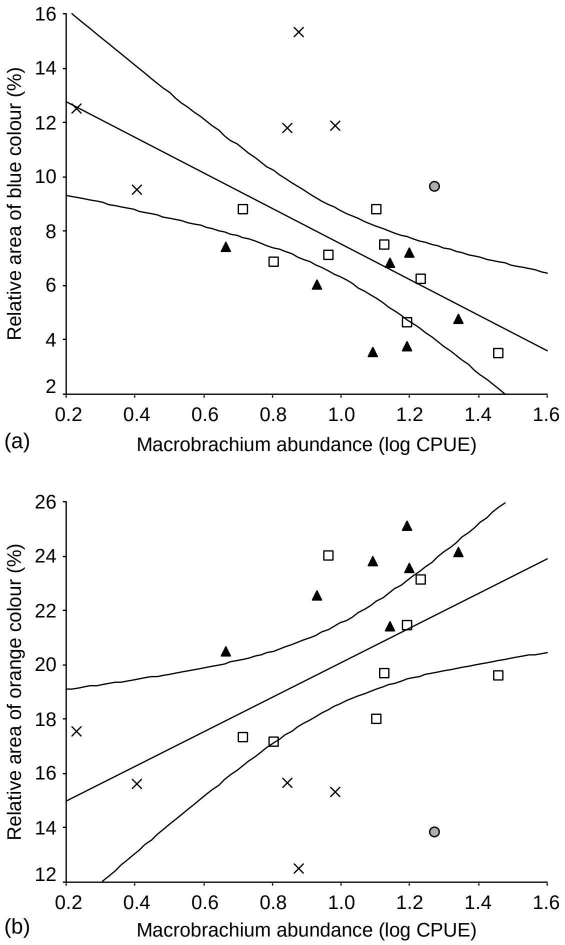

Fig. 3. Relationships between abundance (CPUE) of the prawn *Macrobrachium crenulatum* and male guppy colour: guppies from sites with high *Macrobrachium* abundance have (a) less blue colour, and (b) more orange colour. Sample sites on the Paria are marked with solid triangles, low predation sites on the Marianne with open squares, and high predation sites on the Marianne with crosses. 95% confidence intervals do not include an outlier (Marianne 14; grey circle) that was removed from these analyses.

transmission at both intra-specific (mates and competitors) and inter-specific (predators) levels, ultimately determining how conspicuous or cryptic a given colour pattern appears (Endler 1980, 1991, Reimchen 1989, Boughman 2001). This complexity makes the identification and quantification of individual selective factors difficult, particularly when physical habitat features co-vary with fish predation. The goal of our study was to begin disentangling the effects of these multiple factors for natural populations of guppies.

If we adopt the classic approach based on a high vs low fish-predation contrast, we corroborate the findings of previous investigators: i.e. fish predation has a strong effect on the evolution of guppy colour. The most striking pattern was that high fish-predation sites were characterized by less orange and less total colour, but by more blue colour than low fish-predation sites (Table 1, 3). We can next ask whether physical habitat features co-vary with fish predation and might therefore confound interpretations based on predation alone. Here we confirmed that habitat features potentially important to the evolution of guppy colour correlate with distance from ocean and with fish predation. In particular, sites farther from the ocean in the Marianne (typically low fish-predation) are narrower, shallower, and less tannic than sites closer to the ocean in the Marianne (typically high fish-predation). These results are consistent with previous work (Hynes 1971, Endler 1978, 1983, Grether et al. 2001b, Reznick et al. 2001), and confirm that multiple factors should be considered when interpreting the evolution of male guppy colour.

To begin disentangling the effects of these multiple factors, we fitted regression models that sought to explain variation in male guppy colour as a function of fish predation and physical habitat features. These models revealed that fish predation, distance from the ocean, canopy openness, stream depth and width, and substrate size all explained significant amounts of the variation in particular colour pattern elements (Table 2). However, the effects of physical habitat features often differed between drainages (Fig. 2), suggesting that they do not play a consistent role in the evolution of colour. Interestingly, the spectral properties of the water were not correlated with any aspect of colour pattern, perhaps because water colour varies little throughout the two drainages. Studies where colour pattern is correlated with water colour typically involve comparisons with greater variation in spectral properties (Reimchen 1989, Boughman 2001).

Table 5. AIC$_c$ differences (Δ_i) of four models explaining aspects of male colour from 17 sites on the Marianne and Paria (Marianne 14, an outlier, was removed from the analysis). Models with larger Δ_i values are less plausible (Methods).

	Model 1	Model 2	Model 3	Model 4
	Macrobrachium CPUE+fish predation	*Macrobrachium* CPUE	*Macrobrachium* CPUE+depth	Fish predation
Number of orange spots	0.00	2.50	4.61	0.01
Number of blue spots	2.31	4.52	6.47	0.00
Relative area of orange	2.92	4.25	6.76	0.00
Relative area of blue	2.49	5.17	7.95	0.00
Relative length of orange spots	2.22	1.91	3.80	0.00
Relative length of blue spots	2.72	3.38	6.00	0.00

What then is the relative importance of fish predation within the context of physical habitat features? Based on site means, fish predation was the most important explanatory factor (e.g. $r^2 = 0.23-0.64$ for orange and blue) and its effect was roughly similar regardless of whether or not other factors were considered (Table 4). The importance of fish predation in the evolution of male guppy colour is therefore a robust conclusion. At the same time, however, a substantial amount of the among-site variation could not be explained by fish predation, and some of this variation was associated with physical habitat features. Further consideration of multiple selective factors will undoubtedly improve our understanding of how different factors interact in the evolution of male guppy colour.

Predation by *Macrobrachium*

Freshwater prawns, *Macrobrachium crenulatum*, have been suggested as an important guppy predator (Endler 1978, 1983, 1991, but see Seghers 1990). If this is true, *Macrobrachium* may have interesting effects on colour pattern evolution because their abundance co-varies negatively with the presence of predatory fishes and because their visual system differs from that of most fishes. In particular, selection by prawns should act most strongly against blue and least strongly against orange because prawns are relatively insensitive to long wavelengths of light. Consistent with this expectation, guppies living in sites with high *Macrobrachium* abundance were less blue (Fig. 3a) but more orange (Fig. 3b). Similarly, models explaining variation in orange and blue colour that included *Macrobrachium* abundance had considerable support (Table 5). These apparent effects of *Macrobrachium* on guppy colour evolution can explain some additional patterns in our data.

First, we found a strong negative association between orange and blue colour across sites (arcsine transformed relative areas of colour, 29 sites, Pearson's $r = -0.801$; $p < 0.001$; Fig. 4). This correlation was driven in part by differences between high and low fish-predation sites within the Marianne alone (15 sites; $r = -0.726$; $p = 0.002$), but was also marginally present within the Paria (14 sites, Pearson's $r = -0.529$; $p = 0.052$). The negative correlations between blue and orange may be driven by *Macrobrachium* predation because in sites with high abundance of *Macrobrachium*, selection against blue is strong, but selection against orange is weak. In these sites, orange colour is a "private signal" and may increase if it is favoured for use in communication and mate choice (Cummings et al. 2003). The correlation within the Paria suggests that the negative relationship is caused by prawn predation and not fish predation.

Second, guppies from low fish-predation sites had less blue colour than those from high fish-predation sites (Table 1, 3). These findings initially seem surprising because most authors have found that guppies from low fish-predation sites have more of all colours (Endler 1978, 1983). We suggest the following interpretation based on our *Macrobrachium* results. At high fish-predation sites, predatory fishes select strongly against orange (Endler 1983). At the same time, these fishes may prey on *Macrobrachium* (Phillip 1993, Winemiller and Ponwith 1998), reducing prawn abundance and relaxing selection against blue. Consequently, guppies from high fish-predation sites should evolve more blue but less orange colour. At low fish-predation sites, selection by fishes against orange will be relaxed but *Macrobrachium* abundance will be high, thus increasing selection against blue. These effects are likely more evident at our sites than at those sites used in most previous studies because prawns are common on the north slope but very rare on the south slope (Endler 1983, N. Millar, unpubl.).

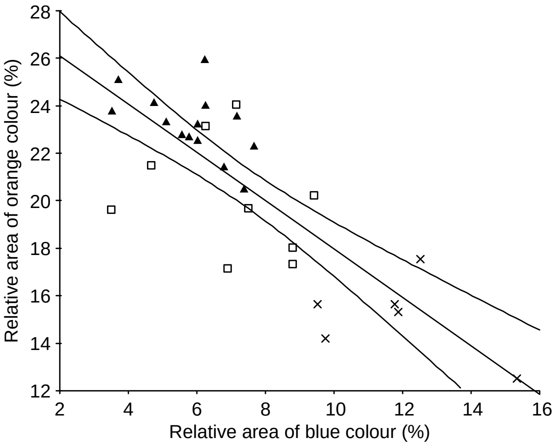

Fig. 4. Negative correlation between the relative area (total area of colour divided by the body area) of blue and orange colour across all sites (n = 29). Sample sites on the Paria are marked with solid triangles, low predation sites on the Marianne with open squares, and high predation sites on the Marianne with crosses. 95% confidence intervals are indicated.

Third, guppies from the Paria, which is entirely low fish-predation, had more orange than guppies from low fish-predation sites on the Marianne (Table 1), a result previously noted by others (Houde 1987, Houde and Endler 1990). Also noted by others (Magurran and Seghers 1990), Paria sites appear to have a very high abundance of prawns which should therefore select against blue and perhaps for orange (as explained above). Our sampling suggested no difference in *Macrobrachium* abundance between the Paria and low fish-predation Marianne sites, but our samples sizes were small (Marianne low fish-predation sites: n = 8, CPUE = 13.48; Paria: n = 7, CPUE = 13.25; P = 0.949). The jury is still out on whether the high amount of orange of Paria guppies is the result of prawn predation. An alternative explanation is that the high amount of orange in the Paria is due to greater female preference for orange in the Paria (Houde and Endler 1990), but this then begs the question of why an elevated preference evolved in the first place.

Summary

We confirmed that predatory fishes are a strong determinant of guppy colour patterns, with guppies in high fish-predation sites having less orange colour and shorter spots than guppies in low fish-predation sites. Physical habitat features were also important, but their specific effects differed between drainages. Predation by *Macrobrachium crenulatum* appeared to increase orange colouration and decrease blue colouration, presumably because these prawns can see blue but not orange. Although the widely-accepted role of predatory fishes is undoubtedly correct, our results show that other factors also contribute to the evolution of male guppy colouration. Specifically, we hypothesize an indirect effect of fish predators on colouration via *Macrobrachium* abundance.

Acknowledgements – Erika Crispo, Swanne Gordon, Meghan Kelly, Amy Schwartz and Dylan Weese provided assistance in the field. Greg Grether and John Endler provided helpful comments and advice. The manuscript was greatly improved by comments from Peter Abrams. This research was supported by the National Science Foundation (DEB 0235605: MTK, DNR, and APH), the Natural Sciences and Engineering Research Council of Canada (Discovery Grant to APH and Postgraduate Scholarship to NPM), the Maine Agricultural and Forest Experiment Station (MTK), the Levinson/LaBrosse Family (NPM), and McGill University (APH and NPM).

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Subject Editor: Lennart Persson