THE INFLUENCE OF HABITAT AND THE VISUAL SYSTEMS OF PREDATORS ON THE EVOLUTION OF MALE COLOUR IN GUPPIES, *POECILIA RETICULATA*

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ABSTRACT

The colour of guppies (*Poecilia reticulata*) evolves as a compromise between sexual selection (favouring conspicuousness) and natural selection (favouring crypsis). However, guppies live in a variety of habitats and with a variety of predators and consequently in a variety of selective environments. I investigated how habitat and predator's visual systems affect the evolution of colour. I used regressions to assess the importance of habitat features on the evolution of colour for 29 guppy populations. I then quantified the colour of guppies living in the presence and absence of two predators. The prawn predator is insensitive to orange light while the fish predator is insensitive to ultraviolet light. Habitat explained some variation in colour, but not in a consistent manner. Guppies living with the prawn were *more* orange and guppies living with the fish had *more* ultraviolet reflectance, providing evidence for the use of these aspects of colour as private signals.

RÉSUMÉ

La couleur des guppys (*Poecilia reticulata*) évolue en tant que compromis entre la sélection sexuelle (favorisant des couleurs voyantes) et la sélection naturelle (favorisant des couleurs cryptiques). Puisque les guppys vivent dans une variété d'habitats parmi une variété de prédateurs, ils sont sujets à une variété d'environnements sélectifs. J'ai étudié comment les systèmes visuels des prédateurs et l'habitat affectent l'évolution de leur couleur. J'ai utilisé des régressions pour évaluer l'importance des particularités de l'habitat sur l'évolution de la couleur de 29 populations de guppys. J'ai ensuite quantifié la couleur des guppys vivant en la présence et l'absence de deux prédateurs. Le premier, la crevette d'eau douce, est peu sensible à la lumière orange tandis que le deuxième, un poisson vorace, est peu sensible à la lumière ultraviolette. L'habitat a expliqué une certaine variation en couleurs, mais pas de façon consistante. Les guppys vivant avec la crevette étaient plus orange et les guppies vivant avec le poisson avaient un reflet plus ultraviolette, fournissant l'évidence concernant l'utilisation de ces colorations en tant que signaux privés.

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CONTRIBUTIONS OF AUTHORS

For the manuscript "*Disentangling the selective factors that act on male colour in wild guppies*" three individuals other than me contributed to the work: David N. Reznick, Michael T. Kinnison, and Andrew P. Hendry. All three were instrumental in establishing collection sites and obtaining samples that were used in the analysis. They also contributed to the design of the study and to revisions of draft manuscripts. For the manuscript "*Population divergence of private and non-private signals: an investigation in wild guppies*" Andrew P. Hendry was intimately involved. He helped with the design of the study and the analysis of data and made many revisions of draft manuscripts.

GENERAL INTRODUCTION

The predominant agent of evolution is selection (Endler 1986). For this reason, an understanding of selection is central to the understanding of evolution and the origin and maintenance of biological diversity. In studies of selection, secondary sexual traits of animals have received a great deal of attention. These traits are molded by both sexual and natural selection. Indeed, the classic explanation for the evolution of such traits is that sexual selection favours increased trait values (conspicuousness) whereas natural selection (e.g., predators) favours reduced trait values (crypsis) (Endler 1980).

This simple, yet powerful, understanding has been nuanced by studies examining the role of other factors. First, the physical environment in which animals live can be both a source and modifier of selection. Second, predation does not select against all secondary sexual traits equally. Predation is stronger on those individuals that are more detectable – that stand out from the background (Endler 1978). A further complication is that the sensory systems of predators are not all the same. Thus, the way in which predators perceive the world determines the nature, or quality, of selection that they impose on their prey. In this thesis, I use a correlational approach to examine how habitat and sensory systems of predators influence the evolution of a secondary sexual trait: colour pattern.

To examine these ideas, I worked with a species of freshwater fish, the guppy (*Poecilia reticulata*; Poeciliidae). Guppies are small fish native to north eastern South America and Trinidad. Males of the species are extremely colourful. Their colour patterns are composed of many spots of different sizes, shapes, and colours. Female guppies, void of any colour, base their choices of mates partially upon colour pattern. The guppy system has been well studied and much is known about their biology and that of their predators. I use information about the sensory systems of guppy predators to frame my hypotheses about colour evolution.

The first manuscript documents natural variation in guppy colour in two Trinidadian streams and investigates the factors responsible for this variation. I quantify the role of predation and the role of habitat (features of the physical environment). I also present evidence for the importance of predator's visual systems in shaping selection. The results from this manuscript prompted a more thorough investigation of predatory visual systems. In the second manuscript, I turn to guppies living with a different set of predators with different visual systems. I ask how the particulars of the visual system influence selection and the evolution of conspecific communication in these populations.

A secondary theme of this thesis is the evolution of animal communication signals. In particular, I examine the evidence for private communication - the use of signals among conspecifics (within a species) that are hard for predators to detect (Cummings et al. 2003). Such signals are not subject to constraining natural selection and can thus be elaborated with less cost to survival. Some theory suggests that these private signals should be favoured over non-private signals. In the first manuscript, I introduce the notion of private signalling in the guppy system, and provide evidence for a private visual signal in long wavelengths (orange and red). In the second manuscript, I provide evidence for a different private visual signal in the short wavelengths (UV).

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MANUSCRIPT 1:

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

Nathan P. Millar, David N. Reznick, Michael T. Kinnison, and Andrew P. Hendry

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Abstract

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.

Introduction

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 (e.g., 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 variation and male-male competition have been invoked as explanations for population differences in sexually selected traits (e.g., Houde 1988, Houde and 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, Endler 1983, Reznick et al. 2001, Grether et al. 2001b), thereby potentially confounding interpretations based solely on fish predation. This co-variation between fish predation and physical habitat features is not perfect, however, because (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 of light (i.e. blue), but insensitive to long wavelengths of light (i.e. orange and red; 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. data). 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), and the 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, 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 (e.g., 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 metres and were set for 40 minutes, 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 (Dorn et al 2005, Collins et al 1983). 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. In our analyses, we excluded rocks of diameter greater than 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 transect and the most downstream transect, 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 aspects of colour 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 spend most of their time 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 (Tables 1 and 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 (Tables 1 and 2). Guppies from high fish-predation sites had shorter spots (all spots combined) than low fish-predation guppies (Tables 1 and 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 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 (Figure 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.

Incorporation of physical habitat features did not eliminate the apparent importance of fish predation in the evolution of male guppy colour. And yet 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).

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 (Figure 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 (Figure 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 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, Reimchen 1989, Endler 1991, 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 (Tables 1 and 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, Endler 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 (Figure 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 (e.g., Reimchen 1989, Boughman 2001).

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, and 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 (see Introduction). Consistent with this expectation, guppies living in sites with high *Macrobrachium* abundance were less blue (Figure 3a) but more orange (Figure 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 $r = -0.801$; $p < 0.001$; Figure 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 a 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 (Tables 1 and 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), thereby 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. data).

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 (e.g., 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 (e.g., Houde and Endler 1990), but this then begs the question of why an elevated preference evolved in the first place (Rodd et al. 2002).

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.

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Tables

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 ± 0.20$^{\alpha}$	2.32 ± 0.28$^{\beta}$	2.50 ± 0.22$^{\beta}$	0.002
Water depth^3 (cm)	1.51 ± 0.17$^{\alpha}$	1.06 ± 0.21$^{\beta}$	1.13 ± 0.18$^{\beta}$	< 0.001
Water flow^3 (m/s)	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 ± 1.39$^{\alpha}$	6.63 ± 2.18$^{\beta}$	4.18 ± 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

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Table 1 continued.

Number of spots				
Blue	1.44 ± 0.26$^{\alpha}$	0.84 ± 0.27$^{\beta}$	0.67 ± 0.19$^{\beta}$	< 0.001
Orange	1.86 ± 0.24$^{\alpha}$	2.44 ± 0.42$^{\beta}$	2.17 ± 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 ± 0.74$^{\alpha,\beta}$	9.18 ± 1.00$^{\alpha}$	8.21 ± 0.50$^{\beta}$	0.011
Relative area (%)				
Blue	11.79 ± 2.12$^{\alpha}$	7.00 ± 1.96$^{\beta}$	5.85 ± 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 ± 1.50$^{\alpha}$	39.97 ± 2.29$^{\alpha,\beta}$	41.28 ± 1.51$^{\beta}$	0.002
Relative spot length (%)				
Blue	18.70 ± 2.32$^{\alpha}$	12.86 ± 3.50$^{\beta}$	11.98 ± 2.36$^{\beta}$	< 0.001
Orange	21.06 ± 2.40	25.24 ± 2.14	29.43 ± 2.04	< 0.001
Black	23.34 ± 2.25$^{\alpha}$	26.07 ± 3.71$^{\alpha,\beta}$	27.98 ± 2.87$^{\beta}$	0.015
Total (all colours)	24.43 ± 0.62	26.11 ± 1.60	27.85 ± 1.01	< 0.001

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[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		B
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	$- 0.453^{b}$

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Table 2 continued.

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	Multiple	Simple	Multiple
	r^2	$(r')^2$	r^2	$(r')^2$
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}

Continued on next page.

Table 3 continued.

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} $P \leq 0.001$

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)	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] $P \leq 0.001$

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 (see 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

Figures

Figure 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.

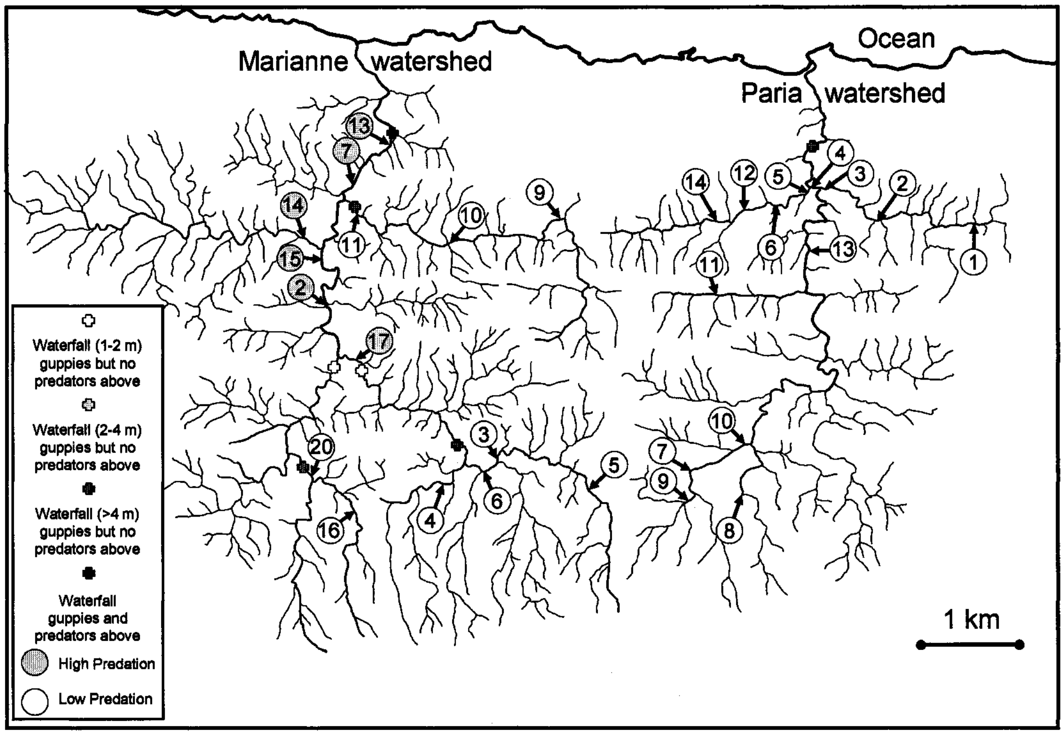

Figure 1

Figure 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 $P = 0.135$).

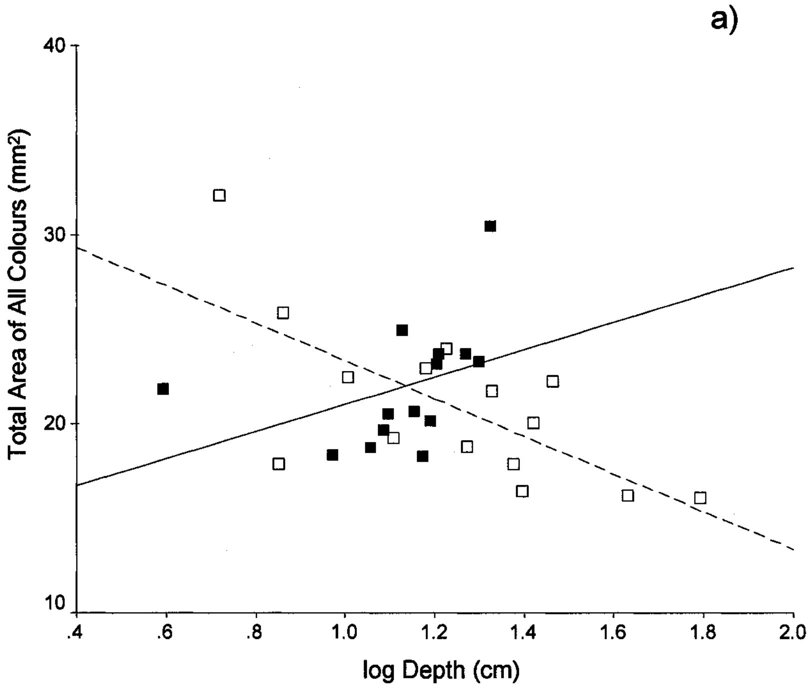

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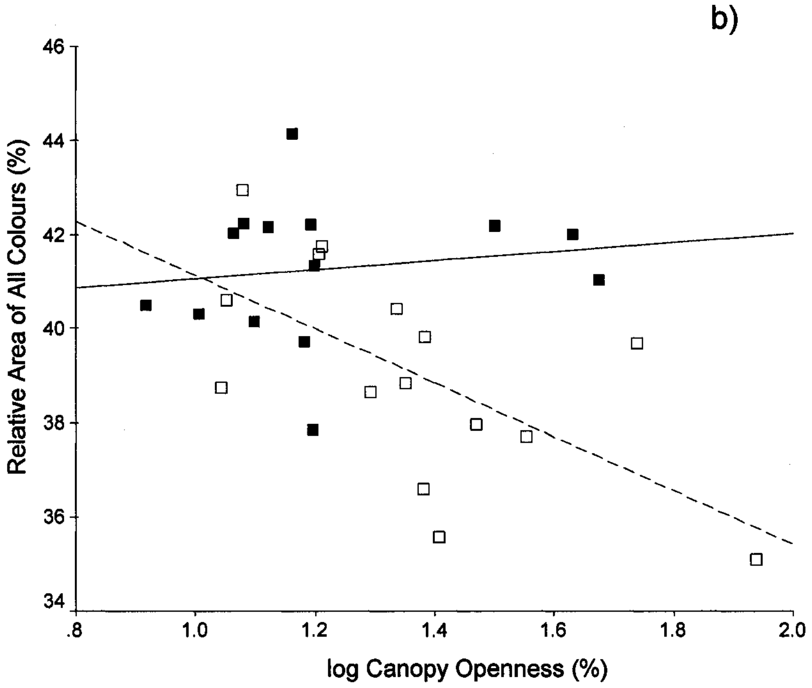
Figure 2 continued.



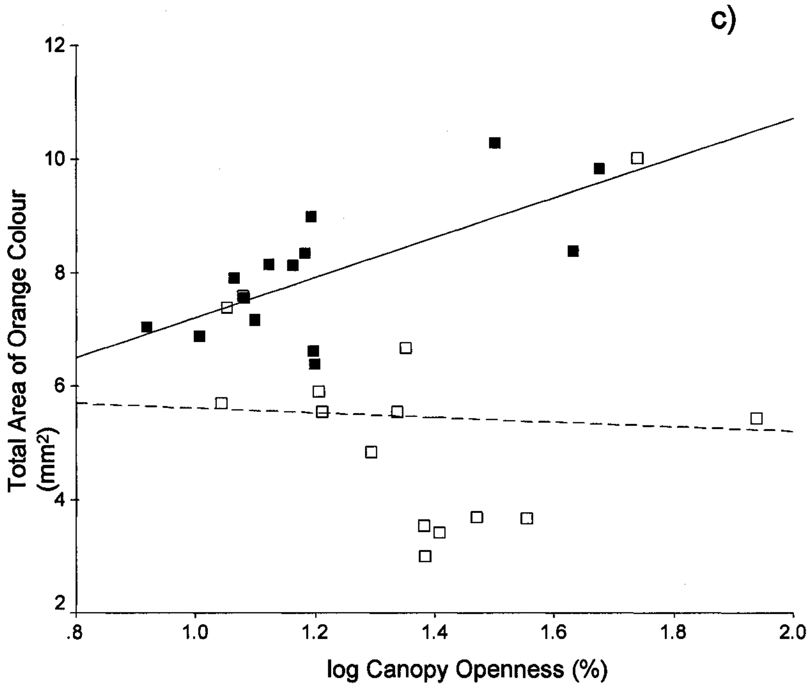
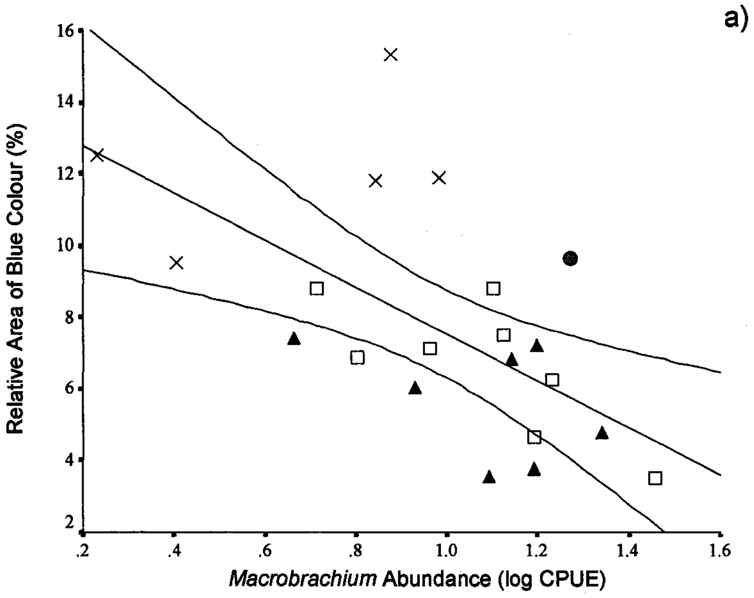


Figure 3. Relationships between abundance (CPUE) of the prawn *Macrobrachium crenulatum* and male guppy colour: guppies from sites with high *Macrobrachium* abundance have **a**) less blue colour, and **b**) more orange colour. Sample sites on the Paria are marked with solid triangles, low predation 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.



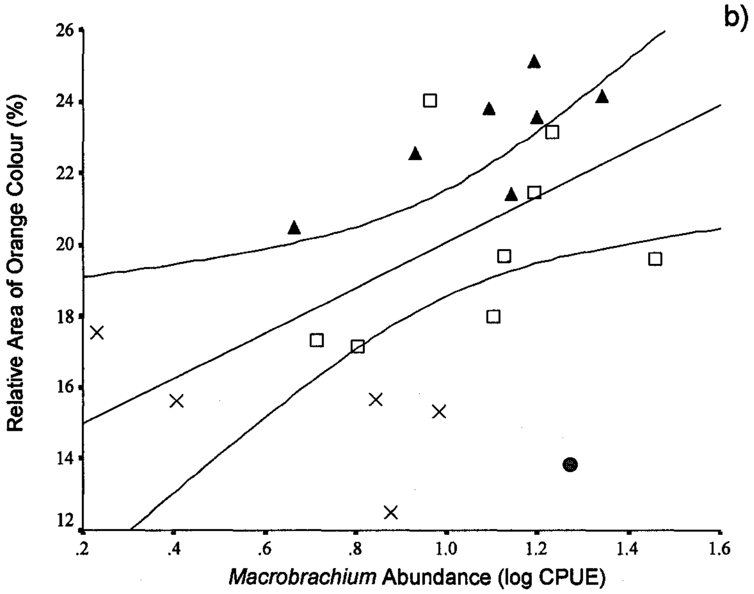
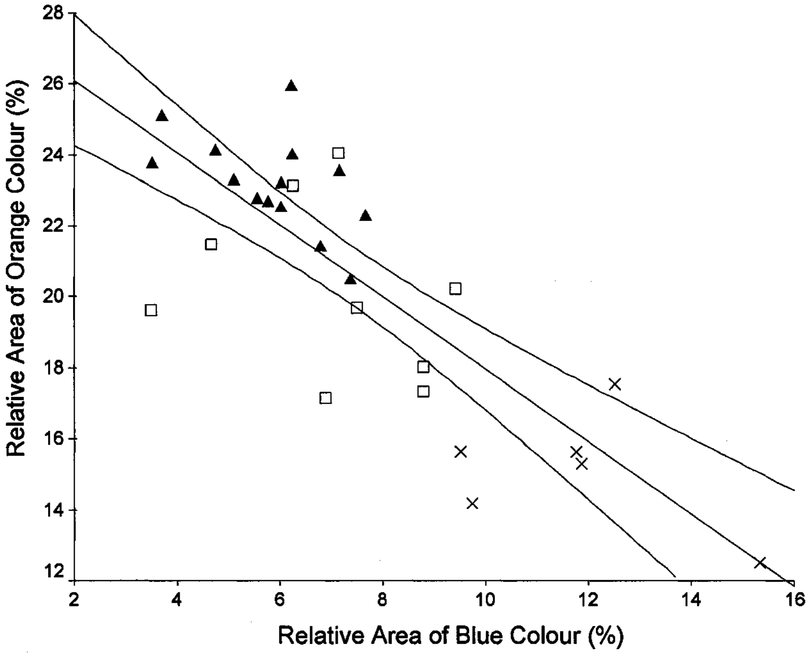


Figure 4. Negative correlation between the relative area (total area of colour divided by the body area) of blue and orange colour across all sites (n = 29). Sample sites on the Paria are marked with solid triangles, low predation sites on the Marianne with open squares, and high predation sites on the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



CONNECTING TEXT

The first manuscript examined the role of predation and physical habitat features in the evolution of guppy colour. I demonstrated that predation is the most important factor but that not all predation is equal: natural selection imposed on guppy colour by one predator can be very different from that imposed by another predator. I also provided evidence for the use of orange colour as a private signal. I found that guppies living with many predatory prawns had *more* orange than those living with few prawns. The second manuscript also investigates private signalling, though with a different predator and a different private signal.

In the first manuscript I examined guppies in rivers on the north slope of Trinidad's Northern Mountain Range. Predators in these rivers belong mostly to marine families. On the south slope of the mountains, guppies live with a different suite of predators, of which many are from families of fish originating from mainland South America. In high-predation sites on the south slope, the major fish predator is the pike cichlid, *Crenichla alta*. Studies have shown that this fish is insensitive to short wavelengths of light (ultraviolet) just as prawns are insensitive to long wavelengths of light (orange and red).

This manuscript investigates whether, in the presence of *C. alta*, the ultraviolet wavelengths of male colour pattern are used as a private signal. To do this, I quantified the reflectance of ultraviolet light on guppies from populations that have evolved in the presence or absence of this predator.

MANUSCRIPT 2:

Population divergence of private and non-private signals: an investigation in wild guppies.

Nathan P. Millar and Andrew P. Hendry

Abstract

Private signalling, where conspecifics use signals that are difficult to detect by predators, avoids the compromise imposed by opposing natural and sexual selection. We investigated the possibility of a private visual signal in the guppy, *Poecilia reticulata*. In some areas, guppies, who can detect ultraviolet (UV) light, coexist with dangerous predators who cannot detect UV. In these populations of guppies, UV could be used as a private signal. We tested this hypothesis by quantifying the UV and non-UV colour of guppies from paired high- and low-predation sites in five Trinidadian rivers. We find evidence in support of the use of UV as a private signal: in some populations male guppies living with the predator had *greater* UV reflectance than male guppies living without the predator. Curiously, we found that the non-UV colour of guppies does not differ as consistently between high- and low-predation environments as previous work had led us to expect.

Introduction

Secondary sexual traits often evolve in response to both natural and sexual selection. These conspicuous traits are used to attract members of the opposite sex or to dominate members of the same sex (Andersson 1994, Maynard-Smith and Harper 2003). And yet these same traits, by virtue of their conspicuousness, may also increase the susceptibility of the bearer to predation (Endler 1980). In general, then, many secondary sexual traits should evolve as a compromise between *opposing* natural and sexual selection (Endler 1980). Differences among populations in either type of selection can thus lead to divergence in secondary sexual traits and mating preferences for them (Schluter 2000). The general prediction for secondary sexual traits perceived by predators is that populations experiencing higher predation should evolve reduced expression of those traits. The first goal of our study is to provide a new test of this prediction in a system where it has ostensibly been well established, Trinidadian guppies (*Poecilia reticulata*).

Given the apparent compromise between obtaining mates and avoiding predators, selection might favour signals that break from this constraint by increasing attractiveness without increasing predation risk. Indeed, this may explain why larger secondary sexual traits are not always associated with decreased adult survival (Jennions et al. 2001). One way to escape the constraint of opposing selection is through the evolution of "private signals" that can be perceived by conspecifics but not (or less so) by predators (Endler 1978 and 1983, Cummings et al. 2003). Private signals are therefore less subject to the conservative action of natural selection imposed by predators.

One clear example of private signalling can be found in northern swordtails (*Xiphophorus spp.*). A major predator of *Xiphophorus* is the Mexican tetra, a fish with little sensitivity to ultraviolet (UV) light. *Xiphophorus spp.* males living with a high density of the tetra have *more* UV reflectance than males living with a low density of the predator. Furthermore, *Xiphophorus spp.* females living with a high density of the tetra prefer males with UV reflectance (Cummings et al. 2003). Another example of private signalling has been advanced in the guppy system: orange colour in the presence of predatory prawns (Millar et al. 2006).

Guppies are a small, neo-tropical freshwater fish native to Trinidad and north eastern South America. Natural guppy populations can be classified into two general types, depending on whether dangerous fish predators are present (high predation) or absent (low predation). High- and low-predation guppies differ in an array of life history, morphological, and behavioural traits (Endler 1995, Houde 1997, Magurran 2005). We are here interested in male colour, which is a complex and highly variable arrangement of spots of varying sizes, shapes, and colours (Endler 1978). Despite high variability among individuals at a given site, guppies in low-predation environments are usually colourful, whereas those in high-predation environments are usually drab (Haskins et al. 1961, Endler 1978, Houde 1997, Millar et al. 2006).

Variation in male guppy colour among populations is thought to reflect a balance between natural and sexual selection. Female guppies, themselves lacking colour spots, generally prefer to mate with more colourful males (Endler 1983, Kodric-Brown 1985, Houde 1987, Brooks and Caithness 1995). Colourful males, however, are also more likely to be seen by predators (Endler 1978 and 1980). Indeed, Endler (1980) has shown that guppies rapidly evolve greater colour in the absence of dangerous fish predators and reduced colour in their presence. But other types of predators are also important.

In a portion of their range, guppies live with the predator *Macrobrachium crenulatum*, a freshwater prawn. *Macrobrachium*, like most decapod crustaceans, are insensitive to long wavelengths of light (orange and red, Endler 1991). Male guppies living with a high abundance of these prawns have *more* orange colour (Endler 1978, 1983, and 1991, Millar et al. 2006). Furthermore, 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). We hypothesized that, under high prawn predation, orange is favoured as a private signal (Millar et al. 2006) because it may be used at a low predation cost to males and to courting females. In the present study, we look for evidence of a second private signal in guppies: an ultraviolet channel.

All 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. Guppies, however, are also sensitive to UV light (Archer et al. 1987, Douglas and McGuigan 1989, Archer and Lythgoe 1990) and the ultraviolet 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, the predatory fishes that would feed on guppies vary in their sensitivity to UV light. For example, the classic weak predator in low-predation sites, *Rivulus hartii*, is sensitive to UV light, whereas the classic strong predator in high-predation sites, *Crenicichla alta*, is not

(Endler 1991). Here then is an opportunity for a private signal (UV) in an environment where other signals are demonstrably costly to survival.

Specific predictions

In the present study, we quantify the non-UV and UV colour of wild male guppies from paired sites with and without *Crenicichla* in five watersheds. If signals visible to predators evolve as a compromise between sexual and natural selection, males from low-predation populations should show a greater expression of non-UV colour than males from high-predation populations, as has been previously shown (Endler 1978). If signals invisible to predators (UV) have escaped the constraint imposed by natural selection, males from high-predation populations should show a greater expression of UV than males from low-predation populations.

Methods

Sampling sites

In March and April of 2004, we visited 10 sites on the south slope of Trinidad's Northern Range Mountains. These sites were paired high- and low-predation locations in five rivers: Guanapo, El Cedro, Turure, Aripo, and Quare (Figure 1, Table 1). Within each of these watersheds, the upstream low-predation populations were most likely colonized independently from the downstream high-predation population of the same river. Three rivers, the Guanapo, El Cedro, and Aripo, are in the westward flowing Caroni drainage, whereas the Quare and Turure are in the eastward flowing Oropuche drainage. These major drainages contain different ancestral lineages of guppies that have been separated for 500,000 to 600,000 years (Fajen and Breden 1992). Specific collection sites were chosen based on accessibility and background knowledge about predation regimes (Endler 1978, Reznick et al. 1996).

Potential predators at each site were recorded in qualitative visual surveys. Minnow traps were then used to assay the abundance of *Rivulus* at each site. The traps were baited with dried dog food and left in the stream for about 45 minutes.

We then calculated catch per unit effort (CPUE) as the estimated number of *Rivulus* caught in an average trap over a one hour period. We also recorded any other organisms captured in the traps.

Physical habitat features can influence the evolution of colour (Endler 1978 and 1983, Grether et al. 2001, Reznick et al. 2001). We therefore quantified potentially relevant habitat features at each site. First, at ten locations per site, we measured the wetted width of the stream. Second, at each of these locations we measured water depth at three equidistant points across the stream. Third, we quantified canopy openness with a concave spherical densiometer (Lemmon 1957). This was done at five locations for each site. We took measurements facing each cardinal direction while standing in the middle of the channel. Fourth, we measured the spectral properties of the water. We collected water samples and held them in the dark until all could be processed on the same day. 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 this 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 contained in each transmission spectra, 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. Relative to the standard, increased attenuation of UV (short wavelengths) decreases I_{UV}. Measurements for stream width, water depth, and percent canopy openness were log 10 transformed.

Fish collection and photography

Twenty-five male guppies were collected from each of the 10 sites and anaesthetized with tricaine methanesulfonate (MS-222). All photographs were then taken at a standard height above a grid-ruled background illuminated with two full spectrum fluorescent lights (Vitalite, Durotest Canada). Colour photographs were taken with a Nikon CoolPix 995 or a Nikon D100 camera, the latter equipped with a Sigma 105 mm macro lens. Two photographs of each fish were taken in the shade, one with and one without a flash. Immediately after taking the colour photographs, we took a UV photograph (Figure 2). Here we used Kodak T-MAX 400 ISO black and white film (sensitivity: 200 – 700 nm) in a Nikon F65 camera equipped with the above Sigma macro lens. To exclude non-UV light from the image, we used a filter (Kodak Wratten 18A) that allowed light transmission only from 300 – 400 nm. In combination, the lens and filter had a transmission from 360 to 400 nm (Figure 3). Because of the great attenuation of light to which the film was sensitive (minimum 80% attenuation), exposure times for the UV photographs were long (~ 30s). All rolls of film were processed in a single batch.

Photo analysis – non-UV

All images were analyzed "blind" with respect to population 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). 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 and 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 as 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.

Colours that individually composed less than 10% of the total colour (yellow, bronze-green, blue, and silver) were not analyzed individually. They were, however, included in composite measures such as structural colour and total colour. Our analyses 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 achieve normality, relative areas were arcsine square-root transformed. Mean values were calculated for each colour measure at each site.

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). We outlined each colour spot and the entire fish with the lasso tool. This was done while simultaneously viewing the colour image, which avoided a potential bias in defining the area of colour spots based solely on UV reflectance. When glare covered part of a spot, UV reflectance was recorded only from the part of the spot that had no glare. For each lassoed area, we examined the luminosity channel of the Histogram and recorded the mean value which ranged from 0 (black) to 255 (white). This is a measure of the average amount of UV reflectance from the selected area.

Luminosity might vary owing to nuances of lighting conditions for a given image, and so we also measured luminosity of the background in each UV photograph (Villafuerte and Negro 1998). The location at which these measurements were taken was representative of the variation in the background, but was never so close to the fish that they fell under the fish's shadow.

From these measurements, we calculated a metric of UV reflectance. This metric was the sum, over every colour spot of a fish's colour pattern, of the product of a spot's mean UV reflectance and its area. This metric depends on the size of the fish, but more specifically on the size (area) of the colour pattern so it was divided by the total area of that fish's colour pattern to give a 'relative UV reflectance of colour pattern'. This metric is used to infer private signalling as it represents the amount of UV reflectance for a given area of colour pattern.

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), predation (fixed), and the river-by-predation interaction as predictor variables, and 10 elements of colour pattern (relative area and number of black, orange, violet-blue, green, structural, and total spots) as response variables. We found a strong interaction between the effects of predation and river (see *Results*), which precluded a straightforward interpretation of differences among rivers and between predation regimes. Our main question was whether high-predation sites have different colour from low-predation sites within each river, and whether this difference varied among rivers. We therefore next used MANOVA to test for the effects of predation independently within each river. These MANOVAs were followed by colour-specific ANOVAs to determine which particular colours drove the observed patterns.

Our analysis of UV first considered correlations between UV reflectance of the colour pattern and the UV reflectance of the fish. This was significant (see *Results*) and so we included UV reflectance of the background as a covariate in subsequent analyses. Therefore, our analysis of UV reflectance was based on ANCOVAs with river and predation as fixed effects, UV reflectance of the background as a covariate, the relative UV reflectance of colour pattern as a dependent variable. We then examined the effect of predation using ANCOVAs within each river separately, again including background UV as a covariate. We also examined the effect of predation on the mean UV reflectance of spots of particular colour. Examining each river separately, we used an ANCOVA for each colour and background UV as a covariate. Finally, to investigate the potential effect of habitat on guppy colour, we examined Pearson's correlations across all 10 sites between non-UV colour and UV reflectance and the following habitat parameters: stream width, depth, canopy openness, and I_{UV}.

Results

Predators

At high-predation sites, we noted the presence of *Crenicichla alta*, another cichlid (*Aequidens pulchur*), and several predatory characins (*Astyanax* bimaculatus and *Hemibrycon dentatum* (see also Haskins et al. 1961, Liley and Seghers 1975, Endler 1978, Reznick et al. 1996). *Crenicichla* is by far the most dangerous predator (Endler 1978) and was observed at all of the high-predation sites. Rivulus was not caught at high-predation sites but had an abundance at low-predation sites that ranged from low (Guanapo, CPUE = 3.94) 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. Only four *Macrobrachium* were captured during our entire sampling period: one in the Aripo low predation (CPUE - number of *Macrobrachium* caught per trap in one hour - 0.08) and three in the Turure low predation (CPUE = 0.39). This is in sharp contrast to the very high CPUEs of *Macrobrachium* on the north slope (Range: 1.69 – 28.61; Mean = 11.89; Millar et al. 2006).

Non-UV colour

Male guppy colour differed significantly among rivers (MANOVA; Wilks' $\lambda = 0.371$, $P < 0.001$) and between predation regimes (Wilks' $\lambda = 0.694$, $P < 0.001$), with a significant interaction between these factors (Wilks' $\lambda = 0.578$, $P < 0.001$). (M)ANOVAs for individual rivers (Table 2) revealed that predation did not have the same effect on colour divergence in the five rivers. In the El Cedro, no differences were evident between high- and low-predation males (Wilks' $\lambda = 0.647$, $P = 0.110$). In the Aripo, low-predation males were more colourful than high predation males (Wilks' $\lambda = 0.460$, $P = 0.001$). In the Guanapo, low-predation males were *less* colourful than high-predation males (Wilks' $\lambda = 0.288$, $P < 0.001$). In the Turure and Quare, high- and low-predation environments differed in colour but the specific colours differed in different ways (Wilks' $\lambda = 0.454$ and 0.342, $P = 0.001$, < 0.001 respectively, Table 2, Figure 4-A, B). These results show that colour differences between paired high- and low-predation environments are not as consistent as previous work has led us to expect.

Non-UV colour was not influenced by habitat or the variation in the intensity of *Rivulus* predation. Though we found significant correlations between some aspects of male guppy colour and habitat (I_{UV} index, canopy openness, and water depth, Table 3), these correlations were driven by the very shallow (Figure 5C) and high I_{UV} (Figure 5A) Aripo low-predation site, which also had very colourful males. When this site is removed from the analyses, no correlations are significant. The correlation between canopy openness and male colour must be interpreted with caution because the canopies of low-predation sites were consistently more closed than those of high-predation sites (Figure 5). We did not find any significant correlations among the abundance of *Rivulus* and aspects of male colour (all $P > 0.05$).

UV reflectance

UV reflectance of the background was correlated with the relative UV reflectance of the fish's colour pattern ($r = 0.265$, $P < 0.001$). We therefore included background UV as a covariate in all further analyses. When all rivers were analyzed together, the relative UV reflectance of colour pattern was influenced by predation (high predation was higher, $F = 11.600$, $P = 0.001$), river ($F = 36.237$, $P < 0.001$), and background UV ($F = 14.321$, $P < 0.001$), and the interaction between river and predation was not significant ($F = 1.941$, $P = 0.104$). We then examined the influence of predation within each river. In four of five rivers, high-predation males had greater UV reflectance than low-predation males and this difference was significant in the Guanapo and Quare (Table 4, Figure 4C).

Despite these differences based on overall UV reflectance we found very few differences between high and low predation in the average UV reflectance of spots of particular colours. We found no differences in the Guanapo, Quare, and El Cedro (all $P > 0.05$). In the Turure, low-predation fish had more UV reflectance from orange spots ($P = 0.001$) and from all spots overall ($P = 0.042$), and in the Aripo, high-predation fish showed more UV reflectance of black ($P <$

0.001) and less UV reflectance of structural spots (P = 0.048). Finally, we did no find any significant correlations between UV reflectance and habitat features (all P > 0.05).

Discussion

Non-private signals

It is repeatedly observed that male guppies in low-predation environments are more colourful than male guppies in high-predation environments (Haskins et al. 1961, Endler 1978, 1980, and 1983, Winemiller et al. 1990, Millar et al. 2006). Here we performed a new test of this hypothesis by pairing high- and low-predation sites within five Trinidadian rivers. This design mimics that usually used to test the hypothesis of parallel evolution (Langerhans and DeWitt 2004). Apart from this study design element, our methods were the same as those in previous work: measuring the number and size of colour spots from photographs of wild-caught males. We were therefore surprised to find that the effect of predation on several aspects of colour differed dramatically among rivers. In relation to high-predation males in the same river, low-predation males were more colourful in the Aripo, similar in the El Cedro, and less colourful in the Guanapo. Differences in male colour within the Turure and Quare depended on the specific colours being considered (Table 2). Several possibilities may explain the discrepancy between the present study and those conducted earlier.

A first possibility is that female preferences vary appreciably among populations (Houde 1988, Endler and Houde 1995, Brooks and Endler 2001) and so sexual selection differ among populations, potentially independently of natural selection. Several studies have found correlations between male traits and female preference for these traits (Houde and Endler 1990, Schwartz and Hendry *in preparation*) indicating a role for sexual selection in shaping male colour. Thus differences among our sites in female preference for particular aspects of male colour may explain our results. Do our results match with what is known about female preference in our study sites? Schwartz and Hendry (*in preparation*) found that low-predation Quare females preferred males with more black and less green while high-predation females preferred less black and had a flat preference function for green. In the Aripo, high-predation females had a preference for less orange and black, while low-predation females had a flat preference function. Also in the Aripo, Stoner and Breden (1988) found that high-predation females preferred drab males to bright males. In the Guanapo, high-predation females preferred orange and discriminated against black (Endler and Houde, 1995). In the rivers for which female preferences are known, there is a general agreement between these preferences and the mean male traits in this study (Table 2).

A second possibility is related to the fact that several of our low-predation populations were recently derived from introduced high-predation guppies. The low-predation site on the El Cedro was established on March 16, 1981, when 100 guppies were introduced from the El Cedro high-predation site (Reznick and Bryga 1987). The low-predation site on the Turure was established in 1967, when C.P. Haskins introduced 200 high-predation guppies from the lower Arima River (Carvalho et al. 1996). Perhaps these populations have not had enough time to evolve the phenotypes characteristic of an equilibrium between natural and sexual selection in low-predation environments. This seems unlikely, however, because work on previous introductions of guppies has shown rapid adaptation of both life history (Reznick et al. 1997) and colour (Endler 1980). On the other hand, increased colour in low-predation populations will depend on sexual selection, which we have already noted does not always favour greater colour, particularly in high-predation populations. It is possible that the introduced females did not have strong preferences for more colour and have not yet evolved this preference in the new low-predation environment.

A third possibility is that colour evolution is influenced by habitat (Endler 1980 and 1991, Grether et al. 1999, Millar et al. 2006) and that the difference in habitat between high- and low-predation environments is variable across rivers. This seems possible given that spectral properties of the water and stream size were correlated with male colour (Table 3). However, the influence of stream size and spectral properties is not robust given that all the correlations between these habitat features and colour are driven by a single site (Aripo low-predation).

Though canopy openness was also correlated with male colour, we cannot disentangle its influence on colour from that of predation because canopy openness is consistently higher at high-predation sites on the south slope (Figure 5D). Finally, nothing about the habitat of the Guanapo would suggest that an opposite pattern of colour evolution would occur in that river (Figure 5). Thus, we here discount the role of habitat differences in explaining our results.

A fourth possibility is that the density of predators, and thus intensity of natural selection, varies among sites. This seems unlikely in low-predation sites because the abundance of *Rivulus* was not correlated with aspects of male colour, though it did vary substantially among sites. This hypothesis cannot, however, be tested in high-predation environments because we were unable to quantify densities of the other fish predators. Though all of our high-predation sites contained *Crenicichla*, the density of this and other predators may have varied among sites. And yet, consistent differences among high- and low-predation sites were apparent in previous studies even when the abundance of predators was not taken into consideration.

A final possibility is that the environment has changed over the 25 years since the last survey of colour on the south slope. For instance, freshwater prawns, once abundant on the south slope (D.N. Reznick, pers. comm.), are now very rare. Indeed, we caught only four individuals in intensive trapping on the south slope, whereas we would catch many hundreds in equivalent sampling on the north slope (Millar et al. 2006). This decrease in prawns on the south slope is likely due to pollution of the Caroni swamp blocking the migration of juvenile prawns back to fresh water. To the extent that selection in sites with a high abundance of prawns leads to greater orange (Millar et al. 2006), this decrease in prawn predation on the south slope may be relaxing former selection for private signalling in the orange channel.

In conclusion, our finding that some rivers do not show the high- vs. low-predation colour difference found in previous work suggests that (1) we sampled in a way (e.g., five paired high- and low-predation populations) that revealed more subtleties than were evident in previous work especially in regard to female preference, or that (2) selection on male colour has changed in the last 25 years and lead to new patterns of adaptive evolution. Either of these possibilities suggests rich opportunities for further use of male guppy colour to understand 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 populations of guppies experiencing higher predation from *Crenicichla alta*, a UV-insensitive predator, would have males with greater UV reflectance, and females that increasingly based their mating decisions on male UV. Our study provides support for the first part of this hypothesis: high-predation males had more relative UV reflectance in four of five rivers, significantly so in two (Table 4, Figure 4C).

Though seemingly consistent across rivers, the divergence in UV reflectance between high and low predation sites was not very strong. Moreover, we found very few differences between high- and low-predation males in the UV reflectance of individual colours. One possible explanation as to why this divergence is weak is that there is only a small selective disadvantage to having UV reflectance in low-predation sites. Upstream sites (low predation) are colonized from downstream (high predation) sites (Carvalho et al. 1991). When guppies from downstream sites, where we hypothesize UV is favoured as a signal, arrive in upstream locations, selection against ultraviolet reflectance may be weak or absent. Though *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), are now largely absent from low-predation sites and so selection against UV may be very slight.

To frame our question, we assumed that a private signal would be favoured because it could be elaborated to a greater degree than a non-private signal. In doing so, we assumed a model of sexual selection wherein traits are used as the basis of mate choice because they are visible (Fisher process and sensory drive). However, some models require that the trait provide information about the quality of the male (indirect and direct benefit). Though private signals can evolve to be more visible than their non-private counterparts, this visibility does not necessarily transmit information about the fitness of the bearer. More work needs to be done to understand what information on fitness, if any, is contained in UV reflectance.

In conclusion, our study provides one piece of evidence that guppies use UV as a private signal when living with *Crenichla alta* – a dangerous, but UV-insensitive predator. The next step would be to demonstrate that high-predation females have an elevated preference for UV reflectance (e.g., Cummings et al. 2003 for *Xiphophorus spp.*). Is UV reflectance used to a greater degree than non-UV colour in mate choice decisions? Studies have shown that high-predation females often have weaker preferences for colour (e.g., Endler and Houde 1995). Perhaps this is because 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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Tables

Table 1. Location of sampling sites on the south slope of Trinidad's Northern Range Mountains. Latitude and longitude readings are from a GPS taken in UTM (WGS 84). Location is based on co-ordinates from UTM grid locations read from 1:25,000 maps (Lands and Surveys Division, Port of Spain, Trinidad).

	Predation	n	Latitude	Longitude	Location
Guanapo	High	25	20 P 0691156	1178883	PS 911 788
	Low	25	20 P 0689526	1184619	PS 893 844
El Cedro	High	25	No reading could be taken		PS 896 788
	Low	25	20 P 0689788	1178724	PS 895 797
Turure	High	25	20 P 0700344	1178573	QS 703 783
	Low	25	20 P 0699964	1181969	PS 999 819
Aripo	High	25	20 P 0695829	1177496	PS 940 781
	Low	25	20 P 0693325	1181913	PS 931 817
Quare	High	25	20 P 0697672	1179461	PS 975 792
	Low	25	20 P 0697317	1181153	PS 969 810

Table 2. Differences in non-UV colour between high- and low-predation sites. P values indicate the significance of ANOVAs comparing means of twelve colour variables. Significant differences are followed by an H (high predation) or an L (low predation) indicating the population with the greater amount of that colour.

	Guanapo		El Cedro		Turure		Aripo		Quare	
Relative area of black	0.534		0.668		0.034	L	0.026	L	<0.001	L
Relative area of orange	0.001	H	0.085		0.031	H	0.035	L	0.339	
Relative area of violet-blue	<0.001	H	0.790		0.176		0.421		0.471	
Relative area of green	0.712		0.195		0.629		0.253		0.001	H
Relative area of structural	0.002	H	0.066		0.008	H	0.927		0.001	H
Relative total area	0.001	H	0.241		0.081		0.002	L	0.282	
No. of black spots	0.442		0.681		0.511		0.022	L	0.004	L
No. of orange spots	0.122		0.884		0.248		<0.001	L	0.029	L
No. of violet-blue spots	0.010	H	0.569		0.020	H	0.333		0.787	
No. of green spots	0.682		0.302		0.385		0.536		0.001	H
No. of structural spots	0.477		1.000		0.075		0.014	L	0.311	
Total number of spots	0.028	H	0.584		1.000		<0.001	L	0.146	

Table 3. Correlations between habitat parameters and aspects of male colour. Pearson's correlation coefficient (r) is displayed for all relationships and significant correlations are indicated. For spectral, width, and depth, n = 9, and for canopy, n = 10. *** P < 0.001, ** P < 0.01, * P < 0.05.

	Spectral	Width	Depth	Canopy
Relative area of black	-0.237	-0.579	-0.495	-0.677 *
Relative area of orange	0.576	-0.037	-0.444	0.330
Relative area of violet-blue	0.316	0.438	0.145	0.478
Relative area of green	-0.460	0.354	0.435	0.321
Relative area of structural	0.090	0.076	-0.137	0.627
Relative total area	0.021	0.174	-0.477	-0.716 *
No. of black spots	0.828 **	-0.533	-0.892 **	-0.243
No. of orange spots	0.777	-0.407	-0.803 **	-0.332
No. of violet-blue spots	0.383	0.248	0.069	0.381
No. of green spots	-0.349	0.415	0.336	0.328
No. of structural spots	0.661	-0.618	-0.795 *	0.139
Total number of spots	0.830 **	-0.527	-0.862 **	-0.085

Table 4. Differences in relative UV reflectance of colour pattern between high- and low-predation males? Least-squared means from each ANCOVA is shown along with F and P values for predation where the model includes background UV as a covariate.

	Low Predation	High Predation	F	P
Relative UV reflectance of colour pattern				
Guanapo	103.06	109.27	9.555	0.003
El Cedro	100.19	98.57	1.531	0.222
Turure	116.14	116.50	0.059	0.809
Aripo	110.22	113.82	1.454	0.234
Quare	101.94	111.89	5.725	0.021

Figures

Figure 1. Map of northern Trinidad showing the locations of the sites used for the study of wild UV colouration. We sampled 25 male guppies from paired high predation (filled stars) and low predation (open squares) sites on five river drainages on the south slope of the Northern Mountain Range.

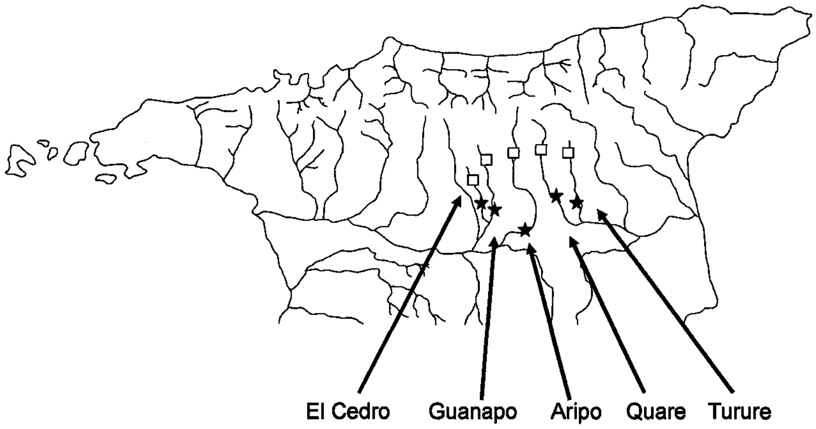

Figure 2. UV (A) and colour (B) photographs of a male guppy from the low predation sampling site in the Aripo River.

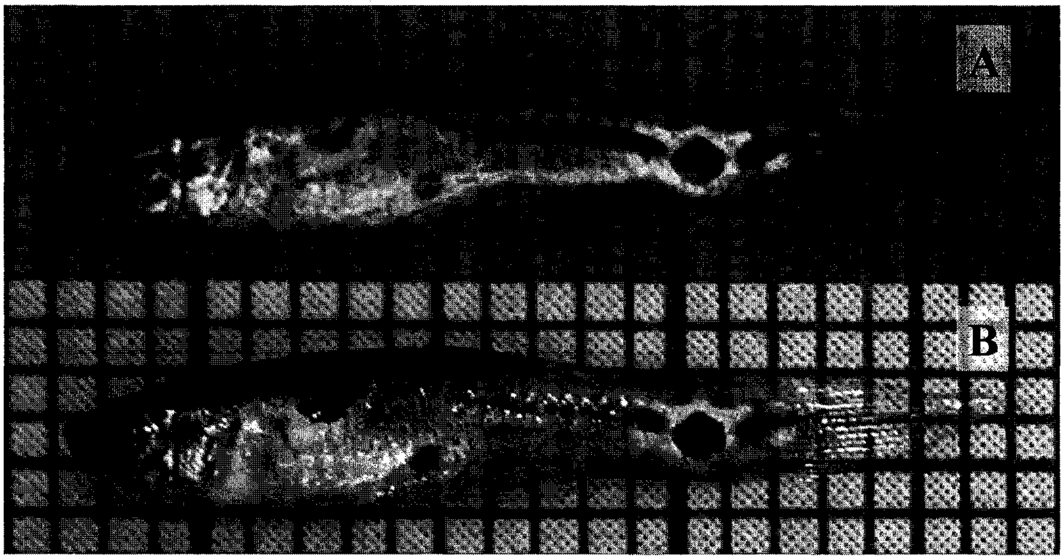

Figure 3. Spectrum of light transmitted through the lens and UV filter measured with an Ocean Optics spectrometer.

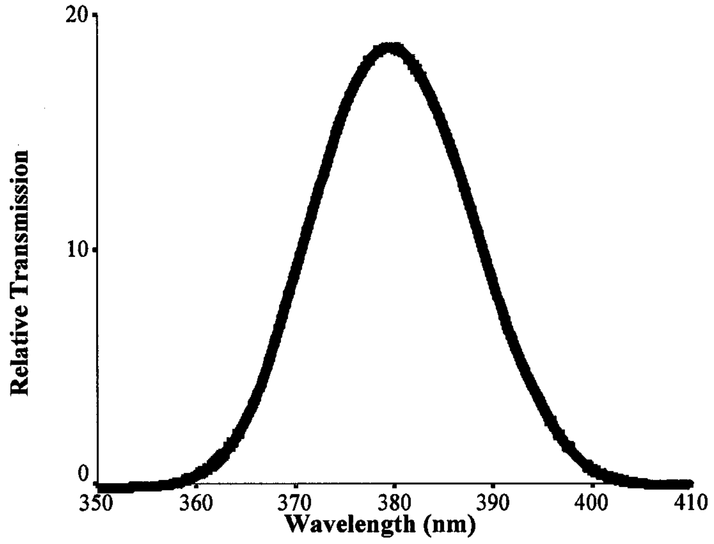

Figure 4: Variation in UV and non-UV components of male colour in low-predation (white bars) and high-predation (black bars) sites in five rivers. Bars represent mean values of the relative area of black (A), the relative area of orange (B), and the relative UV reflectance of the colour pattern (C). Significant difference between high and low predation sites are indicated with asterisks: *** - P < 0.001, ** - P < 0.01, * - P < 0.05.

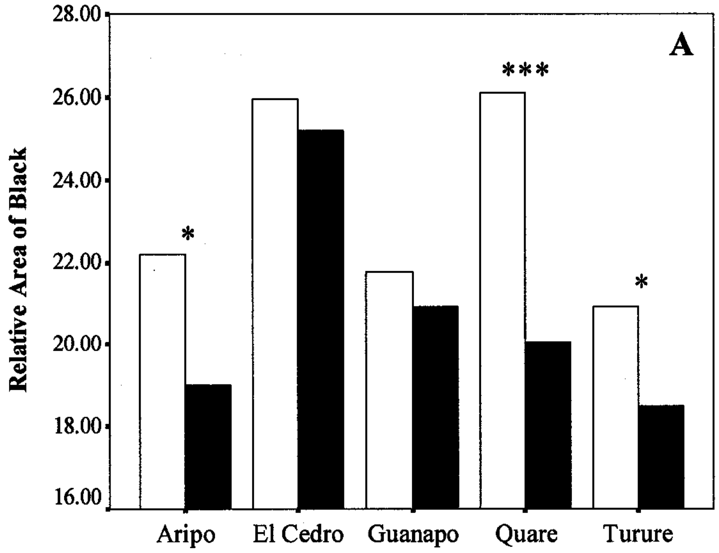

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Figure 4 continued.

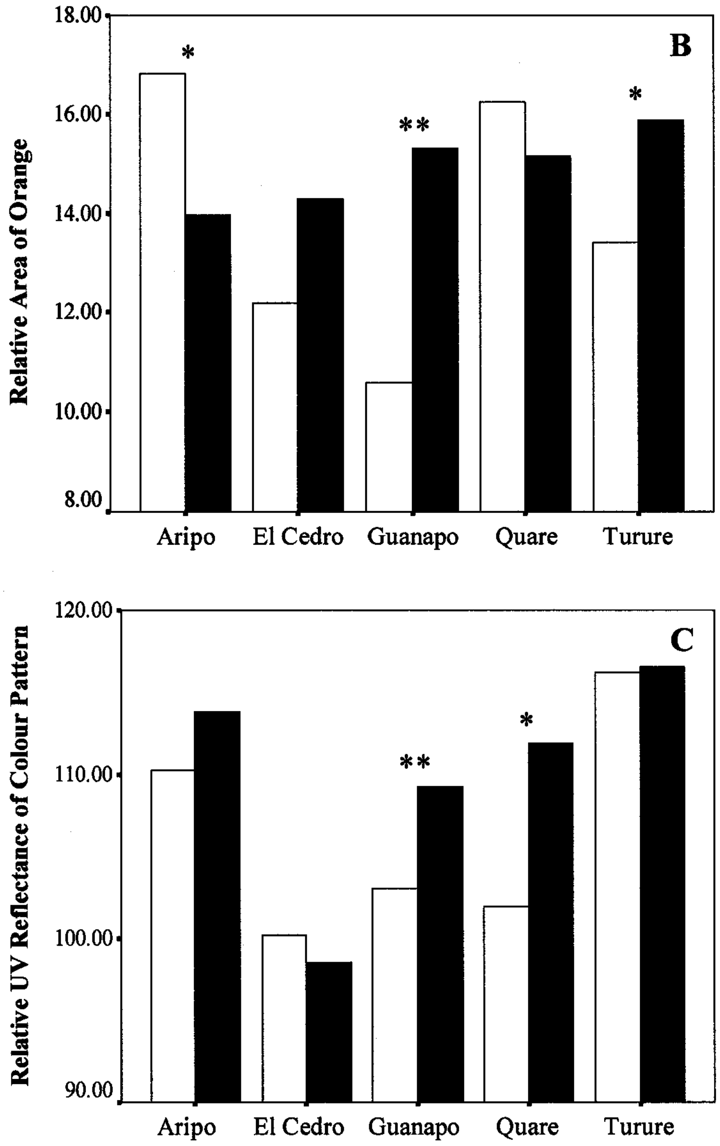
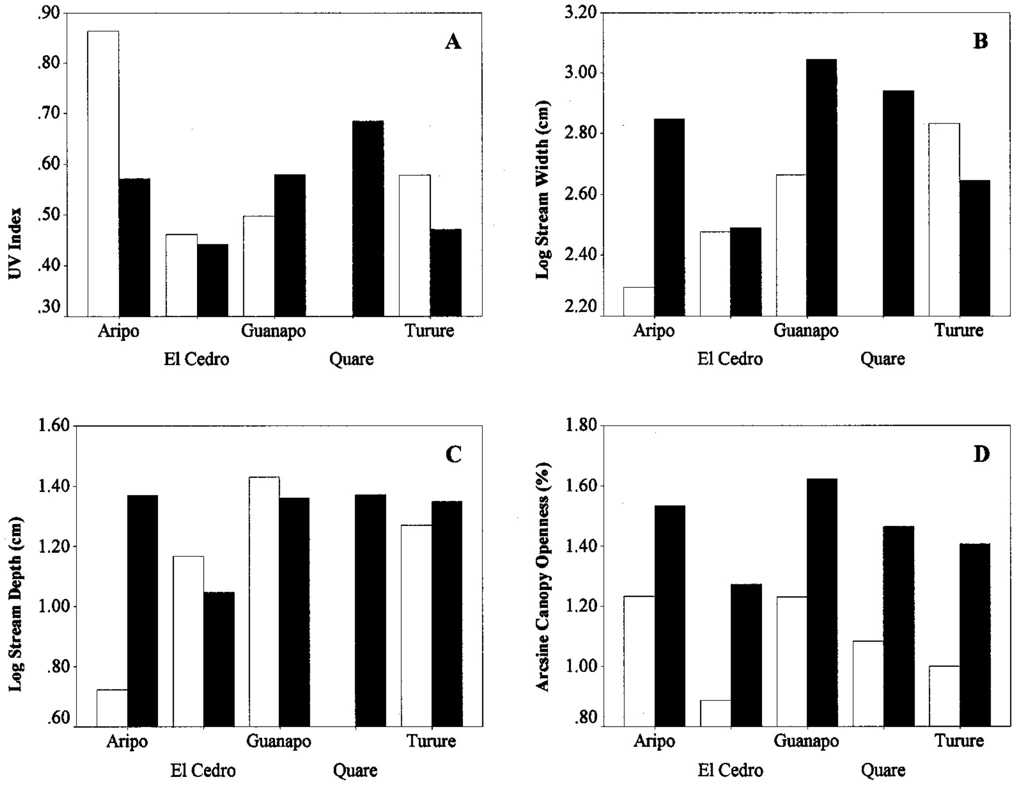


Figure 5. Mean habitat parameters for low predation (white) and high predation (black) on the five rivers.



SUMMARY

Secondary sexual traits evolve as a compromise between natural and sexual selection. The evolution of colour patterns of guppies, *Poecilia reticulata*, is often cited as a classic example of this compromise. Guppies living with dangerous fish predators are less colourful than those living with weak predators. This straightforward understanding of the evolution secondary sexual traits has been expanded to include subtle, yet important, aspects of selection. In this thesis I examined the roles of habitat and the visual systems of predators in the evolution of guppy colour.

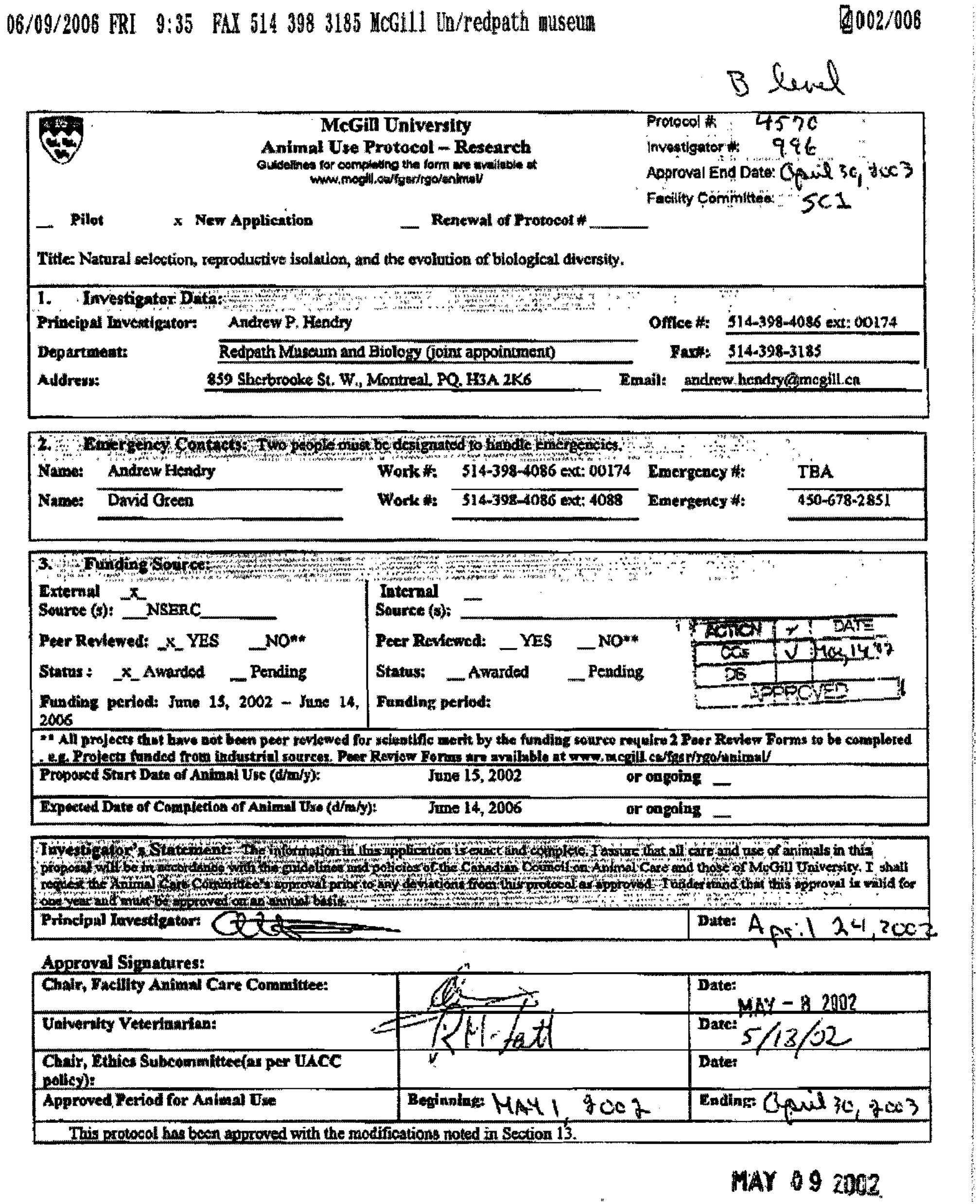
Several studies have shown that aspects of habitat are important to the evolution of male colour. In particular, water colour, canopy openness, and substrate size have been shown, experimentally or empirically, to be important. The habitat in which guppies live varies substantially among and within rivers. To address the question of the role of habitat in the evolution of colour in wild guppies, I sampled guppy populations living in a variety of habitats through two river drainages. Though certain habitat parameters did correlate with aspects of male colour, these correlations were not consistent across drainages. In this study system, habitat appears to play only a minor role, if any, in the evolution of male colour. Predation, on the other hand, plays a prominent role.

Guppies live with a variety of predators and each one may have different sensory capabilities. In regards to colour pattern evolution, the sensitivity of the visual system of predators to certain wavelengths of light is particularly important. Selection by predators should be strongest on colours that reflect those wavelengths of light to which the predator is *most* sensitive. In the presence of these predators, guppies should evolve colour patterns that contain these highly visible colours. Selection by predators is weakest on colours that reflect wavelengths of light to which the predator is *least* sensitive. These colours are not subject to the conservative action of natural selection and can be elaborated. These private signals should therefore be favoured by sexual selection over non-private signals.

The visual sensitivity of guppies is broad; they see wavelengths of light from the short (ultraviolet) to the long (orange-red). However, two of their predators do not see all of these wavelengths of light. The freshwater prawn, *Macrobrachium crenulatum*, is not sensitive to orange-red light whereas the pike cichlid, *Crenicichla alta*, is not sensitive to UV light. I examined whether, in the presence of these predators, male guppies exhibited more of the private signal (orange colour for *Macrobrachium* and UV reflectance for *Crenicichla*) than in the absence of these predator. I found evidence for the use of both private signals. Though the use of these signals must be confirmed by examining whether female preference for the private signal is greater when in the presence of the predator, these results are strong evidence that predatory visual systems are very important in determining selection on colour patterns in guppies.

APPENDIX A: Animal care committee compliance certificates (2002 – 2006)

2002 - 2003



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B level

McGill University
Animal Use Protocol – Research
Guidelines for completing the form are available at
www.mcgill.ca/fgsr/rgo/animal/

Protocol #: 4570
Investigator #: 996
Approval End Date: April 30, 2003
Facility Committee: SC1

__ **Pilot** x **New Application** __ **Renewal of Protocol #** ______

Title: Natural selection, reproductive isolation, and the evolution of biological diversity.

1. Investigator Data:

Principal Investigator: Andrew P. Hendry
Office #: 514-398-4086 ext: 00174
Department: Redpath Museum and Biology (joint appointment)
Fax#: 514-398-3185
Address: 859 Sherbrooke St. W., Montreal, PQ, H3A 2K6
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2. Emergency Contacts: Two people must be designated to handle emergencies.

Name:	Andrew Hendry	**Work #:**	514-398-4086 ext: 00174	**Emergency #:**	TBA
Name:	David Green	**Work #:**	514-398-4086 ext: 4088	**Emergency #:**	450-678-2851

3. Funding Source:

External _x_	**Internal** __
Source (s): __NSERC______	**Source (s):** ______
Peer Reviewed: _x_ YES __NO**	**Peer Reviewed:** __ YES __NO**
Status: _x_ Awarded __ Pending	**Status:** __ Awarded __ Pending
Funding period: June 15, 2002 – June 14, 2006	**Funding period:**

ACTION	✓	DATE
CCs	✓	May 14 '02
DS		
APPROVED		

** All projects that have not been peer reviewed for scientific merit by the funding source require 2 Peer Review Forms to be completed, e.g. Projects funded from industrial sources. Peer Review Forms are available at www.mcgill.ca/fgsr/rgo/animal/

Proposed Start Date of Animal Use (d/m/y): June 15, 2002 **or ongoing** __

Expected Date of Completion of Animal Use (d/m/y): June 14, 2006 **or ongoing** __

Investigator's Statement: The information in this application is exact and complete. I assure that all care and use of animals in this proposal will be in accordance with the guidelines and policies of the Canadian Council on Animal Care and those of McGill University. I shall request the Animal Care Committee's approval prior to any deviations from this protocol as approved. I understand that this approval is valid for one year and must be approved on an annual basis.

Principal Investigator: **Date:** April 24, 2002

Approval Signatures:

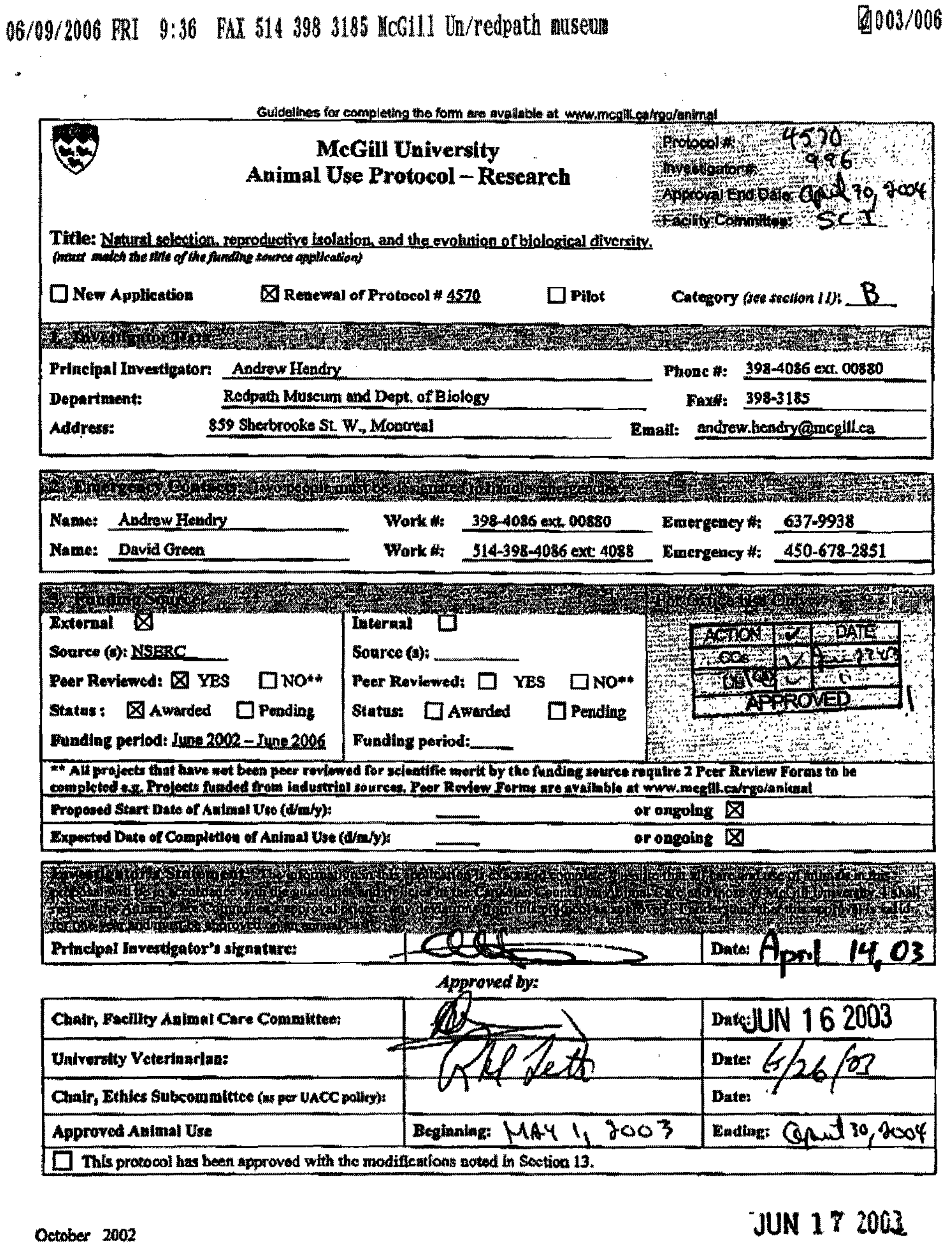
Chair, Facility Animal Care Committee:		**Date:** MAY - 8 2002
University Veterinarian:		**Date:** 5/13/02
Chair, Ethics Subcommittee(as per UACC policy):		**Date:**
Approved Period for Animal Use	**Beginning:** MAY 1, 2002	**Ending:** April 30, 2003

This protocol has been approved with the modifications noted in Section 13.

MAY 09 2002

April 2001

2003 – 2004



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McGill University
Animal Use Protocol – Research

Protocol #: 4570
Investigator #: 996
Approval End Date: April 30, 2004
Facility Committee: SCI

Title: Natural selection, reproductive isolation, and the evolution of biological diversity.
(must match the title of the funding source application)

☐ **New Application** ☒ **Renewal of Protocol #** 4570 ☐ **Pilot** **Category** *(see section 11)*: B

1. Investigator Data

Principal Investigator: Andrew Hendry **Phone #:** 398-4086 ext. 00880
Department: Redpath Museum and Dept. of Biology **Fax#:** 398-3185
Address: 859 Sherbrooke St. W., Montreal **Email:** andrew.hendry@mcgill.ca

2. Emergency Contacts: two people must be designated to handle emergencies

Name: Andrew Hendry	**Work #:** 398-4086 ext. 00880	**Emergency #:** 637-9938
Name: David Green	**Work #:** 514-398-4086 ext: 4088	**Emergency #:** 450-678-2851

3. Funding Source

External ☒	**Internal** ☐
Source (s): NSERC	**Source (s):**
Peer Reviewed: ☒ YES ☐ NO**	**Peer Reviewed:** ☐ YES ☐ NO**
Status: ☒ Awarded ☐ Pending	**Status:** ☐ Awarded ☐ Pending
Funding period: June 2002 – June 2006	**Funding period:**

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ACTION	✓	DATE
CCs	✓	[illegible]
DB	✓	[illegible]
APPROVED		

**** All projects that have not been peer reviewed for scientific merit by the funding source require 2 Peer Review Forms to be completed e.g. Projects funded from industrial sources. Peer Review Forms are available at www.mcgill.ca/rgo/animal**

Proposed Start Date of Animal Use (d/m/y): ____ **or ongoing** ☒
Expected Date of Completion of Animal Use (d/m/y): ____ **or ongoing** ☒

Investigator's Statement: [illegible]

Principal Investigator's signature: **Date:** April 14, 03

Approved by:

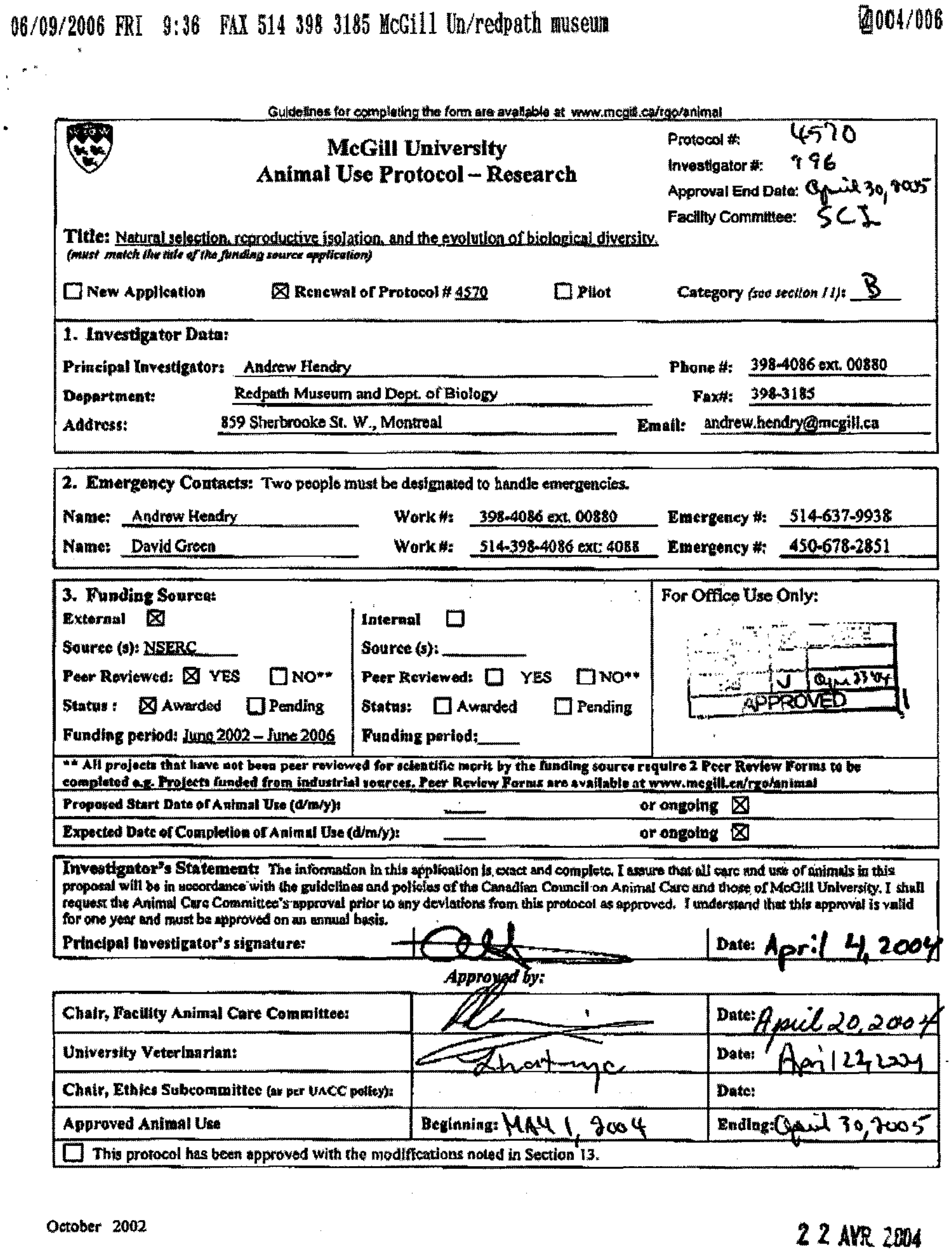
Chair, Facility Animal Care Committee:		**Date:** JUN 16 2003
University Veterinarian:		**Date:** 6/26/03
Chair, Ethics Subcommittee (as per UACC policy):		**Date:**
Approved Animal Use	**Beginning:** MAY 1, 2003	**Ending:** April 30, 2004

☐ This protocol has been approved with the modifications noted in Section 13.

October 2002

JUN 17 2003

2004 – 2005



06/09/2006 FRI 9:36 FAX 514 398 3185 McGill Un/redpath museum 004/006

Guidelines for completing the form are available at www.mcgill.ca/rgo/animal

McGill University
Animal Use Protocol – Research

Protocol #: 4570
Investigator #: 196
Approval End Date: April 30, 2005
Facility Committee: SCI

Title: Natural selection, reproductive isolation, and the evolution of biological diversity.
(must match the title of the funding source application)

☐ **New Application** ☒ **Renewal of Protocol #** 4570 ☐ **Pilot** **Category** *(see section 11)*: B

1. Investigator Data:

Principal Investigator: Andrew Hendry **Phone #:** 398-4086 ext. 00880
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3. Funding Source:

External ☒	**Internal** ☐	**For Office Use Only:**
Source (s): NSERC	**Source (s):**	APPROVED
Peer Reviewed: ☒ YES ☐ NO**	**Peer Reviewed:** ☐ YES ☐ NO**	
Status: ☒ Awarded ☐ Pending	**Status:** ☐ Awarded ☐ Pending	
Funding period: June 2002 – June 2006	**Funding period:**	

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Proposed Start Date of Animal Use (d/m/y): ____ **or ongoing** ☒
Expected Date of Completion of Animal Use (d/m/y): ____ **or ongoing** ☒

Investigator's Statement: The information in this application is exact and complete. I assure that all care and use of animals in this proposal will be in accordance with the guidelines and policies of the Canadian Council on Animal Care and those of McGill University. I shall request the Animal Care Committee's approval prior to any deviations from this protocol as approved. I understand that this approval is valid for one year and must be approved on an annual basis.

Principal Investigator's signature: **Date:** April 4, 2004

Approved by:

Chair, Facility Animal Care Committee:		**Date:** April 20, 2004
University Veterinarian:		**Date:** April 22, 2004
Chair, Ethics Subcommittee (as per UACC policy):		**Date:**
Approved Animal Use	**Beginning:** MAY 1, 2004	**Ending:** April 30, 2005

☐ This protocol has been approved with the modifications noted in Section 13.

October 2002

22 AVR 2004

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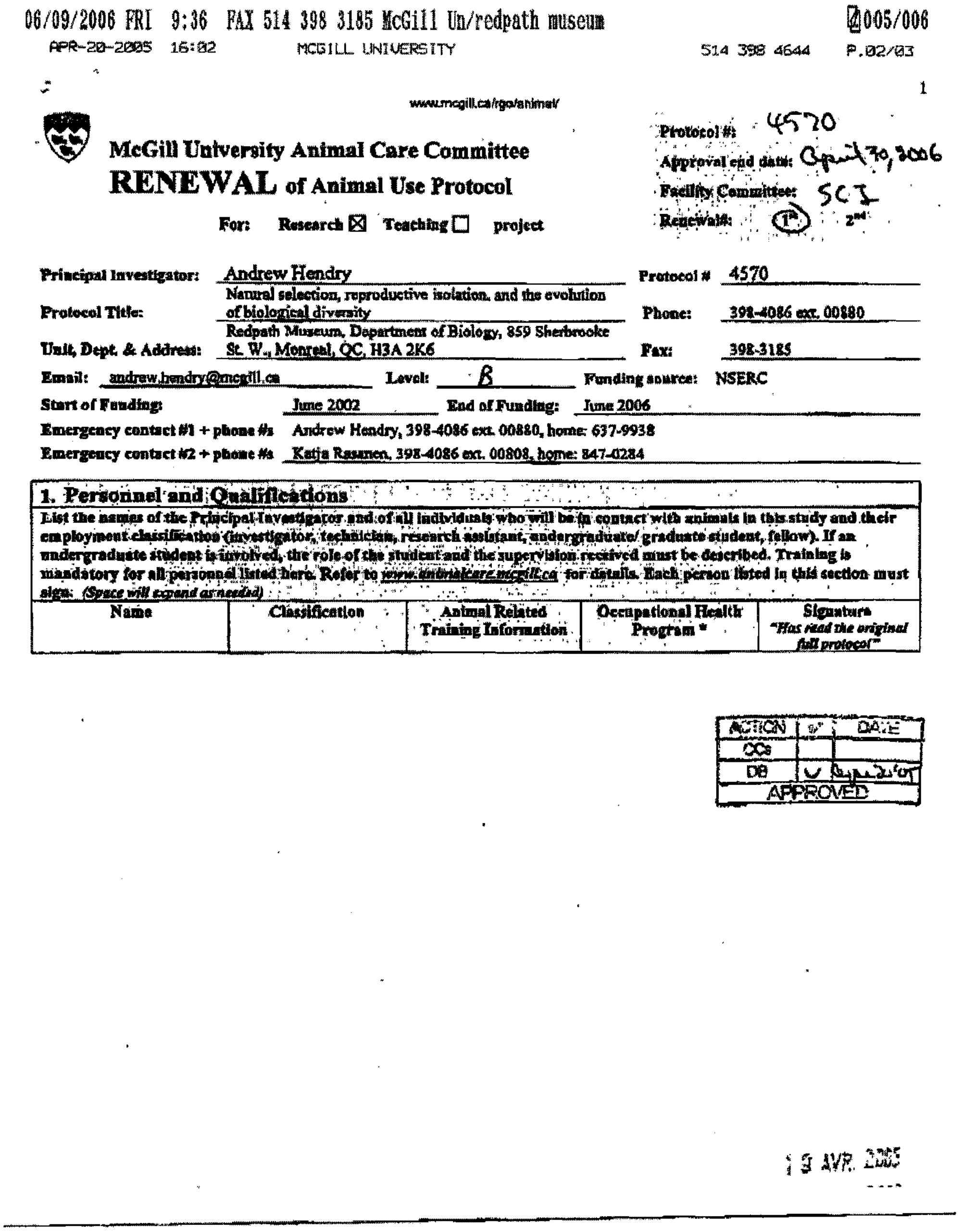

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www.mcgill.ca/rgo/animal/

McGill University Animal Care Committee
RENEWAL of Animal Use Protocol

For: Research ☒ Teaching ☐ project

Protocol #: 4570
Approval end date: April 30, 2006
Facility Committee: SCI
Renewal#: [✓ 1st] 2nd

Principal Investigator: Andrew Hendry **Protocol #** 4570
Protocol Title: Natural selection, reproductive isolation, and the evolution of biological diversity **Phone:** 398-4086 ext. 00880
Unit, Dept. & Address: Redpath Museum, Department of Biology, 859 Sherbrooke St. W., Montreal, QC, H3A 2K6 **Fax:** 398-3185
Email: andrew.hendry@mcgill.ca **Level:** B **Funding source:** NSERC
Start of Funding: June 2002 **End of Funding:** June 2006
Emergency contact #1 + phone #s Andrew Hendry, 398-4086 ext. 00880, home: 637-9938
Emergency contact #2 + phone #s Katja Rasanen, 398-4086 ext. 00808, home: 847-0284

1. Personnel and Qualifications

List the names of the Principal Investigator and of all individuals who will be in contact with animals in this study and their employment classification (investigator, technician, research assistant, undergraduate/ graduate student, fellow). If an undergraduate student is involved, the role of the student and the supervision received must be described. Training is mandatory for all personnel listed here. Refer to *www.animalcare.mcgill.ca* for details. Each person listed in this section must sign. *(Space will expand as needed)*

Name	Classification	Animal Related Training Information	Occupational Health Program *	Signature *"Has read the original full protocol"*

ACTION	✓	DATE
CCs		
DB	✓	Apr 21/05
APPROVED		

1 9 AVR. 2005

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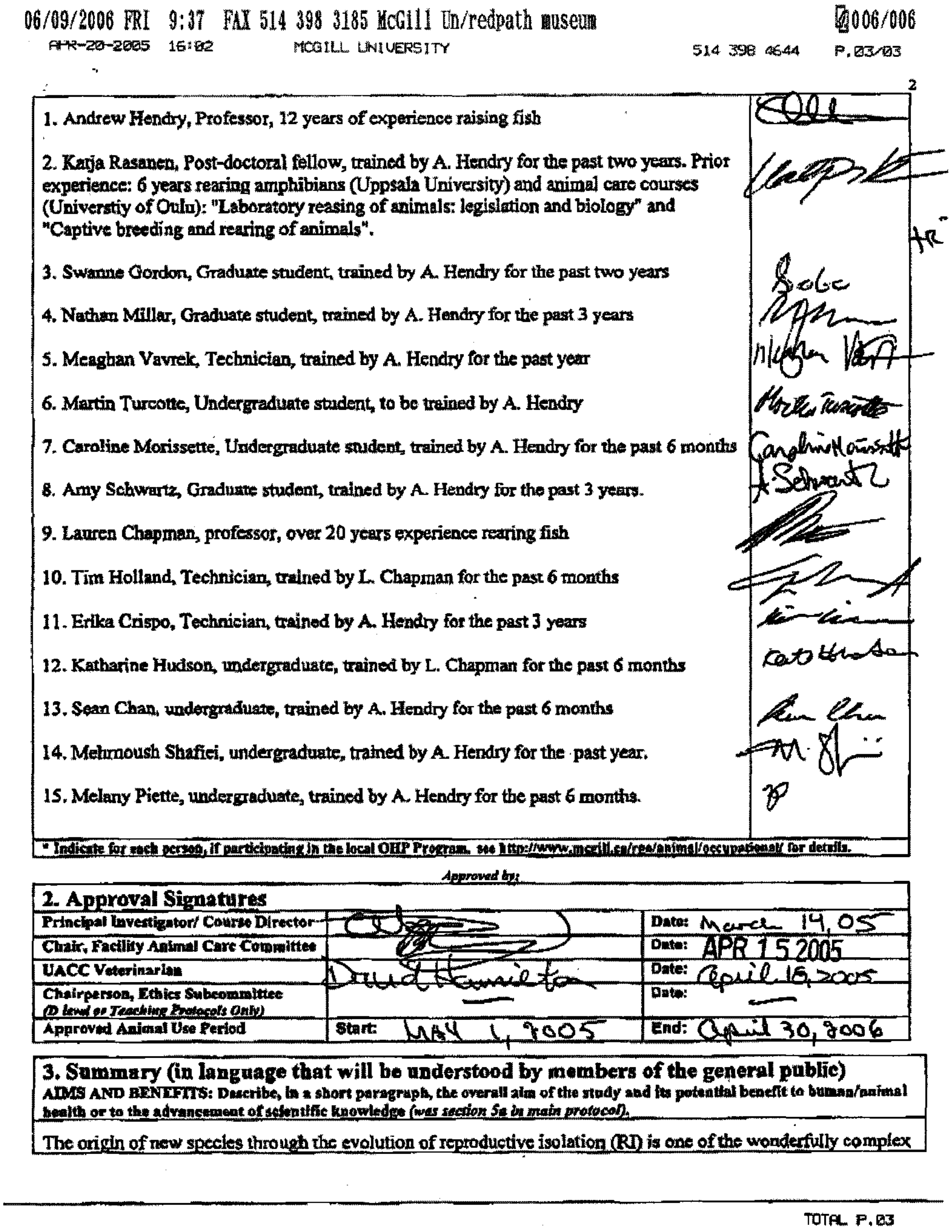

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2

1. Andrew Hendry, Professor, 12 years of experience raising fish	
2. Katja Rasanen, Post-doctoral fellow, trained by A. Hendry for the past two years. Prior experience: 6 years rearing amphibians (Uppsala University) and animal care courses (Universtiy of Oulu): "Laboratory reasing of animals: legislation and biology" and "Captive breeding and rearing of animals".	
3. Swanne Gordon, Graduate student, trained by A. Hendry for the past two years	
4. Nathan Millar, Graduate student, trained by A. Hendry for the past 3 years	
5. Meaghan Vavrek, Technician, trained by A. Hendry for the past year	Meghan V
6. Martin Turcotte, Undergraduate student, to be trained by A. Hendry	Martin Turcotte
7. Caroline Morissette, Undergraduate student, trained by A. Hendry for the past 6 months	Caroline Morissette
8. Amy Schwartz, Graduate student, trained by A. Hendry for the past 3 years.	A. Schwartz
9. Lauren Chapman, professor, over 20 years experience rearing fish	
10. Tim Holland, Technician, trained by L. Chapman for the past 6 months	
11. Erika Crispo, Technician, trained by A. Hendry for the past 3 years	
12. Katharine Hudson, undergraduate, trained by L. Chapman for the past 6 months	Kate Hudson
13. Sean Chan, undergraduate, trained by A. Hendry for the past 6 months	Sean Chan
14. Mehrnoush Shafiei, undergraduate, trained by A. Hendry for the past year.	
15. Melany Piette, undergraduate, trained by A. Hendry for the past 6 months.	

* Indicate for each person, if participating in the local OHP Program. see http://www.mcgill.ca/rpe/animal/occupational/ for details.

Approved by:

2. Approval Signatures

Principal Investigator/ Course Director		**Date:**	March 14, 05
Chair, Facility Animal Care Committee		**Date:**	APR 15 2005
UACC Veterinarian	David Hamilton	**Date:**	April 15, 2005
Chairperson, Ethics Subcommittee *(D level or Teaching Protocols Only)*		**Date:**	
Approved Animal Use Period	**Start:** May 1, 2005	**End:**	April 30, 2006

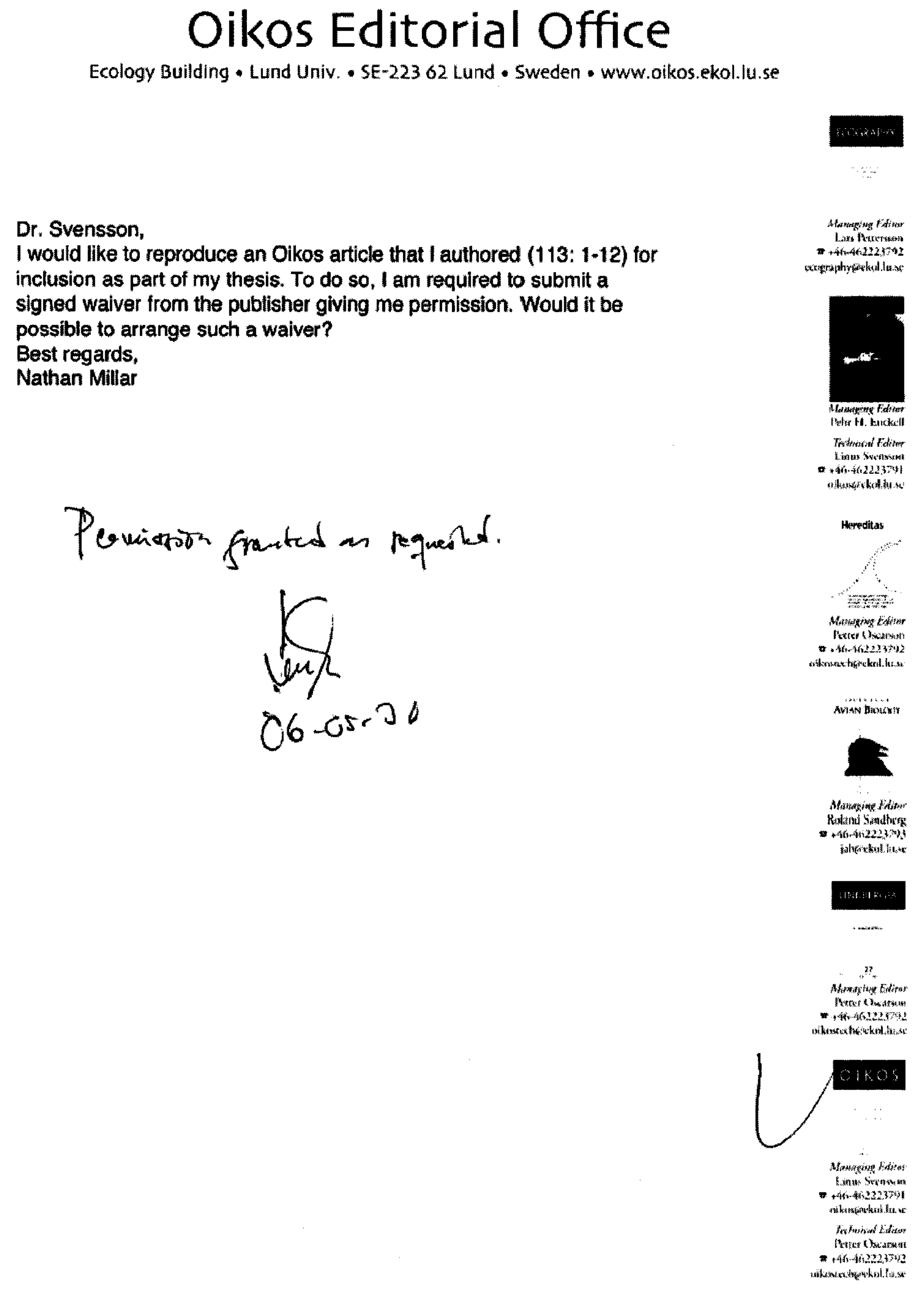
3. Summary (in language that will be understood by members of the general public)

AIMS AND BENEFITS: Describe, in a short paragraph, the overall aim of the study and its potential benefit to human/animal health or to the advancement of scientific knowledge *(was section 5a in main protocol).*

The origin of new species through the evolution of reproductive isolation (RI) is one of the wonderfully complex

TOTAL P.03

APPENDIX B: Signed waiver to reproduce Oikos article



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Dr. Svensson,
I would like to reproduce an Oikos article that I authored (113: 1-12) for inclusion as part of my thesis. To do so, I am required to submit a signed waiver from the publisher giving me permission. Would it be possible to arrange such a waiver?
Best regards,
Nathan Millar

Permission granted as requested.

06-05-30

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APPENDIX C: *Poecilia*, the story of how one guppy found and then lost love

Poecilia
To the tune of Cecilia (P. Simon 1969)

Poecilia, you're breaking my heart
You lower my fitness daily
Oh *Poecilia*, I'm making displays
And looking for sigmoid answers

Poecilia, you're breaking my heart
You lower my fitness daily
Oh *Poecilia*, I'm making displays
And looking for ways to get action
Glide to me

Guppy love in aquaria
With *Poecilia* down in my weed bed
I went up to surface-feed
When I come back to mate
Someone's planted their seed

Poecilia, you're breaking my heart
You lower my fitness daily
Oh *Poecilia*, I'm making displays
And looking for ways to get action
Glide to me

Copulation,
I got coloured spots
I'm making displays and I'm waiting

Sex. Selection,
She pays me no heed
I gave up on her so I'm sneaking