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Predicting the direction of ornament evolution in Trinidadian guppies (*Poecilia reticulata*)

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Sexual selection is thought to be opposed by natural selection such that ornamental traits express a balance between these two antagonistic influences. Phenotypic variation among populations may indicate local shifts in this balance, or that different stable ‘solutions’ are possible, but testing these alternatives presents a major challenge. In the guppy (*Poecilia reticulata*), a small freshwater fish with male-limited ornamental coloration, these issues can be addressed by transplanting fish among sites of varying predation pressure, thus effectively manipulating the strength and nature of natural selection. Here, we contrast the evolutionary outcome of two such introductions conducted in the Trinidadian El Cedro and Aripo Rivers. We use sophisticated colour appraisal methods that account for full spectrum colour variation and which incorporate the very latest visual sensitivity data for guppies and their predators. Our data indicate that ornamentation evolved along different trajectories: whereas Aripo males evolved more numerous and/or larger orange, black and iridescent markings, El Cedro males only evolved more extensive and brighter iridescence. Examination of the El Cedro experiment also revealed little or no ornamental evolution at the control site over 29 years, which contrasts markedly with the rapid (approx. 2–3 years) changes reported for introduction populations. Finally, whole colour-pattern analysis suggested that the greatest visual difference between El Cedro introduction and control fish would be perceived by the two most salient viewers: guppies and the putatively dangerous predator *Crenicichla alta*. We discuss whether and how these evolutionary trajectories may result from founder effects, population-specific mate preferences and/or sensory drive.

Keywords: colour; iridescence; mate choice; predation; ultraviolet; vision

1. INTRODUCTION

In animals that bear exaggerated secondary sexual characters, evolutionary theory predicts that the expression of the sexual trait will represent a balance between sexually selected benefits and naturally selected costs (Fisher 1930; Endler 1980; Andersson 1994). Whereas more highly ornamented or armoured males should win out in competition for mates, and thus be favoured by sexual selection, they should also suffer increased predation or other naturally selected costs associated with developing and bearing an exaggerated phenotype. In the case of visually conspicuous ornamental traits, populations exposed to visually hunting predators (or subject to less intense sexual selection) are therefore expected to exhibit less exaggerated ornaments than populations exposed to reduced levels of predation (Endler 1980).

The interplay between natural and sexual selection in shaping sexual ornamentation can be examined correlatively (e.g. among populations experiencing different

selective regimes; Endler 1978; Millar *et al.* 2006) or, more elegantly, using introduction experiments. As part of a classic set of experiments, Endler (1980) transplanted Trinidadian guppies (a promiscuous freshwater fish in which the males exhibit polymorphic ornamental colour patterns) from a site on the Aripo River, in which they co-occurred with several visually hunting guppy predators, to an upstream location in which both guppies and their primary predators were previously excluded. The result, which was subsequently replicated under laboratory conditions, was that males in the transplanted population evolved more conspicuous ornamental coloration (estimated as an increased number and size of colour spots) in just two years, or approximately four generations (Endler 1980; refer to Reznick *et al.* 1997 for a formal estimate of generation time in low-predation environments). Because the measured colour pattern elements are putative targets of sexual selection in the study population (Endler 1980; Endler & Houde 1995; Brooks & Endler 2001), this result indicates that a reduction of visual predation pressure can rapidly alter the cost–benefit balance of ornament expression. As compelling as these data are, however, introduction experiments of this nature have rarely been employed in

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studies of sexual ornamentation, nor have such experiments been applied to other populations of guppies (although see Carvalho *et al.* 1996 and references therein). The nature of the interplay between natural and sexual selection is likely to be complex and population-specific (e.g. Pocklington & Dill 1995; Stuart-Fox *et al.* 2004; Millar *et al.* 2006); hence, considerably more remains to be learned about the factors that drive ornament evolution under a shifting natural/sexual selection balance.

Given that Endler's (1980) experiment produced rapid and significant phenotypic evolution, one point of interest is whether different guppy populations would show similar evolutionary responses following upstream transplantation; that is, whether evolution is repeatable. Guppy ornamentation represents a complex mosaic of colour patches varying in shape, size and spectral qualities, and there is extraordinary variation both within and among different populations. Ornamental differences among populations have been correlated with differences in female preference (Houde & Endler 1990; Endler & Houde 1995), and both have a strong genetic basis (Houde 1994; Grether 2000; Brooks & Endler 2001; Grether *et al.* 2005). These factors imply that the evolutionary trajectories of male ornamentation following transplantation may vary depending on the population 'starting point', in terms of male ornamentation and/or female preference (including the pattern of covariances within and among these traits; e.g. Hughes *et al.* 2005). Similarly, the nature of the change in predation following transplantation should influence among-population differences in whether and how male coloration evolves to a more exaggerated state. Guppies coexist with a suite of variously dangerous vertebrate and invertebrate predators (Endler 1978), each of which possesses a different visual system (Endler 1991; see below); hence, changes in both the overall intensity of predation and the composition of the predator community should promote characteristic changes in male coloration. Finally, changes in ambient light and transmission properties of the environment should also alter the appearance of guppy colour patterns to both other guppies and their visually hunting predators (Endler 1991), and are therefore also expected to influence their evolution (Endler 1992).

In this paper we report and contrast the outcome of a second Trinidadian guppy introduction experiment, conducted 28 years ago in the El Cedro River. The reduction in predation experienced by fish in this introduction is equivalent to that of Endler's (1980) classic Aripo experiment (see §2); hence, we first set out to compare the nature of ornamental trait evolution between these two 'replicate' introductions. Our goal here is to ask whether the direction of evolution is repeatable, given two similar experiments conducted on different stream populations. To this end, we have resurrected the coloration data from Endler's (1980) Aripo experiment and compared them directly with equivalent variables measured upon El Cedro fish. If evolution is perfectly repeatable, then El Cedro introduction fish would be expected to possess more and larger black, orange and iridescent markings than their control site counterparts (the pattern seen in the Aripo). However, given that the El Cedro introduction was conducted nearly 30 years ago, it is also

possible that fish at the control site evolved throughout the intervening time period, which would affect our ability to assess the trajectory of ornament evolution. We addressed this possibility by comparing the ornamentation of present day (2005) El Cedro control guppies with that of control site fish captured and photographed over 30 years ago (by J.A.E.).

Our second aim here is to expand considerably upon prior studies of guppy ornamentation, in which only the number and size of colour spots are typically compared, by measuring the spectral characteristics of these spots. This approach affords a more sophisticated, whole colour-pattern analysis of population differences, and allows incorporation of the visual sensitivities of guppies and their primary visually hunting predators (Endler & Mielke 2005; see below). In doing this, we draw upon the most cutting-edge estimates of guppy and predator visual sensitivity, including, for example, provision for a long-wave cone polymorphism in the guppy eye (Archer & Lythgoe 1990) and an additional short-wave cone in the eye of *Crenicichla alta* (C. J. Weadick, H. Rodd, B. Chang & E. Loew 2008, unpublished data). Our second main goal is therefore to explore what additional insights might be gained from moving beyond the traditionally simple classifications of colour spot size and number in the appraisal of guppy sexual ornamentation.

2. MATERIAL AND METHODS

(a) *Aripo introduction experiment*

The Aripo introduction experiment is fully detailed in Endler (1980). Briefly, in July 1976 a sample of 200 guppies were transplanted from a main stream site (the 'control' site), where they coexisted with the predatory cichlids *Crenicichla alta* and *Aequidens pulcher*, the characids *Hemibrycon dentatum* and *Astyanax bimaculatus*, and other species of predatory fish including *Hoplias malabaricus*, *Rhambdia sebae*, *Synbranchus marmoratus* and *Gymnotus carapo*, then introduced into a previously guppy-free tributary containing only the cyprinodont *Rivulus hartii*. Given that *Crenicichla* is a dangerous predator, whereas *Rivulus* preys mainly upon juvenile guppies (Seghers 1973; Mattingly & Butler 1994; Reznick *et al.* 1996), the change in predation experienced by introduction fish was likely to be considerable. In May 1978, 49 control and 60 introduction fish were sampled, photographed and measured for colour using the protocols detailed by Endler (1978).

(b) *El Cedro introduction experiment and fish sampling*

This introduction was performed in 1981, when one of us (D.N.R.) transplanted approximately 200 guppies from the main stream of the El Cedro River above several successive barrier waterfalls to an upstream site previously devoid of guppies and most of their predators. Fish experienced a similar change in predation as in Endler's experiment: the high predation 'control' site contained the same suite of predators as the Aripo control, and the upstream 'introduction' site contained only *R. hartii*. Studies over the subsequent 7 years demonstrated rapid life-history evolution, as also observed in the Aripo (see Reznick & Bryga 1987; Reznick *et al.* 1990, 1997); however, these fish have never been investigated for changes in ornamental coloration. Mature individuals were collected for this purpose from control

($n = 19$) and introduction ($n = 29$) sites in April 2005 and maintained in laboratory stock tanks prior to measurement four to six weeks later.

In addition to measurements of live fish, we used photographs of 21 adult males captured from the El Cedro control site by J.A.E. in 1976 to assess changes in this population from when the introduction was initially conducted (1981) to when fish were sampled for colour assessment (2005). Photography protocols were as used for Aripo fish (detailed in Endler 1978), and assessment protocols were identical to those used on 2005-sampled El Cedro fish (outlined below). However, owing to glare that sometimes obscured some areas of iridescence, we could not extract data on iridescent markings from the 1976 photographs.

(c) Measurement and summary analysis of colour

(i) Colour measurement

We quantified the coloration of live El Cedro males using digital photography and reflectance spectrometry. The techniques are fully described by Kemp *et al.* (2008) and will be outlined briefly here. Reflectance characteristics of individual colour patches were measured using an Ocean Optics USB-2000 spectrometer in which the detector was situated overhead and focused (Ocean Optics 74-UV lens) to sample a 1-mm-diameter circular region. Illumination was provided at 45° using a PX-2 pulsed xenon light source, with the light beam focused through a 72-UV lens to provide homogeneous illumination over a 5-mm-diameter circular region. Fish were anaesthetized using ethyl 3-aminobenzoate methane sulphonic acid salt (MS-222) and measured on a piece of moistened black card placed upon a multi-axis universal stage. Perfect alignment of the light source and detector beams at the sample surface was confirmed using a standard laser pointer device shone down the collector optical fibre. The spectrometer was recalibrated against a magnesium oxide standard after every three fish. Following spectrometry, we photographed both sides of each fish against a matte black background using an Olympus C-755 digital camera situated at 90° to the lateral plane of the flank. Illumination was provided at 45° from a tungsten-halogen fibre-optic light source.

(ii) Summary analysis of coloration

We averaged reflectance data for specific colour patches across the right and left sides of each individual, then calculated guppy- and predator-specific visual cone excitation estimates (E_j) using the formula

$$E_j = \frac{(K_j P_j)^n}{[(K_j P_j)^n + 1]}, \quad (2.1)$$

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 (see Endler 1990) and K_j is the reciprocal of the photon catch required to produce a half-maximal excitation in cones of class j (Chittka 1992). The value of K_j is assumed to depend upon the light spectrum to which the viewer's eyes are adapted, such that all cones reach half-maximal excitation when the receiver's eyes are illuminated by the adaptation light (Laughlin 1981). We assumed that the illumination light was the same as the adaptation light, which is realistic for the conditions under which guppies normally view each other. The exponent, n , relates to specific (but unknown) properties of each viewer's sensory system, and is set to 1.0 (as per Chittka 1992).

We subsequently used the cone excitation estimates to calculate colour patch luminance (also known as brightness, defined as the amount of light reflected from that colour patch as perceived by the viewer) and chroma (also known as colour saturation), and to compare entire colour pattern differences (see below).

The estimation of photon catch (P_j) requires data on the viewer's visual sensitivity, which in turn requires knowledge of the absorbance function and wavelength of peak sensitivity (i.e. λ_{\max}) for each of their photoreceptors. Absorbance functions were calculated using Govardovskii *et al.*'s (2000) pigment absorbance template, 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 (see citations in Archer & Lythgoe 1990). We defined the peak sensitivities (λ_{\max}) of guppy visual pigments as the published values of $U = 389$ nm, $S = 410$ nm, $M = 465$ nm and $L = 533/543/572$ nm. As explained in detail below, three values for the long-wave (L) photoreceptor were used because guppies have a known polymorphism for the position of this photoreceptor (Archer & Lythgoe 1990). After Endler (1991), we estimated the peak cone sensitivities for *R. hartii* as $S = 410$ nm, $M = 511$ nm and $L = 566$ nm, and for *C. alta* as $M = 545$ nm and $L = 606$ nm, but we also accounted for recent suggestions of a short-wave *C. alta* cone with a λ_{\max} ranging in position from 415 to 475 nm (C. J. Weadick, H. Rodd, B. Chang & E. Loew 2008, unpublished data). Finally, for *A. pulcher* we used Kröger *et al.*'s (1999) published peak cone-sensitivity estimates of $S = 453$ nm, $M = 530$ and $L = 570$ nm. We calculated photon catch using an 'open-cloudy' irradiance spectrum, which would approximate the spectrum of ambient light under both open canopy and overcast conditions (Endler 1993). We obtained similar results (at $\alpha = 0.05$) using the other light environments characterized by Endler (1993), which suggests our conclusions are robust to changes in the colour of ambient light, at least under the simplifying assumption that all cone classes contribute equally to colour and luminance perception under all environments.

From the photographs we calculated the areal coverage of orange, fuzzy black (indistinct black markings, according to Brooks & Endler 2001) and iridescent colour using SCION IMAGE (Scion Corporation; available at <http://www.scioncorp.com>). We also counted the number of discrete orange, blue and black spots on the body of each fish. Kemp *et al.* (2008) provide a schematic indicating the approximate location of all colour pattern elements, but we also note that the precise positioning of colour patches across the flank is highly variable. For simplicity we grouped all colours of putatively structural provenance (i.e. blue, violet/blue and blue/green) as 'iridescent' coloration. We did not measure colour patches on the fins and the tail fin itself, which proved less amenable to spectrometry. All measurements of El Cedro fish were made by D.J.K. blindly with respect to population.

(d) Statistical analyses

(i) Comparison of Aripo and El Cedro introduction experiments

We compared the effect of each introduction upon the number and size (area in mm²) of ornamental colour spots using a generalized linear/nonlinear modelling approach. In each model we included 'experiment' (Aripo versus El Cedro), 'site' (control versus introduction) and the experiment \times site interaction, with the latter term directly

testing whether the focal colour trait changed equivalently with response to each introduction. For analyses of spot number we specified a multinomial ordinal distribution, whereas for analyses of spot size we specified a lognormal distribution (colour patch area is lognormally distributed; see Endler 1980). Because Endler (1980) assessed iridescence by counting iridescent and blue spots, we pooled the total area of all these spots for each fish in order to compare with our areal coverage measure for iridescence in El Cedro fish. We also conducted within-experiment contrasts of population differences (i.e. control versus introduction fish, and El Cedro control fish in 1976 versus 2005) using multinomial ordinal- and lognormal-based models. In all analyses of spot size and areal coverage, we included body size (area) as a covariate in order to control for differences in mean body size between sites. As found by Endler (1978, 1980) and Kemp *et al.* (2008), the significant population differences remained in contrasts of absolute spot size and areal coverage (i.e. in a model not including body size). These analyses were conducted using STATISTICA v. 7.1.

One notable difference between the Aripo and El Cedro colour assessments was that Endler (1980) included spots on the tail fins in his assessments of Aripo fish, whereas we did not. The El Cedro data consequently underestimate the total number and coverage of discrete colour patches. We accounted for this by subtracting the tail spots from Endler's (1980) data, and therefore standardized our assessments to use spots on the body only. However, the location of size-measured spots could not be resolved from Endler's (1980) data, which means that tail spots contributed to the estimates of mean spot size in Aripo fish but not El Cedro fish.

(ii) *Spectral analyses and whole colour-pattern contrasts*

For El Cedro fish we also assessed site differences in entire guppy colour patterns using Endler & Mielke's (2005) LSED-MRPP program. This is a non-parametric multivariate approach broadly equivalent to a hierarchical (nested) analysis of variance, which in our case contrasts overall colour pattern differences between the two populations (in which the colour pattern of individual fish is nested within population). The contrast takes into account differences in means, variances and distribution shapes of aggregate sets of colour spectra (i.e. the elements that comprise an individual's 'colour pattern'). The program generates an effect size for the difference between two groups (populations), called the 'disparity index' (d ; for a comprehensive treatment of how this index is derived, the reader is referred to Endler & Mielke 2005). The method can be used to compare the visual disparity between animals and their visual backgrounds (e.g. Endler *et al.* 2005), or—as in our case—between two groups of animals. Greater disparity values indicate greater visual differences between the colour patterns of the two groups, and the index is accompanied by a p -value denoting the statistical significance of the difference. We used relative photon capture values expressed as coordinates in tetrahedral space based upon Chittka's (1992) cone excitations (as explained above; refer to Endler & Mielke 2005) and analysed luminance and colour differences separately using the data derived for the visual systems of each viewer (i.e. guppies, *C. alta*, *R. hartii* and *A. pulcher*).

The LSED-MRPP program also accounts for differences due to the relative size (or coverage) of discrete colour-pattern elements. This is achieved by weighing the data describing each

discrete colour element according to its proportional coverage across the fish's flank (as measured from the photographs). In practice, weights are applied by varying the number of times the spectral data for each element is represented in the individual's dataset. Because we did not measure 'background' colours (i.e. the head, eyes, gills, fins, etc.), and because many of these colours are largely achromatic (D. J. Kemp 2005, personal observation), we 'mapped' the arrangement of measured colour spots onto an assumed achromatic (i.e. $x = 0$; $y = 0$; $z = 0$) background, which (at least in colour terms) is analytically indistinguishable from the black spots and fuzzy black. This means that the disparity analysis accounts primarily for differences stemming from the non-black colour elements, which are most generally implicated in guppy mate choice (Endler & Houde 1995; Brooks & Endler 2001) and likely to contribute most to visual conspicuousness (Endler 1978; see §4).

In addition to the whole colour-pattern contrasts, we analysed population differences in chromaticity and luminance separately for each of the most common non-black colour patches (the black markings were too dark to accurately measure). Chromaticity, as perceived by guppies and their predators, was estimated as the Euclidean distance between the position of each colour patch in tetrahedral colour space and the achromatic 'origin' (i.e. x , y and $z = 0$; Endler & Mielke 2005). Luminance was estimated as the summed excitation values (ΣE_i) for all visual cones, so calculated because luminance contrast for stationary objects is thought to be a function of the envelope of all cone classes. However, in fish, large-scale movement detection may be mediated by the long-wave cone only (Schaerer & Neumeier 1996), whereas small objects are tracked using input from the mid-wave cone (Gehres & Neumeier 2007). Thus, luminance could be perceived via the summed output of all cone classes, or as the sum of red and green cone classes, or as the output of either individual class. This situation is further complicated for guppies, which are known to possess polymorphic long-wave cones, with absorbance peaks at 533, 543 and 572 nm (Archer & Lythgoe 1990). We accounted for this complexity by calculating luminance using each of these absorbance peaks separately and in combination with each other, with the mid-wave cone, and with all other cones, in order to bracket the possible values that luminance may take (see electronic supplementary material). Our results remained unchanged in all cases (at $\alpha = 0.05$; see §3), which suggests that the principal conclusions are robust to the idiosyncrasies of exactly how luminance may be perceived. On this basis, and in the interests of brevity, we hereafter report luminance results calculated using the sum of all cone classes.

We used Mann–Whitney tests for contrasts involving the non-normally distributed chromaticity data, and t -tests for luminance data, which proved in all cases to be normally distributed.

3. RESULTS

(a) *Long-term stability at the El Cedro control site*

The El Cedro introduction was conducted in 1981, but fish were not captured for colour measurement until 2005, some 24 years later. This means that our estimation of control site coloration is based upon the descendants of the 1981 fish. Differences between control and introduction populations in 2005 could therefore exist due to

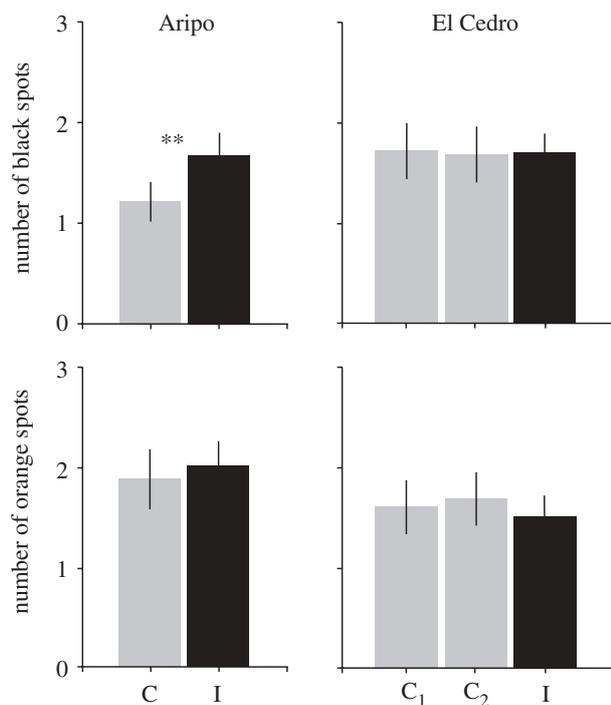


Figure 1. Mean ($\pm 95\%$ CI) spot numbers for the control sites (grey columns, 'C') and introduction sites (black columns, 'I') for each introduction experiment. El Cedro sites C₁ and C₂ represent control fish sampled in 1976 and 2005, respectively. ** $p < 0.01$.

evolution in one or both populations, but we are primarily interested in the evolutionary trajectory of introduction fish. We sought to examine whether control fish also evolved by comparing males captured from this site in 1976 versus 2005. Although slight differences in photography methods precluded a meaningful comparison of iridescence, we found no difference in either the number or size of black or orange spots ($n = 40$ for all contrasts; black spot number: $G_1 = 0.0179$, $p = 0.893$; black spot size: $G_1 = 0.0699$, $p = 0.792$; orange spot number: $G_1 = 0.113$, $p = 0.737$; orange spot size: $G_1 = 1.211$, $p = 0.271$; figures 1 and 2). Thus, at least in terms of simple black and orange spot characteristics, no detectable evolution in male ornamentation occurred at the control site in the 29 years since 1976. This validates our use of 2005 control fish to estimate the coloration of fish at this site in 1981.

(b) Comparison of the Aripo and El Cedro introductions

In these two experiments we first compared colour pattern characteristics between the El Cedro and Aripo control populations in order to address whether the two populations of 'colonizing' fish differed to begin with. Although there was no population difference in the number ($G_1 = 0.0811$, $n = 68$, $p = 0.776$) or size ($G_1 = 0.938$, $n = 33$, $p = 0.333$) of orange spots, and no difference in black spot size ($G_1 = 0.00192$, $n = 33$, $p = 0.965$), El Cedro fish possessed a greater number of black spots ($G_1 = 6.38$, $n = 68$, $p < 0.05$) and a markedly larger area of iridescence ($G_1 = 36.3$, $n = 33$, $p < 0.0001$; figures 1 and 2) than their Aripo control counterparts.

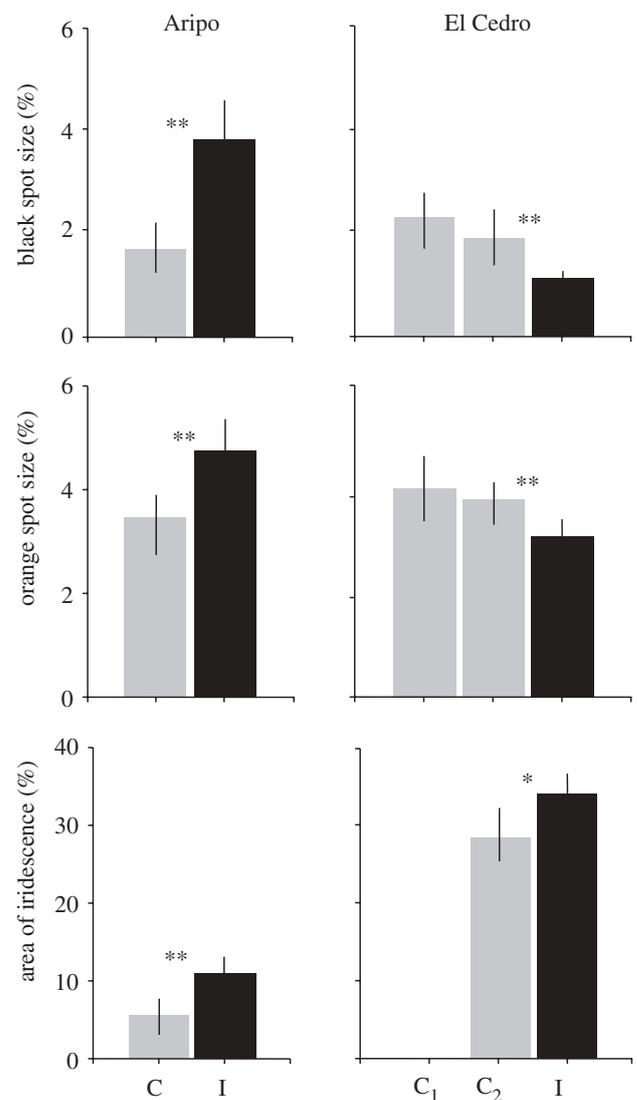


Figure 2. Mean ($\pm 95\%$ CI) spot size and iridescent area for the control sites (grey columns, 'C') and introduction sites (black columns, 'I') for each experiment. El Cedro sites C₁ and C₂ represent control fish sampled in 1976 and 2005, respectively. The values are a percentage of overall lateral body area. * $p < 0.05$; ** $p < 0.01$.

In addition to these differences in the 'starting point', there were also significant differences in the apparent evolutionary trajectories of male ornamentation following each introduction (given by the experiment \times site interaction terms in table 1). The main differences concerned the number and/or size of black and orange spots. Whereas Aripo introduction fish evolved to have more black spots, and larger black and orange spots (figures 1 and 2; electronic supplementary material, table S1; see also Endler 1980), El Cedro introduction fish did not differ from their control population in spot number (black: $G_1 = 0.207$, $n = 48$, $p = 0.649$, orange: $G_1 = 0.636$, $n = 48$, $p = 0.425$), and their spots were actually smaller than those of control fish (black: $G_1 = 7.97$, $n = 48$, $p < 0.005$, orange: $G_1 = 5.80$, $n = 48$, $p < 0.05$; figures 1 and 2). Fish in both experiments evolved greater coverage of iridescence, and El Cedro introduction fish also exhibited significantly less fuzzy black than their control counterparts ($G_1 = 13.3$, $n = 48$, $p < 0.0005$; a trait not measured for the Aripo fish). Thus, whereas Aripo introduction fish

Table 1. Generalized linear/nonlinear contrasts of the results of each introduction experiment. The interaction term formally tests whether the strength and direction of evolution in each colour parameter was equivalent across both experiments. n.a., not applicable.

colour parameter	experiment (Aripo versus El Cedro)	population (control versus introduction)	interaction	body size (covariate)
black spot number	$G_1 = 3.74, p = 0.053$	$G_1 = 6.84, p < 0.01$	$G_1 = 5.24, p < 0.05$	n.a.
orange spot number	$G_1 = 24.2, p < 0.0001$	$G_1 = 2.88, p = 0.090$	$G_1 = 3.55, p = 0.060$	n.a.
black spot size	$G_1 = 8.06, p < 0.005$	$G_1 = 7.05, p < 0.01$	$G_1 = 23.9, p < 0.0001$	$G_1 = 7.16, p < 0.01$
orange spot size	$G_1 = 3.16, p = 0.075$	$G_1 = 0.739, p = 0.390$	$G_1 = 18.3, p < 0.0001$	$G_1 = 15.8, p < 0.0001$
area of iridescence	$G_1 = 42.3, p < 0.0001$	$G_1 = 4.25, p < 0.05$	$G_1 = 1.25, p = 0.263$	$G_1 = 9.64, p < 0.005$

evolved more extensive black, orange and iridescent markings, the El Cedro introduction only promoted the evolution of more extensive iridescence. The magnitudes of the differences between colour parameters of control versus introduction fish (i.e. the 'effect sizes') were also generally greater for the Aripo population (figures 1 and 2; electronic supplementary material, table S1).

(c) *Spectral properties of male ornamentation in the El Cedro*

Reflectance spectra captured from the four most common colour pattern elements of El Cedro fish (orange spots, iridescent blue spots, blue/violet iridescence and iridescent green/blue) are represented in figure 3. All colour elements are characterized by a strong reflectance peak in the region of 370–390 nm, thus indicating a strong UV component. In perceptual terms, the largest and most consistent difference between the populations is that all viewers would perceive brighter iridescent blue/violet markings in introduction site fish ($t_{45} > 2.013, p < 0.05$; electronic supplementary material, tables S2–S4). No other luminance differences were evident, and differences in chromaticity were limited to slight increases in the orange spot and iridescent tail green/blue of introduction fish, as viewed by guppies and *Crenicichla*, respectively (Mann–Whitney $U < 173, p < 0.05$; electronic supplementary material, table S4). However, these chromaticity differences were not consistent across the various formulations of each of these viewers' visual systems (e.g. across all positions of the guppy long-wave cone polymorphism) and therefore do not present as robust findings.

(d) *Disparity analysis of entire El Cedro colour patterns*

Disparity analysis of overall colour patterns revealed significant differences between El Cedro control and introduction fish in terms of both colour (i.e. hue and chroma: $d > 0.0106, p < 0.005$) and luminance (i.e. brightness: $d > 0.0302, p < 0.0001$), as seen by all viewers (figure 4). Even given the uncertainty regarding the precise positions of photoreceptors (indicated by the hatched regions of columns in figure 4), it is clear that guppies and *C. alta* would perceive by far the greatest colour difference between populations (i.e. the colour contrast between control and introduction fish would be largest as viewed by guppies and *C. alta*). Luminance

differences would be perceived similarly across all studied viewers.

4. DISCUSSION

Introduction experiments have much to teach us about the potential rates and directions of evolution in the wild, and about how different selective factors might act in concert to shape such evolution (Endler 1980; Reznick & Bryga 1987; Reznick *et al.* 1997; Losos 2007). This approach has been used with great success to examine the evolution of life histories and ornamentation in guppies (e.g. Endler 1980; Reznick & Bryga 1987; Reznick *et al.* 1990, 1997). Here we build upon this legacy by quantifying colour evolution in an El Cedro River introduction, comparing these results with the classic Aripo experiment (Endler 1980) and appraising ornamental colour using a sophisticated, sensory processing-based approach. The results express three key features. First, the populations of fish that were used in each introduction (i.e. the control populations, which in the El Cedro case was estimated using the 2005 descendants from the 1981 population) differed in ornamental colour composition to begin with; the most obvious difference being a fivefold greater coverage of iridescence in El Cedro males (i.e. 28.5 versus 5.5% iridescent coverage in El Cedro versus Aripo control males). The extent of iridescence in El Cedro fish contrasts markedly with estimates made recently for Aripo, Marianne and Quare populations (8.2–13.4%; Kemp *et al.* 2008), which suggests that El Cedro fish are unusually exaggerated in this regard. Second, ornament exaggeration following the El Cedro introduction was evidenced primarily as an increase in the size and brightness of iridescent markings, which contrasts with the broader changes observed in the Aripo fish (figure 1). By comparing photographs of El Cedro control fish sampled in 1976 versus 2005, we also revealed striking evolutionary stability in key ornamental colour parameters, which contrasts with the cases of relatively rapid evolution seen when guppies are transplanted from high predation to reduced predation environments (e.g. Endler 1978, 1980; this study). Third, sensory processing-based contrasts of entire colour patterns suggested that the extent of ornament exaggeration in El Cedro introduction fish would be most apparent to guppies and their most salient predator, *C. alta*. We discuss these findings in turn, and in relation to prior work in guppies and other visually ornamented animals.

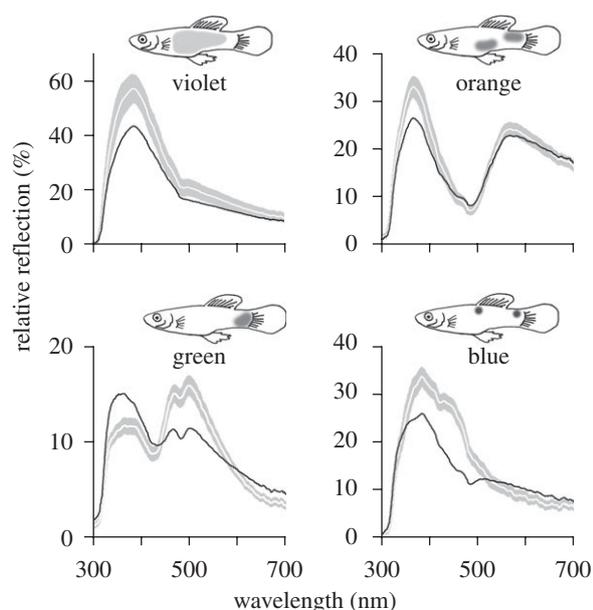


Figure 3. Reflectance spectra for the four most common measurable colour pattern elements of El Cedro males from the control (solid line) and introduction populations (greyscale bands indicating the mean \pm 1 s.e.). Error bands for the control curves are not shown to preserve clarity but are similar in magnitude to the introduction bands. The shaded regions in the guppy schematics indicates the distribution of each colour element across the flank (although the positions and extent of these markings varied considerably among individuals). In the case of the blue spots, the control site curve is based upon measurement from a single individual; $n > 17$ in all other cases.

Endler (1980) reported that, following introduction into an upstream tributary, Aripo guppies evolved more and larger orange, black and iridescent markings (figure 1), a result that was replicated in artificial stream-based experiments. Our El Cedro results differed in that introduction fish only appeared to evolve relatively more iridescence. The extent of orange and black markings (including fuzzy black) actually decreased in the introduction population (measured relative to control site fish, which exhibited no significant evolution over almost three decades). We therefore conclude that despite fish in each experiment experiencing a similar change in predator community composition, male ornamentation evolved along unique trajectories. What factors could determine this difference? One possibility is that differences in the direction of evolution were shaped by differences in the male coloration and/or mate preferences of the guppies that were introduced (i.e. the 'starting' point in terms of ornamentation and/or choice). Endler & Houde (1995) demonstrated that female preferences are based on numerous colour characters that vary extensively in strength and sign among populations. If El Cedro females collectively possess a particular penchant for bright iridescent markings then this would explain both the enriched iridescence in the control population and the subsequent evolutionary trajectory taken by introduction fish. A strong female preference for iridescent colours could also account for the observed reduction in the area of non-iridescent colours because different colours, in effect, compete for space on the bodies of male

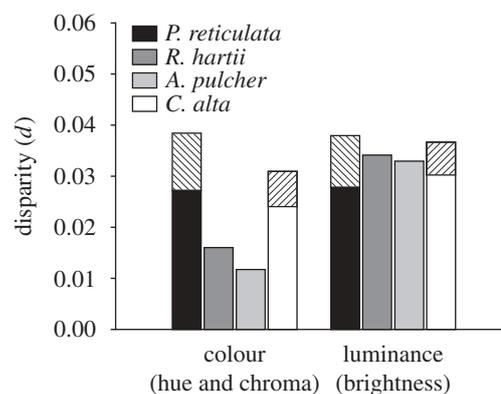


Figure 4. Visual differences between El Cedro control and introduction guppies in terms of the colour (hue and chroma) and luminance (brightness) of their entire colour pattern. Each column indicates the visual difference (i.e. 'disparity') between the two populations as perceived by a particular viewer. These values do not necessarily indicate directionality with respect to conspicuousness, as contrast measures against the visual backgrounds at each site would be ultimately required to make this judgement; they simply represent the magnitude of the visual difference between fish of each population viewed under standard lighting and against standard backgrounds. The hatched regions in the *P. reticulata* and *C. alta* columns indicate the range of values obtained under different scenarios of defining positions of polymorphic long-wave cones (*P. reticulata*: 533, 543 or 572 nm) and short-wave cones (*C. alta*: 415 or 475 nm, as explained in the text. All effects are significant ($p < 0.005$).

guppies (i.e. it would not be necessary for females to prefer smaller orange and black spots). A further, non-mutually exclusive possibility, given that individual females have specific preferences (see Brooks & Endler 2001), is the influence of genetic 'founder effects'. In this case, the evolutionary trajectory of male colour patterns could vary idiosyncratically among introduction populations according to the specific preferences of founding individuals. Such effects could also apply in the case of male ornamentation; in fact, genetic linkages between male ornamentation and female preferences (Houde & Endler 1990; Houde 1994) would see founder effects apply on both counts. Last, sensory drive theory (Endler 1992) predicts that the specifics of viewing conditions (visual backgrounds, water colour and/or ambient lighting, as affected by canopy cover, etc.) should influence colour evolution. Elucidation of these possibilities would require data on both the viewing conditions and female preferences at each site, which are presently unavailable.

Regardless of the underlying cause, the slight decrease in orange spot size in El Cedro fish is interesting because much work in guppies has focused on this element of male ornamental coloration (e.g. Kodric-Brown 1989; Houde & Endler 1990; Grether 2000; Grether *et al.* 2005). In fact, it has been suggested that a general preference for orange coloration in guppies may have arisen as a pleiotropic consequence of a pre-existing visual bias for orange-coloured food items (Rodd *et al.* 2002). Although there are other explanations (as above), our data imply that orange markings need not always be most strongly targeted by sexual selection, and that if pre-existing

biases are present and shape overall mating preferences then they may vary idiosyncratically among populations. This is consistent with Endler & Houde's (1995) finding that average female preferences differ significantly across different guppy populations, and that orange markings are not strongly favoured in all studied streams.

The second main goal of this study was to investigate further insights that can be gained by measurement of the spectral properties of the colour elements and from using a more sophisticated, visual modelling-based analysis. Reflectance spectrometry indicated that all measured colour components, including the 'orange' spots, contained strong ultraviolet–blue reflectance peaks (figure 2; see also White *et al.* 2003), which is interesting in light of guppies having two photoreceptors in this region of the spectrum (i.e. $\lambda_{\max} = 389$ and 410 nm). Ultraviolet reflectance has been shown to contribute to male attractiveness (Kodric-Brown & Johnson 2002). Analysis of spectral data indicated that El Cedro introduction fish evolved brighter iridescent violet/blue and more chromatic iridescent green/blue (figure 2), both of which are major constituents of the iridescent markings (as classified during areal assessments). Such markings are very bright and chromatic, show a high degree of colour 'flicker' (i.e. rapid changes in reflectance with subtle changes in viewing orientation; Vukusic *et al.* 2001), and are therefore likely to contrast highly against in-stream objects such as gravel, sand and vegetative debris. The colour flicker of these iridescent colours would particularly enhance the sigmoidal courtship display, which contains a long axis rotational movement as well as a vertical component. We therefore interpret our findings as demonstrating an increase in conspicuousness in the introduction population, although an assessment of background contrast would ultimately be required to quantify this increase (see below). The fact that both the extent and brightness of iridescence increased following introduction also strengthens the case for this component of male ornamentation as the focus of female preferences in the El Cedro.

Finally, our visual modelling approach allowed appraisal of whether and how changes in guppy ornamentation might be explained by the visual sensitivities of each viewer. Along these lines, we would generally expect the introduction fish to differ mostly from their control counterparts as viewed by the most relevant excluded predator—which in the El Cedro (and Aripo) is *C. alta* (Endler 1978). This is simply because if the coloration of control site guppies is constrained by *C. alta* predation then sexual selection following release from such predation would be free to promote ornament exaggeration in directions that were previously penalized. Such a signature was clearly evident in relation to colour (hue and chroma), but not luminance (figure 4). This may reflect the relative importance of colour versus brightness in determining predation risk (i.e. predation due to *C. alta* constrains the hue/chroma, but not the luminance, of guppy ornamentation at the control site). It is also notable that guppies would perceive a similar pattern of colour versus luminance differences across these two populations (figure 4), which raises the possibility that sexual selection is primarily responsible for the nature of ornamental exaggeration in the introduction population. This would be consistent with the scenario in which the direction of

evolution was prescribed by the collective set of founder female preferences. Ultimately, however, guppy colour patterns would have to be compared against the visual backgrounds at each site in order to directly appraise how conspicuousness (i.e. the inverse of crypsis) actually changed according to each viewer. An evaluation of visual backgrounds, coupled with studies of female mate preferences, offers excellent possibilities for elucidating how and why ornamental coloration responds to a shift in the balance between natural and sexual selection.

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