

Ornamental evolution in Trinidadian guppies (*Poecilia reticulata*): insights from sensory processing-based analyses of entire colour patterns

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The evolution of exaggerated sexual ornamentation is classically thought to proceed as a compromise between opposing vectors of sexual and natural selection. In colour-based ornamentation, as exhibited by guppies (*Poecilia reticulata*), heightened trait expression may be beneficial in promoting attractiveness, but costly in terms of predation. Opportunities to reconcile this compromise will exist if there are differences between conspecifics and predators in their sensory systems; in such situations guppies should evolve to exploit the ways in which their ornamentation would appear maximally conspicuous to conspecifics. In the present study, we addressed this hypothesis via a study of geographic variation employing the most sophisticated colour analysis yet attempted for Trinidadian guppies. We made two paired contrasts, one between two Aripo populations that vary in the presence of the potential predator *Aequidens pulcher*, and another between Quare and Marianne populations that vary in exposure to a predatory prawn, *Macrobrachium crenulatum*. We predicted that, if ornamentation is constrained by the presence of either predator, then guppy conspicuousness should change most markedly across each of the two paired populations as viewed by that predator. Although disparity analysis of entire colour patterns indicated significant differences in both contrasts, this prediction was most clearly supported for the Marianne/Quare contrast. Marianne fish, which co-exist with prawns, exhibited larger black spots coupled with less extensive, less bright flank iridescence. The brightness reductions are notable because, as the only potential guppy predator with a dedicated ultraviolet (UV) photoreceptor, prawns may detect passing male guppies via their UV-bright blues, violets and 'UV/oranges'. We discuss our findings in light of the additional insights that might be obtained by combining spectral assessments and visual modeling with more traditional methods of colour pattern appraisal. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, 95, 734–747.

ADDITIONAL KEYWORDS: iridescence – mate choice – predation – ultraviolet – vision.

INTRODUCTION

Wild animal populations are subject to multiple, sometimes opposing, vectors of selection. In species with males that bear conspicuous secondary sexual characters, sexual selection for continued trait exaggeration is classically thought to be opposed by natural selection for predator avoidance (Fisher, 1930; Andersson, 1994). Prevailing thought is that sexual trait expression in these cases represents a

local balance between sexually selected benefits on the one hand and naturally-selected costs on the other (Zuk & Kolluru, 1998). Variation in either source of selection, either within a single population over time, or among different populations, should affect this balance, and thereby contribute to variation in the population mean level of trait expression. Thus, with all other things being equal, populations subject to reduced predation risk are expected to exhibit more conspicuous ornaments and/or sexual behaviours than populations exposed to high predation risk.

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In the case of colour-based ornamental traits, variation among populations may be promoted by consistent population-wide differences in the intensity of predation, differences in the target of female preference, and/or differences in the conditions under which the trait is typically viewed (e.g. ambient light regimes, transmission and background properties; Endler 1992, 1993a). Of these factors, perhaps the most research effort has gone into trying to understand the effects of predation. The balance of this work has generally upheld the prediction that colour patterns should evolve to be less conspicuous under increasing predation risk (Endler, 1978, 1980, 1983). However, until recently, studies have proceeded with little specific reference to how visual signals are processed by animal sensory systems, which may have limited their power for making evolutionary inferences (Endler *et al.*, 2005). Given that a single colour pattern may appear differently depending upon the visual sensitivity of specific viewers (Endler, 1978; Lythgoe, 1979), sensory processing is expected to be of prime importance to interpreting the likely strength of natural selection via predation. Furthermore, if colour sensitivity (i.e. the ability to detect specific light wavelengths) differs between predators and conspecifics, then sexual ornamentation should evolve in directions that increase conspicuousness to conspecifics while remaining relatively inconspicuous to predators. This is the 'sensory drive' hypothesis of Endler (1992, 1993b); for empirical support, see Endler (1991); Stuart-Fox *et al.* (2004); Håstad, Victorsson & Ödeen (2005); and Heinsohn, Legge & Endler (2005). Such cases pose an evolutionary 'opportunity' for the reduction of the costs associated with signalling and/or bearing potentially conspicuous ornamentation; however, this opportunity is ultimately mediated by the extent to which conspecific visual systems vary from those of their most ecologically relevant predators.

In the present study, we address these issues by examining colour variation among different populations of Trinidadian guppies (*Poecilia reticulata*). This sexually promiscuous and highly male-ornamented species has presented a classic model system for exploring the tension between natural and sexual selection (Houde, 1997; Magurran, 2005, and references therein; see below). Prior work has demonstrated evolution in guppy colour patterns in response to changes in predation (Endler, 1978, 1980), geographic variation in female preferences and male coloration (Endler & Houde, 1995; Millar *et al.*, 2006), and geographic variation in predator recognition/avoidance behaviour (Magurran & Seghers, 1990). Intrapopulation studies have also incorporated data regarding guppy and predator vision and viewing conditions to reveal that male

colour patterns are likely to be selectively conspicuous to guppies, at least under the conditions in which courtship most often occurs (Endler, 1987, 1991). All of these lines of evidence suggest that predation has posed an important agent of natural selection upon guppy colour patterns, and that the ornamental colour patterns of different populations are likely to be fine tuned according to the visual systems of the local guild of predators. However, although component parts of this hypothesis have been tested (Millar *et al.*, 2006), no study has yet incorporated sensory processing data of guppies and their relevant predators to shed light on patterns of geographic variation in male ornamentation. Recent advances in the measurement and analysis of animal colour patterns (Endler & Mielke, 2005) also provide excellent opportunities for refinement of prior efforts to relate colour pattern conspicuousness and predation risk. The aims of the present study are therefore two-fold: (1) to explore and contrast the use of more sophisticated, whole-colour pattern analyses with the traditional techniques used to classify guppy coloration and (2) to use these updated approaches to test specific predictions (detailed below) regarding how guppy coloration should differ between populations differing in specific predator species.

STUDY SYSTEM, SAMPLING SITES, AND SPECIFIC PREDICTIONS

Guppies are small fish native to northeastern South America, where they occur in clear, shallow montane forest streams with sandy or gravely substrates. Their mating system is well described (Houde, 1997; Magurran, 2005); briefly, it is characterized as a promiscuous and nonresource based system where males sequentially court females and where female acceptance determines male mating success (along with male sneak copulation behaviour). Male attractiveness is largely based upon aspects of ornamental coloration, which although highly polymorphic, is comprised of three principal elements: (1) orange, red, and yellow spots based upon the presence of carotenoid and/or pteridine pigments (Grether, Cummings & Hudon, 2005); (2) black and brown spots, bars and fuzzy markings based upon the presence of melanin pigment; and (3) iridescent and structurally-produced blues, violets, greens, and silver, which may occur in semi-discrete patches or as a 'background' refugence. Female preferences have been demonstrated for all of these colour components (Kodric-Brown, 1989; Endler & Houde, 1995; Brooks, 2000; Brooks & Endler, 2001a,b) and ornament expression and female preferences co-vary genetically and geographically (Houde, 1994; Endler & Houde, 1995; Grether, 2000). As noted above, this ornamentation also makes

guppies more conspicuous to their primary visually-hunting predators, which include the piscivorous fish *Crenicichla alta*, *Rivulus hartii*, and *Aequidens pulcher*, and the freshwater prawn *Macrobrachium crenulatum* (Endler, 1978).

We sampled fish from four different sites (two sites on the Aripo river, one site on each of the Marianne and Quare rivers), yielding two separate contrasts to formulate and test sensory drive-based predictions regarding male coloration. In the first contrast, we compared fish from Aripo pool 14 (hereafter 'Aripo control'), a moderate predation upstream site in the Caroni drainage containing the guppy predators *R. hartii* and *A. pulcher*, with fish from a low predation upstream tributary of the Aripo river (hereafter 'Aripo introduction'). In 1981, approximately 50 fish from the control site were liberated into the introduction site (by DNR), which previously contained *R. hartii* but no guppies. Thus, a comparison of these two sites represents an introduction experiment, similar to the classic experiments conducted by Endler (1980, 1983); in this case, introduction site fish (which are genetically very similar to 'control' fish) have experienced reduced predation due to the excluded predator, *A. pulcher*. In the second contrast, we compared fish from a tributary to the Marianne River (Petit Marianne, from the north slope of Trinidad's northern range mountains) with fish from the Quare river, which is part of the Oropuche drainage, on the south slope of the Northern Range Mountains. Guppies from these two sites will be strongly genetically differentiated (Magurran, 2005), which suggests they may express genetic differences in coloration (and possibly also female preference; Houde, 1988). Moreover, although both sites contain *R. hartii*, only the Marianne supports moderate population densities of the predatory freshwater prawn, *M. crenulatum*. The prawn is interesting on two vision-related counts: first, because this is the only predator with visual sensitivity well into the ultraviolet (UV) wavelengths and, second, because it is relatively less sensitive to red light (i.e. light of wavelengths longer than 600 nm; Endler, 1991). This pattern of sensitivity would appear to offer good opportunities, in populations of guppies exposed to prawns, for male ornamentation to become elaborated in ways conspicuous to other guppies but not entirely evident to the prawn. With this type of reasoning as a basis, we formulated the following three-step predictions regarding each of our two specific contrasts:

1. If guppies in the sites with other predators in addition to *Rivulus* (i.e. the Aripo control and Marianne) are subject to significant predation, but also significant sexual selection by guppies, then

they should evolve conspicuous coloration in ways that are most apparent to guppies and least apparent to the predators. This fine-tuning could proceed to the extent that there are differences between guppies and predators in their visual sensitivities, such that male guppy coloration would represent a local compromise between these opposing sources of selection (Endler, 1978, 1991).

2. In each corresponding *Rivulus*-only site (i.e. the Aripo introduction and Quare), guppies would be free to respond to sexual selection for colour elaboration in ways otherwise penalized by the predators at the other sites. Thus, male guppies may evolve more conspicuous markings overall (as a result of a shift in the relative intensity of natural versus sexual selection), but the change in ornamental conspicuousness should occur mostly in ways apparent to the visual system of the excluded predator (i.e. *A. pulcher* in the Aripo contrast, and *M. crenulatum* in the Marianne/Quare contrast). Note here that we do not assume *Rivulus* predation would be similar in each *Rivulus*-only site; merely that guppy inhabitants would experience reduced predation relative to individuals from each paired, higher predation site.
3. Comparison of the coloration of paired guppy populations 'through the eyes' of each viewer should therefore reveal that they differ most as viewed by the excluded predator, followed by guppies themselves. Both differences should occur in the direction of increased guppy conspicuousness in the reduced predation populations (i.e. the Aripo introduction and Quare fish). With regard to *Rivulus*, which is a potential agent of natural selection in all populations, specific a priori predictions are difficult to formulate, primarily because *Rivulus* abundance and/or life history will also vary between sites due to differences in overall predation. Thus, predation by *Rivulus* will probably also vary across all sites. The simplest expectation would be that *Rivulus* should pose greater danger to guppies in Aripo introduction and Quare; in which case these guppy populations should evolve coloration that appears less conspicuous to *Rivulus*.

This reasoning assumes general similarities among populations in the female visual system and the way in which females place visually mediated sexual selection (refer to the Discussion for a more detailed treatment of this issue). We evaluated these predictions by quantifying colour patterns from each of the four guppy populations and contrasting differences in whole-colour patterns, as seen by each of the relevant viewers, using the disparity index of Endler & Mielke (2005). As noted earlier, this method is far

more sophisticated than techniques used previously to study guppy colour pattern evolution, i.e. measures of the size, number and areal coverage of individual colour patches; Endler (1978, 1980, 1983), and we employ it as the primary analytic tool in this study. However, we also perform more 'traditional' analyses of spot size and number in an effort to pinpoint the specific source and direction of population differences, and to see whether and how the disparity approach allows for unique explanatory insights into the evolution of colour patterns.

MATERIAL AND METHODS

FISH SAMPLING

Fish were collected from both Aripo sites (Universal Transverse Mercator Grid, Zone 20 coordinates: PS 939 802, PS 937 803) and from the Petite Marianne river (PS 862 916) in February 2003 (by G.F.G.), and from the Quare river in January 2002 (by D. N. R.). All of the laboratory stocks were set up identically, and maintained in the laboratory for several generations prior to measurement (in May/June 2005). We began with 20–25 wild-caught, adult females. Because adult females store sperm, and are generally multiply inseminated, they produce many litters of young when taken out of the field and kept in isolation. We used equal numbers of offspring from each female to set up our open stocks and maintain them in large numbers in multiple 40-L aquaria. All of this was intended to sustain a high level of genetic diversity and to minimize bias in holding conditions prior to colour measurement.

MEASUREMENT AND SUMMARY ANALYSIS OF COLOUR

Reflectance spectrometry

We measured the reflectance characteristics of individual colour patches (Fig. 1) using the 'beam method' of spectrometry (Endler, 1990: fig. 6a). We used an Ocean Optics USB-2000 spectrometer with the detector situated overhead and focused (Ocean Optics 74-UV lens) to sample from a circular 1 mm region. Illumination was provided at 45° to one side of the fish using a PX-2 pulsed xenon light source fitted with a 400 µm fibre-optic cable. The light beam was focused to coincide with the area of spectrometric capture at the sample surface using a 74-UV lens. The PX-2 light source has adequate output across the 'entire' visually-relevant range of 300–700 nm. Each fish was removed from its holding tank and anaesthetized using ethyl 3-aminobenzoate methane sulfonic acid salt (MS-222) then placed on a moistened piece of matte black cardboard on a dual axis goniometer. The goniometer was positioned so that the spectrometer probe was sampling from the approxi-

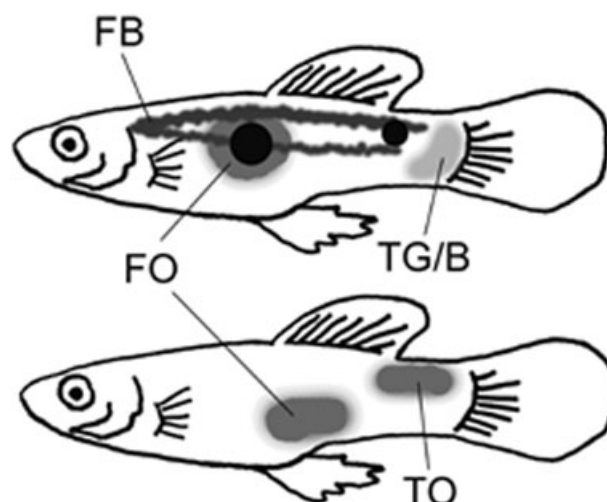


Figure 1. Guppy schematic diagrams showing the approximate location of the various colour pattern elements quantified using digital photography and spectrometry. All colour elements are labelled except for the black spots in the top panel. FB, fuzzy black; FO, flank orange; TG/B, tail green/blue; TO, tail orange. Other iridescent coloration (aside from tail green/blue) was present across a highly variable area of most of the body, and is not indicated. Colour elements such as the fuzzy black and black and orange spots were also highly variable in location and size. Not all elements were present in all fish.

mate centre of the colour patch of interest. Micro-adjustments were made to the height of the stage so that the working distance from detector to sample was controlled, and alignment of the light source and detector beams at the sample surface was confirmed prior to all measurements by directing a laser beam down the detector fibre.

The iridescent blue, green violet and UV colours are (by definition) highly directional in their appearance, which means that their brightness, chroma and/or hue vary according to the angle from which they're viewed. There can be up to a 14-fold difference in reflectance of some guppy structural coloration over an angular range of 40° (J.A. Endler, pers. comm., 1990). Measurements of these colours from a fixed arbitrary viewing angle are therefore unlikely to provide an accurate representation; what is required are multiple measures, or at the very least, a measure of peak brightness/chroma [refer also to the work of Kemp & Rutowski (2007) regarding iridescent butterfly coloration]. We therefore rotated each sample less than 10° along two orthogonal axes in order to locate the orientation conducive to maximum brightness of the colour peaks of interest. In the case of the blue and violet patches, we endeavored to maximize the height of the roughly unimodal reflectance peak that

occurred in the short wavelengths (300–450 nm). For the ‘orange’ spots, we attempted to maximize the height of the short wave (300–450 nm) and long wave (500–700 nm) reflectance, while simultaneously minimizing mid-wave reflectance. In the case of the iridescent blue/green immediately adjacent to the tail of the fish, we endeavored to maximize the height of the mid-wave peak (450–500 nm). Via extensive preliminary measurements we determined that an angular range of 10° (i.e. ±5° from normal) was always sufficient to locate these maxima. The brightness of longer wavelengths (i.e. > 500 nm) varied little with these minor changes in orientation, as would be expected because this reflectance is diffuse and modified primarily (among individuals) by the presence of pigments (Grether *et al.*, 2005). Although our approach meant that the angle of the sample surface relative to the detector varied slightly (±5°) among scans and from the angle used to calibrate against the white standard, our micro-adjustments reduced any error variation due to misalignment of the beams at the sample surface. When checked against the white standard, the range of sample incidence angles we used results in no more than 5% brightness reduction from 100% reflectance. This suggests that any negative effect introduced by varying the sample angle will be outweighed by the advantage of measuring the iridescent colour from its orientation of ‘peak’ reflectance. However, we also note that a single measurement is insufficient to characterize the full angle-dependency of these colours. More complex future analyses may incorporate multiple measurements to characterize the full range of hue, chroma and brightness variation that occurs with changes in viewing/illumination angle.

We measured all non-black colour patches (approximately > 2 mm diameter) on the right flank, then reanaesthetized the fish for approximately 1 min in the MS-222 solution before measuring the left side. The spectrometer was recalibrated against a Magnesium Oxide standard after every three fish; in all cases, drift from the prior calibration was seen to be less than 5%. Glare from the wet surface of the fish was evident at some orientations as a dramatic and spectrally indistinct increase in reflectance. In ‘practice’ scans, we quickly learned to identify this effect and exclude it from measurements. All fish fully recovered from the measurement process within 10 min of placement back in the holding tank. Because the fish received a relatively large dose of the anaesthetic MS-222, its effects upon coloration did not begin to subside until well after all measurements were taken (thus minimizing any bias due to differential exposure). We also randomly chose five fish and remeasured all their colour patches completely anew to estimate measurement error.

SPECTRAL ANALYSIS

Reflectance data were averaged for specific colour patches across both sides of the fish and then converted into guppy- and predator-specific visual cone excitation estimates (E_j) according to the equation:

$$E_j = (K_j P_j)^n / [(K_j P_j)^n + 1] \quad (1)$$

where E_j is the excitation level of cone class j , expressed as a proportion of the maximum receptor voltage, P_j is the photon catch for cone class j (Endler, 1990; Endler & Mielke, 2005) and K_j is the reciprocal of the photon catch required to produce a half-maximal excitation in cones of class j (Chittka, 1992). Exponent n was set to 1.0, *sensu* Chittka (1992). These cone excitation estimates were subsequently used to calculate colour patch luminance and chroma, and to compare entire colour pattern differences.

Estimates of photon catch (P_j) require data on the visual sensitivities of the viewer, which can be estimated from knowledge of the absorbance function for each of the viewer’s photoreceptors, which in turn is modeled as a visual pigment absorbance template (Govardovskii *et al.*, 2000). Absorbance functions were calculated using the formulae of Govardovskii *et al.* (2000), assuming that guppies possess A1 (rhodopsin) visual pigments (Archer & Lythgoe, 1990), and their predators possess A2 (porphyropsin) pigments, as is more usual for freshwater fish (Archer & Lythgoe, 1990). Our principal results were identical (at $P < 0.05$) regardless of whether A1 or A2 based pigments were used for the predators. For these calculations, we defined the peak sensitivities (i.e. λ_{\max}) of guppy visual pigments as the published values of 389, 410, 465, and 543 nm (Archer & Lythgoe, 1990). For *R. hartii*, we estimated the peak cone sensitivities as 410, 511, and 566 nm, based upon an extrapolation of Levine & MacNichol’s (1979) cyprinodontid visual sensitivity data. For *A. pulcher*, we used peak cone sensitivity estimates of 453, 530 and 570 nm (Kröger, Bowmaker & Wagner, 1999) and, for the freshwater prawn, *M. crenulatum*, we used λ_{\max} values of 350, 440, and 500 nm (Endler, 1991). Based on these data, and available absorbance templates (Govardovskii *et al.*, 2000), we estimated the visual sensitivities of guppies and their predators as illustrated in Figure 2.

We calculated photon catch using an ‘open-cloudy’ irradiance spectrum, which would approximate the spectrum of ambient light under an open canopy or under overcast conditions (Endler, 1993a). We obtained similar results (at $P < 0.05$) using any combination of Endler’s (1993a) other light environments; these data are omitted for brevity.

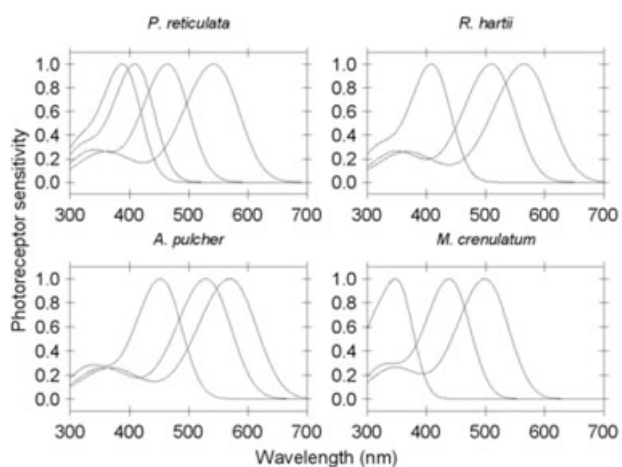


Figure 2. Photoreceptor absorption spectra for guppies and their three relevant predators, as used to model the appearance of guppy ornamentation. Curves were constructed using literature-derived values for photoreceptor peaks and using the visual pigment absorbance templates of Govardovskii *et al.* (2000).

PHOTOGRAPHY

Following spectral measurement, we photographed each side of the fish against the same matte black background using an Olympus C-755 digital camera situated at 90° to the lateral plane of the flank (refer to the representative images in Fig. 3). Illumination was provided at 45° to the top of the fish by a tungsten-halogen fibre-optic light source, which has relatively little output below 400 nm, but is appropriate for all colour patches of interest here. Digital images were subsequently imported into the analysis package Scion Image (available at <http://www.scioncorp.com>), and the areal coverage of orange, black and iridescent colour calculated using a distance scale included in each image. We also counted the number of discrete orange and black spots on the body of each fish, but did not assess fin coloration, which proved less amenable to spectrometry. Colour patch parameters were strongly correlated across the right and left sides of individual fish [Pearson's r -values: the number (0.901) and size (0.927) of orange spots, the number (0.675) and size

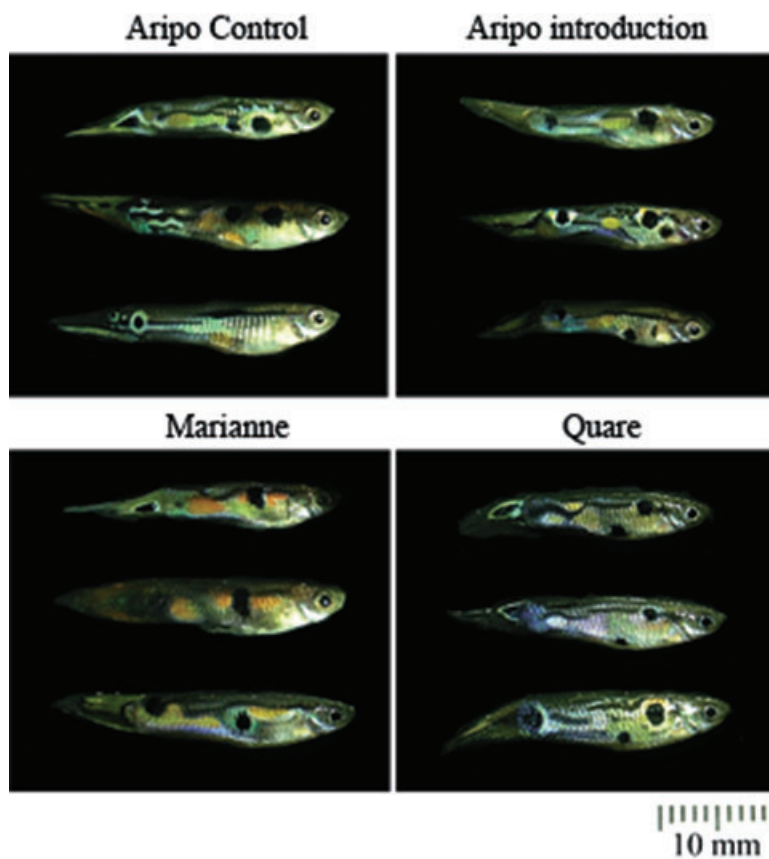


Figure 3. Representative photographs of guppies from the four studied populations, as used to quantify the number and size of spots, and areal coverage of fuzzy black and iridescence.

(0.924) of black spots, and the areas of fuzzy black (0.805) and iridescence (0.783)], and we averaged them. In the case of black spot number, there was among-population variation in the strength of the left- versus right-side correlation (Aripo control: 0.702; Aripo introduction: 0.805; Marianne: 0.900; Quare: 0.651). Measurements were made by D.J.K. who was blind with respect to population.

STATISTICAL ANALYSIS

Colour analysis

We assessed population differences in male ornamentation both from the point of view of entire colour patterns and of specific elements (e.g. the number, size and reflectance characteristics of discrete colour spots). We analysed differences in entire colour patterns between pairs of populations using LSED-MRPP (Endler & Mielke, 2005), which is a nonparametric multivariate approach broadly equivalent to a hierarchical (nested) analysis of variance. This program compares the means, variances and distribution shapes of aggregate sets of colour spectra (i.e. colour patterns) and generates an effect size measure called the 'disparity index' (d). Greater disparity values indicate larger between-group differences in visual appearance, and the index is accompanied by a P -value for statistical significance. This analytic approach is most appropriate for comparing relative photon capture values that are expressed as coordinates in tetrahedral space; hence, we used tetrahedral x , y , and z coordinates for the reflectance spectra of individual colour elements based upon cone excitations reported by Chittka (1992); see also Endler & Mielke (2005). We ran the disparity analysis separately using data derived for the guppy visual system and those of their most important visually-hunting predators for which appropriate visual data are available (Fig. 2). Given that LSED-MRPP also accounts for differences due to the relative size (or areal coverage) of each colour pattern element, we represented each spectrum in each individual's data set according to that colour element's proportional coverage, as assessed via the areal measurements. We did this by entering multiple data points for each colour element such that the number of multiple additions was proportional to relative patch area. Because we did not measure the 'background' colours of each guppy (i.e. the head, eyes, gills, fins, etc.), we 'mapped' the arrangement of measured colour spots onto an assumed achromatic (i.e. tetrahedral coordinates: $x = 0$, $y = 0$, $z = 0$) background, which is analytically indistinguishable from the black and fuzzy black markings (in terms of tetrahedral colour, but not brightness). This means that

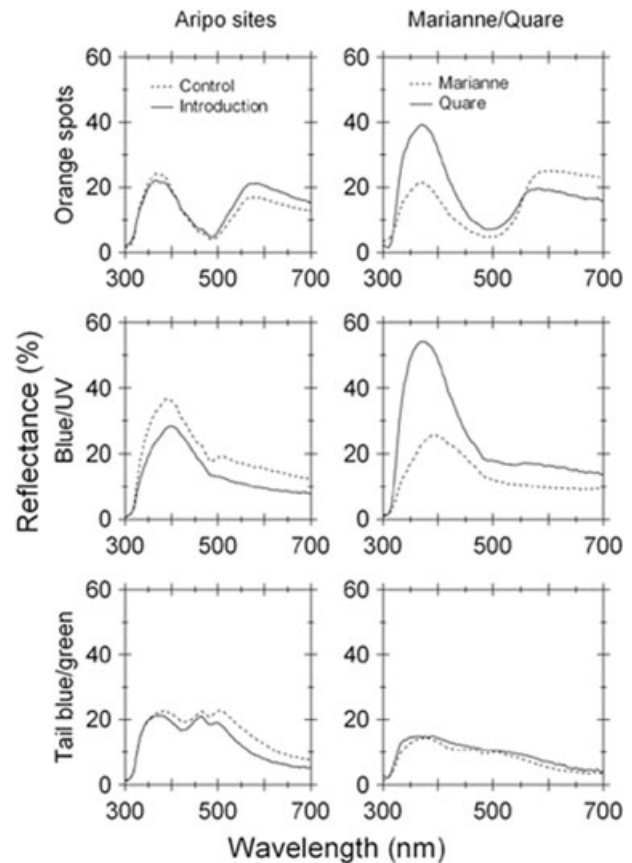


Figure 4. Reflectance scans of three common guppy colour patch elements, averaged for each population: orange spots ('FO' in Fig. 1), blue/ultraviolet and tail green/blue (TG/B in Fig. 1). Reflectance of tail orange ('TO' in Fig. 1) was indistinguishable from flank orange, and is omitted here for brevity. UV, ultraviolet.

the disparity analysis accounts primarily for differences stemming from the non-black colour elements. We ran two separate disparity analyses, the first dealing with differences in 'colour' (i.e. hue and chroma: the 'shape' of spectral curves; Fig. 4), and the second dealing with differences in overall luminance (brightness).

We supplemented the disparity analysis of overall colour pattern differences (which provided the primary test of our predictions) with more specific analyses aiming to identify the sources of variation among populations. Here, we compared the number and size of black and orange spots, and the areal coverage of iridescence and fuzzy black, using independent t -tests. All of these parameters were normally distributed ($P < 0.05$) except for the areal coverage of fuzzy black, which was normalized using the log transformation [the results proved to be identical ($P < 0.05$) to those obtained using the

nonparametric Mann–Whitney test]. For simplicity in the analysis of areal parameters we grouped all colours of structural provenance (i.e. blue, violet, and bluish–green) as ‘iridescent’ coloration. We analysed each parameter separately, because we were interested in exploring differences at the level of individual colour elements. There was also significant intercorrelation among parameters [e.g. iridescence was negatively correlated with black spot size ($r = -0.42$, $N = 90$, $P < 0.001$), the area of fuzzy black ($r = -0.30$, $N = 90$, $P < 0.005$), and the number of orange spots ($r = -0.39$, $N = 90$, $P < 0.001$)]. The full nature of these relationships and their implications for sexual selection are to be explored elsewhere.

We also analysed population differences in luminance and chromaticity separately for each of the most common non-black colour patches. Luminance as perceived by guppies (and their predators) was estimated as the summed excitation values (ΣE_j) for all visual cones. Chromaticity was estimated as the Euclidean distance between the position of each colour patch in tetrahedral colour space and the achromatic centre of the colour space (i.e. x , y , and $z = 0$; Endler & Mielke, 2005). Colour data are known to often violate the assumptions of parametric statistics (Endler & Mielke, 2005); in our case, we found evidence for departures from normality (and homoscedasticity) only among the tetrahedral chroma data. We therefore use nonparametric Mann–Whitney tests to evaluate population chromaticity differences. We also estimated the repeatability of these parameters by comparing the first and second measurements across the five fish that were measured twice. With data from all colour patches pooled (flank blue/UV, flank and tail orange, tail green/blue), correlations were high for luminance ($r = 0.896$) and chromaticity ($r = 0.753$). For the luminance of individual colour patterns, repeatability was moderately high for flank blue/UV ($r = 0.652$), moderate for flank and tail orange ($r = 0.452$), but weak for tail blue/green ($r = 0.066$). Repeatability of chromaticity estimates was very high for flank blue/UV (0.905) and tail green/blue ($r = 0.989$), but low for flank and tail orange ($r = 0.169$). These correlations are for the guppy visual system under an open/cloudy light environment, but are very similar to those obtained for other viewers and light environments. They suggest caution in the appraisal of luminance estimates for tail blue/green and chromaticity estimates for the orange markings.

In all of these analyses, we also calculated a sample size-independent measure of effect size, d , as the difference between the two means expressed as a proportion of the respective grand mean, which is used to interpret the strength of population differences among our data.

RESULTS

COLOUR PATCH SPECTRAL CHARACTERISTICS

Mean reflectance scans for the three most common non-black colour elements (flank blue/UV, flank orange and tail green/blue) are presented in Figure 4. All elements exhibited relatively strong reflectance in the UV wavelengths, and characteristic shapes in the ‘human visible’ range (i.e. 400–700 nm), which determine their appearance to a human observer. In the case of the flank markings (blue/UV and orange), there were also strong unimodal peaks in the 350–400 nm range which changed depending upon the viewing angle (i.e. this reflectance was ‘iridescent’). The position of these reflectance peaks corresponds well with the shortest-wave guppy cone at 389 nm, which would maximize their guppy-perceived luminance. Of all studied populations, Quare fish exhibited the highest short-wave reflectance peaks (i.e. highest luminance), whereas Marianne and Aripo introduction fish exhibited characteristically low reflectance in this range. The relatively low UV peaks in Marianne fish colours are notable given that this population is the only one to co-exist with a strongly UV-sensitive predator (*M. crenulatum*). Tail blue/green, which was also iridescent, was most variable in spectral shape among populations, with tri-modal reflectance peaks observed in the Aripo populations, and a single short-wave peak in the Marianne and Quare.

FORMAL AMONG-POPULATION CONTRASTS

Aripo control versus Aripo introduction

Disparity analyses of overall colour patterns indicated population differences ($P < 0.005$) in both colour (hue/chroma) and luminance, as seen by all viewers (Fig. 5A, B). This analysis suggested that population differences in colour, and to a lesser extent, luminance, would be most apparent to both *R. hartii* and *A. pulcher*, and then to *P. reticulata*. Closer examination of the source of these differences indicated that introduction site fish possessed larger orange spots, but the two populations were otherwise indistinguishable in terms of spot size and number and the areal coverage of fuzzy black and iridescence (Table 1). Analyses of colour patch luminance and chromaticity (Table 2) indicated brighter flank blue/UV in control fish, as viewed by *R. hartii* and *A. pulcher*, but not *P. reticulata*. This suggests that population differences in overall colour pattern (Fig. 5A, B) are primarily driven by the introduction fish having larger orange spots, which would be visible to all viewers, and duller blue/UV markings, which would be most apparent to *A. pulcher* and *R. hartii* (and which runs counter to our initial predictions; see Discussion).

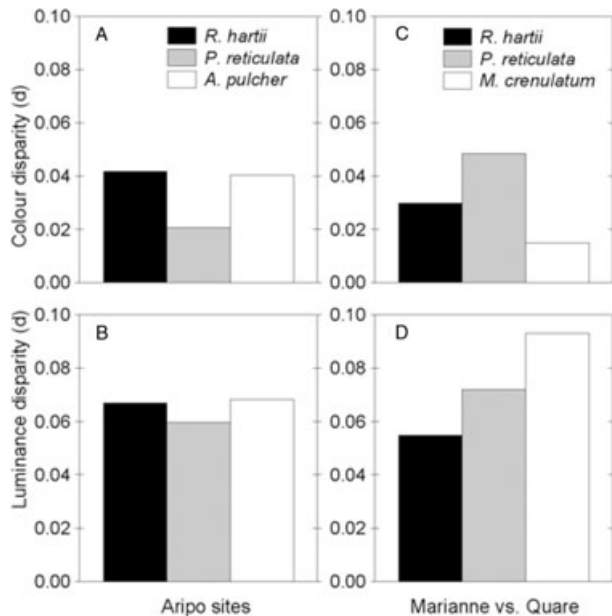


Figure 5. Results of the disparity analyses for whole colour pattern differences in each of the two specific two-population contrasts. A, B, contrast between the Aripo control and introduction populations. C, D, contrast between the Marianne and Quare populations. The *y*-axes depict *d*-values for the size of colour pattern differences between each of the two populations, as viewed by guppies and each of their relevant predators. All contrasts were performed for Endler's (1993a) open/cloudy ambient light environment.

Marianne versus Quare

As with the Aripo contrast, disparity analysis indicated significant differences ($P < 0.005$) between the Marianne fish and the Quare fish in both colour (hue/chroma), and luminance, as seen by all viewers (Fig. 5C, D). In terms of whole colour pattern hue/chroma (Fig. 5C), differences between the two populations were clearly largest as viewed by *P. reticulata*, followed by *R. hartii* and *M. crenulatum*. The notable finding here is the lack of a relatively large difference as viewed by *M. crenulatum*, given our a-priori prediction that guppies should differ most markedly as viewed by this potential predator (which is found only at the Marianne site). In terms of whole colour pattern luminance, however, the observed pattern of differences was consistent with initial prediction (i.e. differences as viewed by *M. crenulatum* are greater than differences as viewed by *P. reticulata*; Fig. 5D). To determine the source of these differences, and their direction with respect to perceived conspicuousness, we ran more specific analysis of the number, size, aerial coverage and spectral characteristics of individual colour elements. These analyses revealed several interesting differences (Tables 1, 2, 3). First,

Marianne fish possessed larger black spots and less extensive iridescent markings than Quare fish (Table 1). Second, the luminance of flank orange and blue/UV markings was lower in Marianne fish (as seen by all viewers), with reasonable effect sizes for these differences ($0.295 < |d| < 0.469$; Table 3). Third, the chromaticity of flank orange was greater in Marianne fish, only as viewed by *R. hartii*, and the chromaticity of blue/UV was greater as viewed by *M. crenulatum* (Table 3). Although highly statistically significant, however, the two latter effects were relatively small ($0.030 < |d| < 0.097$; Table 3), especially in relation to the observed luminance effects. The overall differences between Marianne and Quare fish (Fig. 5C, D) therefore appear to be driven by the Marianne fish having larger black spots, less extensive iridescent markings and duller flank orange and blue/UV colour patches reflect significant UV (Fig. 4), these results are consistent with the signature of predation in the Marianne due to the UV-sensitive prawn (*Macrobrachium*).

DISCUSSION

Traditional analyses of animal colour patterns have simply scored the number, position and/or size of specific colour patch elements (Endler, 1978; Whitfield, 1986; Wiernasz, 1989; Gustafsson, Qvarnstrom & Sheldon, 1995). However, although this approach underpins a large research program into the evolution of male ornamentation and female mating biases (for guppies, see Houde, 1997; Magurran, 2005), it glosses over considerable detail. Discrete colour patches, such as those due to orange-red carotenoid pigment coloration, vary considerably in the amount of light reflected, both overall and in specific wavelengths (i.e. they vary in brightness, chroma and hue; Grether *et al.*, 2005). The same colour patch will also appear differently depending upon viewing conditions (Endler, 1991). Because these factors will influence the nature and strength of sexual and natural selection (Endler *et al.*, 2005), there is considerable scope for researchers to gain enhanced resolution in explaining colour patch evolution. In the present study, we applied more sophisticated methods of colour measurement and analysis to shed light on patterns of geographic variation in male guppy ornamentation. Our findings revealed that measuring the spectral reflectance of colour patches (Fig. 4) provides unique insights regarding the differences in overall colour pattern as seen by specific viewers (Fig. 5). To use the Marianne versus Quare contrast as an example, whereas 'traditional' analyses revealed that Quare fish differ only by having more iridescence and smaller black spots, our analyses revealed additional

Table 1. Descriptive statistics and planned among-population contrasts for each colour parameter

Parameter	Contrast 1: Aripo sites			Contrast 2: Marianne/Quare		
	Control (<i>N</i> = 10)	Introduction (<i>N</i> = 7)	Contrast	Marianne (<i>N</i> = 10)	Quare (<i>N</i> = 10)	Contrast
Number of black spots	4.30 ± 0.62	4.14 ± 0.59	<i>t</i> ₁₅ = 0.205, <i>P</i> = 0.838 (<i>d</i> = 0.037)	3.40 ± 0.31	4.70 ± 0.50	<i>t</i> ₁₈ = 1.873, <i>P</i> = 0.070 (<i>d</i> = -0.321)
Number of orange spots	4.50 ± 0.56	3.86 ± 0.63	<i>t</i> ₁₅ = 0.769, <i>P</i> = 0.447 (<i>d</i> = 0.152)	5.80 ± 0.33	5.00 ± 0.67	<i>t</i> ₁₈ = 1.054, <i>P</i> = 0.299 (<i>d</i> = 0.148)
Size of black spots (mm ²)	1.47 ± 0.22 (2.69 ± 0.39%)	1.23 ± 0.19 (2.54 ± 0.38%)	<i>t</i> ₁₅ = 0.062, <i>P</i> = 0.951 (<i>d</i> = 0.012)	3.06 ± 0.27 (4.76 ± 0.31%)	1.39 ± 0.14 (2.42 ± 0.20%)	<i>t</i>₁₈ = 5.347, <i>P</i> < 0.0001 (<i>d</i> = 0.682)
Size of orange spots (mm ²)	1.57 ± 0.18 (2.85 ± 0.30%)	2.55 ± 0.83 (5.17 ± 1.45%)	<i>T</i>₁₅ = 2.095, <i>P</i> < 0.05 (<i>d</i> = -0.645)	3.81 ± 0.61 (5.83 ± 0.74%)	2.43 ± 0.32 (4.27 ± 0.52%)	<i>t</i> ₁₈ = 1.428, <i>P</i> = 0.163 (<i>d</i> = 0.321)
Area of fuzzy black (mm ²)	4.12 ± 0.65 (8.05 ± 1.45%)	2.81 ± 0.52 (5.87 ± 1.05%)	<i>T</i> ₁₅ = 0.925, <i>P</i> = 0.362 (<i>d</i> = 0.286)	4.11 ± 0.87 (6.50 ± 1.23%)	4.78 ± 0.73 (8.14 ± 1.08%)	<i>t</i> ₁₈ = 0.850, <i>P</i> = 0.402 (<i>d</i> = -0.207)
Area of iridescence (mm ²)	11.44 ± 1.14 (20.83 ± 1.32%)	8.24 ± 0.77 (17.27 ± 1.33%)	<i>T</i> ₁₅ = 0.986, <i>P</i> = 0.331 (<i>d</i> = 0.152)	10.29 ± 0.65 (16.31 ± 1.10%)	13.42 ± 1.83 (23.05 ± 2.62%)	<i>t</i>₁₈ = 2.919, <i>P</i> < 0.01 (<i>d</i> = -0.412)

Data are the means ± SE and, in the case of size measures, the proportional coverage across the fish's flank is given below in parentheses. Spot numbers are given for both sides of the fish, and areal values are per-side means. Significant contrasts are shown in bold. The *d*-values are sample size-independent effect size estimates (see text); a negative value indicates a smaller value in the first population.

Table 2. Planned contrasts of colour patch differences between the Aripo control and introduction sites

Viewer	Colour patch	Luminance	Chromaticity
<i>Poecilia reticulata</i>	Flank orange	$t_{14} = 0.811, P = 0.430$ ($d = 0.068$)	$U = 27, z = 0.325, P = 0.745$ ($d = 0.087$)
	Flank blue/UV	$t_{15} = 2.045, P = 0.059$ ($d = 0.215$)	$U = 21, z = 1.366, P = 0.172$ ($d = -0.286$)
	Tail green/blue	$t_{12} = 0.599, P = 0.560$ ($d = 0.121$)	$U = 21, z = 0.200, P = 0.841$ ($d = 0.135$)
<i>Rivulus hartii</i>	Flank orange	$t_{14} = 0.098, P = 0.923$ ($d = -0.008$)	$U = 24, z = 0.651, P = 0.515$ ($d = -0.022$)
	Flank blue/UV	$t_{15} = 2.229, P < 0.05$ ($d = 0.235$)	$U = 16, z = 1.854, P = 0.064$ ($d = -0.018$)
	Control brighter Tail green/blue	$t_{12} = 0.723, P = 0.483$ ($d = 0.147$)	$U = 18, z = 0.600, P = 0.548$ ($d = 0.017$)
<i>Aequidens pulcher</i>	Flank orange	$t_{14} = 0.519, P = 0.612$ ($d = -0.041$)	$U = 20, z = 1.085, P = 0.278$ ($d = -0.007$)
	Flank blue/UV	$t_{15} = 2.278, P < 0.05$ ($d = 0.231$)	$U = 16, z = 1.854, P = 0.064$ ($d = -0.036$)
	Control brighter Tail green/blue	$t_{12} = 0.748, P = 0.469$ ($d = 0.149$)	$U = 16, z = 0.867, P = 0.386$ ($d = -0.021$)

The d -values (parentheses) represent a sample size-independent measure of effect size, with negative values indicating brighter or more chromatic markings in the introduction site. The direction of significant effects is shown in bold. Mann–Whitney U -tests are used to evaluate the non-normally distributed chromaticity data. UV, ultraviolet.

Table 3. Planned contrasts of colour patch differences between the Marianne and Quare sites

Viewer	Colour patch	Luminance	Chromaticity
<i>Poecilia reticulata</i>	Flank orange	$t_{18} = 4.143, P < 0.001$ ($d = -0.442$)	$U = 44, z = 0.456, P = 0.650$ ($d = 0.067$)
	Quare brighter Flank blue/UV	$t_{18} = 3.343, P < 0.005$ ($d = -0.441$)	$U = 34, z = 1.209, P = 0.226$ ($d = -0.196$)
	Quare brighter Tail green/blue	$t_{13} = 0.536, P = 0.601$ ($d = -0.099$)	$U = 24, z = 0.122, P = 0.902$ ($d = -0.047$)
<i>Rivulus hartii</i>	Flank orange	$t_{18} = 2.781, P < 0.05$ ($d = -0.295$)	$U = 15, z = 2.646, P < 0.01$ ($d = 0.030$)
	Quare brighter Flank blue/UV	$t_{18} = 3.150, P < 0.01$ ($d = -0.400$)	Marianne more chromatic $U = 47, z = 0.227, P = 0.821$ ($d = 0.002$)
	Quare brighter Tail green/blue	$t_{13} = 0.588, P = 0.566$ ($d = -0.111$)	$U = 22, z = 0.367, 0.713$ ($d = -0.008$)
<i>Macrobrachium crenulatum</i>	Flank orange	$t_{18} = 4.208, P < 0.001$ ($d = -0.469$)	$U = 37, z = 0.983, P = 0.326$ ($d = 0.007$)
	Quare brighter Flank blue/UV	$t_{18} = 3.445, P < 0.005$ ($d = -0.426$)	$U = 6, z = 3.326, P < 0.001$ ($d = 0.097$)
	Quare brighter Tail green/blue	$t_{13} = 0.519, P = 0.612$ ($d = -0.095$)	Marianne more chromatic $U = 24, z = 0.122, P = 0.902$ ($d = -0.011$)

The d -values (parentheses) represent a sample size-independent measure of effect size, with negative values indicating brighter or more chromatic markings in the Quare. The direction of significant effects is shown in bold. Mann–Whitney U -tests are used to evaluate the non-normally distributed chromaticity data. UV, ultraviolet.

differences, primarily regarding the brightness of orange and blue/UV patches. Disparity analysis, which integrates all potential sources of colour pattern variation (Endler & Mielke, 2005), indicated that, although *P. reticulata* would perceive the greatest difference in overall coloration (i.e. hue & chroma), the greatest difference in colour pattern brightness would be viewed by the excluded predator, *Macrobrachium*. This detail has high potential relevance to the action of sexual and natural selection across these different guppy populations.

The disparity index of Endler & Mielke (2005) has been applied with some success to the study of avian

plumage evolution (Endler *et al.*, 2005; Heinsohn *et al.*, 2005). Because our study system has well-defined predators with well-defined visual systems (Levine & Macnichol, 1979; Archer & Lythgoe, 1990; Endler, 1991; Kröger *et al.*, 1999), we were able to formulate specific predictions based upon the sensory drive hypothesis (Endler, 1992, 1993b). These predictions were partly supported, most clearly regarding the Marianne/Quare contrast (as above). These findings could indicate the signature of prawn predation in the Marianne, particularly upon the UV-bright colour patches such as flank blue/UV and flank orange. Notably, Marianne fish did not possess more

extensive orange markings (which has been previously reported in contrasts against a range of populations; Endler, 1978; Millar *et al.*, 2006); instead, we found qualitative differences in the reflectance characteristics of the orange patches among these two populations. Because Endler (1978) and Millar *et al.* (2006) utilized multiple population contrasts, this discrepancy may indicate a difference specific to the single Marianne population studied here. We also found that Marianne fish possessed orange spots that reflect less short-wave (UV) light and relatively more long-wave light (Fig. 4). Because prawns see well into the UV but less well in longer wavelengths, this is exactly the type of population difference expected if prawn predation were selecting significantly upon colour patch brightness (see Fig. 4 with reference to the differences in visual sensitivity of guppies versus prawns, as shown in Fig. 2). This suggests that prawn predation has led to Marianne fish evolving orange spots that would appear less conspicuous to prawns, rather than leading to a reduction in orange spot size (as previously reported).

Remaining with the Marianne/Quare contrast, it was interesting that the disparity results based upon 'colour' itself (i.e. hue and chroma) did not proceed as predicted, with population differences actually smallest as perceived by the prawn. This could be indicative of differences between the predatory prawn and other viewers in the relative importance they place upon luminance- versus colour-based visual information. Work on the relative utility of luminance versus colour in animal vision has suggested that luminance contrast may be more relevant to the detection of movement (Persons *et al.*, 1999). Other studies suggest that colour and brightness information are at least processed separately and contribute additively to stimulus detection (Fleishman & Persons, 2001). On these grounds, it is therefore possible that prawns, as ambush hunters who detect animal prey primarily via movement, rely more heavily upon information contained in the brightness channel (i.e. the brightness contrast between guppies and their background). Reductions in the bright UV flank markings of Marianne guppies would reduce the likely brightness contrast against freshwater stream backgrounds typically low in UV (Endler, 1978). Furthermore, because the structural UV markings are highly iridescent, peak brightness (as measured in the present study) provides a proxy measure of within-colour patch brightness contrast (i.e. changes in the brightness of individual colour patches as the guppy switches between orientations when the iridescence is visible versus when it is not). Guppies with brighter iridescent markings will therefore be more detectable to a stationary predatory prawn cueing to movement (especially if prawns are also likely to be

operating well into the twilight), which may explain why colour patch luminosity (brightness), not colour *per se*, was most reduced in Marianne fish.

In comparison with the Marianne/Quare, the most obvious finding with regard to the Aripo contrast was the relative dearth of individual colour pattern differences (Tables 1, 2, 3), which may reflect the fact that the excluded predator, *A. pulcher*, does not pose great danger to guppies (Endler, 1978). Nevertheless, it is notable that disparity effect sizes for colour and brightness differences were similar in magnitude across each of the paired-population contrasts (Fig. 5). The high Aripo disparity values could have been driven by differences in colour patch hue, a component of colour which we did not examine separately (Tables 2, 3); however, appraisal of the population mean reflectance scans (Fig. 4) suggests otherwise. Given that disparity integrates differences in means, variances and distribution shapes of aggregate sets of colour spectra (i.e. colour patterns), this result emphasizes how overall colour pattern differences may be considerably greater than suggested by the sum of an incomplete set of individual contrasts (as is traditionally undertaken in studies of colour evolution). In any event, the Aripo population differences in overall brightness and (particularly) colour were larger as likely viewed by *A. pulcher* than as viewed by guppies, which agrees with our original prediction. Because the only revealed individual differences are for introduction fish to possess larger orange spots but less bright flank blue/UV, it is difficult to say whether introduction fish have evolved in the predicted direction of increased visual conspicuousness. Disparity analyses appraising the difference between fish and their visual background would be ultimately required to shed light on this issue (see below).

Although our present approach to measuring coloration and contrasting population differences is an advance upon prior methods, an increasingly accurate appraisal of the effects of natural and sexual selection would require several additional layers of information. From a perceptual perspective, the most important present omission concerns data on the visual backgrounds at each site. Without such data, one cannot directly assess actual conspicuousness as seen by specific viewers but, instead, one must make the simplifying assumption that brighter and more chromatic colour patches are more conspicuous [which is at least qualitatively likely, see Endler (1978) as well as our earlier arguments pertaining to bright UV-rich structural colour]. If the average visual background against which guppies are usually seen (either by other guppies or by predators) varies among sites, then this might contribute to differences in male ornamentation, above and beyond any differences in

predation (or sexual selection; see below). Second, from the point of view of natural selection, we lack quantitative estimates of the relative danger of each predator, which compels the assumption that all predators present at each site are equally dangerous to guppies. Also along these lines, our analysis does not account for other visual predators, although Endler (1978) argues convincingly for the lack of any additionally relevant source of diurnal guppy predation in our study populations. Third, from the point of view of sexual selection, researchers have demonstrated variation among populations in the ornamental target(s) of female preference (Endler & Houde, 1995). These preferences have a genetic basis (Houde, 1988; Brooks & Endler, 2001b) and could stem from among-population differences in female visual sensitivity. The strength and nature of sexual selection may therefore vary among populations, especially more the genetically disparate ones (such as the Marianne and Quare), which may in turn drive inter-population differences in ornamental colour.

In conclusion, the present study has indicated how more detailed measurement and analysis of coloration can yield insights not provided by traditional assays of colour patch size and number. Spectrometric measurements indicated a strong UV component of reflectance, even in colour patches previously considered 'orange' by the human visual system (see also White, Partridge & Church, 2003). For example, the orange markings of Quare fish contain more UV than orange reflectance (Fig. 4), which emphasizes the complexity that has been largely ignored in prior analyses. Our results also indicate how such information is relevant to our understanding of colour pattern evolution. We have shown how inter-population differences in colour appear differently to depending upon the viewer's visual system, which implicated prawn predation in the Marianne as having most significant effects upon whole colour pattern luminance (brightness). This suggests that predation need not only act upon the size of conspicuous colour patches, as previously reported (Endler, 1978; Millar *et al.*, 2006), but also upon their spectral properties. Future work should incorporate the multiple axes along which colour traits may vary, and would ideally include additionally-relevant information (e.g. background spectra) for a truly holistic appraisal of colour pattern evolution, *sensu* Endler *et al.* (2005).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Luminance (summed cone excitations) and chromaticity (tetrahedral chroma) of colour patch elements as viewed by guppies and their predators under open/cloudy conditions.

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