JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.13342

Colour pattern component phenotypic divergence can be predicted by the light environment

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Keywords:

colour patterns;
light environment;
Poecilia reticulata;
sensory drive;
vision;
visual communication.

Abstract

The sensory drive hypothesis predicts that across different light environments sexually selected colour patterns will change to increase an animal's visual communication efficiency within different habitats. This is because individuals with more efficient signal components are likely to have more successful matings and hence produce more offspring. However, how colour pattern signals change over multiple generations under different light environmental conditions has not been tested experimentally. Here, we manipulated colour pattern signal efficiency by providing different ambient light environments over multiple generations to examine whether male colour pattern components change within large replicated populations of guppies (*Poecilia reticulata*). We report that colour patches change within populations over time and are phenotypically different among our three different light environments. Visual modelling suggests that the majority of these changes can be understood by considering the chroma, hue and luminance of each colour patch as seen by female guppies under each light environment. Taken together, our results support the hypothesis that different environmental conditions during signal reception can directly or indirectly drive the phenotypic diversification of visual signals within species.

Introduction

Animals often use complex signals to communicate with conspecifics and heterospecifics across diverse environments for a variety of reasons (reviewed in Hebets *et al.*, 2016). While animal signalling and communication are an active research area with a long and rich history (e.g. Bradbury & Vehrencamp, 2011), investigating how and why complex signals are phenotypically different across contexts, including signalling environments, remains to be fully explored.

Male colouration patterns that are sexually selected are good candidates for exploring the reasons for complex signal diversity. First and foremost, their functions are relatively easy to identify [i.e. to send an unambiguous signal to receivers that favour a mating response (Bradbury & Vehrencamp, 2011)]. This makes it possible to relate multivariate form and function to fitness and, in turn, predict change from function (Arnold, 1983, 2003; Endler, 1986). Second, how colour pattern signal efficiency changes across different signalling environments is well understood; the efficiency of a colourful visual signal jointly depends on the interaction between environmental light and visual conditions, all the elements of the visual signal and the visual system of the individuals receiving the signals (Lythgoe, 1979; Endler, 1990, 1993a,b). These interactions profoundly affect both reception and perception of visual signals (Lythgoe, 1979; Endler, 1990, 1993a,b; Vorobyev & Osorio, 1998; Kelber *et al.*, 2003). Consequently, if the ambient light spectrum changes due to factors such as climate, habitat type, season, time of day and canopy cover, an organism's colour pattern may change from inconspicuous to conspicuous or vice versa (Endler, 1991, 1993a, 1997). This yields the prediction that a single species living in habitats with different light environments and signal transmission properties should have different colour pattern parameters to signal effectively in each habitat that it uses intensely.

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Studies that examine the relationship between colour patterns and the lighting environment within species typically take two different approaches (reviewed in Fuller, 2002). The first approach is to examine the irradiance (light environment) that a given species signals in and ask whether those light environments are a specialized subset of those available to the signaller (e.g. Endler, 1991; Endler & Thery, 1996). The second method is the comparative approach, where colour pattern differences among populations are investigated with reference to the lighting environment and phylogeny (e.g. Fuller, 2002; McNaught & Owens, 2002; Marshall *et al.*, 2015). Empirical evidence from both approaches supports the idea that the light environment plays a critical role in shaping both the phenotypic diversity and evolution of animal colour patterns and visually based behaviours. However, while these comparative approaches have identified the light environment as a possible mechanism driving male colouration differences, this empirical research is not conclusive because colour pattern variation in the wild is usually associated with correlated variation in several environmental factors. For example, light environment variation is typically associated with other variables such as temperature, predator communities, and food types and their availability. Here, we test directly for changes in male colouration patterns and pattern components using a large-scale multigeneration experiment in which only the light environment varies among populations.

Guppies (*Poecilia reticulata*) are an excellent model system to examine the effects of different light environments on the phenotypic divergence of complex visual signals. Adult male colouration traits are highly heritable, genetically polymorphic and critical for mate signalling and choice (Endler, 1978; Houde, 1997). To attract females, male guppies exhibit an outstanding degree of polymorphic variation that consists of a complex mosaic of colour patches, varying in shape, size, position and spectral shape (colour). Guppy colour patches can be classed (from a human perspective) as black, fuzzy black (facultatively expressed, see Baerends *et al.*, 1955), orange, gold, silver, green, green-blue and violet (see Table S1). In addition, colour patches on the tail are often a combination of yellow, silver or black reticulation (Endler, 1980; Endler & Houde, 1995; Houde, 1997). Furthermore, *P. reticulata* is one of the premier model organisms for the study of phenotypic variation, owing to rapid evolutionary responses to both predation and sexual selection (Houde, 1997).

When assessed by females, male guppy colour components may be considered individually (i.e. as signal components) or assessed together as a functional multiple-component signal. To examine multiple-component signals together, visual modelling approaches that estimate visual contrast (i.e. a measure of the variation in colour or luminance across a male's body) have been used (e.g. Endler, 1990). In guppies, colour pattern components that increase a males' visual contrast diverge among clear and tea-stained water as well as under different intensities of predation balanced by sexual selection (Endler & Houde, 1995). Colour patterns evolve increased visual contrast by sexual selection over multiple generations when released from predation (Endler, 1980; Kemp *et al.*, 2009). A male's visual contrast changes with environmental light and significantly affects mating success (Long & Houde, 1989; Endler, 1991; Smith *et al.*, 2001; Gamble *et al.*, 2003; Kemp *et al.*, 2008, 2009). Moreover, female guppies favour different combinations of colour patches on males under different light conditions (Cole & Endler, 2015). One overall explanation for these observations is that those colour patches with reflectance spectra that match the ambient light spectra are more efficient reflectors than those that mismatch (except for highly reflective white). The highest conspicuousness, and hence most attractive to females, should be achieved by colour patterns containing efficient reflectors of the ambient light present during courtship (Endler, 1991, 1993a). In addition, to further enhance visual contrast, colour patch reflectance spectra should also reflect light that stimulates the most sensitive photoreceptors in a guppies' retina. Different photoreceptors will have different sensitivities as a result of chromatic adaptation (see Vorobyev & Osorio, 1998; Gamble *et al.*, 2003). In sum, these results lead us to predict that male colour pattern components that are both efficient reflectors and that enhance visual contrast will be differentially favoured under different light environments.

Here, we use an evolutionary experimental approach to manipulate colour pattern signal efficiency to examine how male colour pattern components change phenotypically over multiple generations using large replicated populations of guppies. By examining foundation populations and different light environment populations after 2 years (or approximately 6–8 overlapping generations), we can capture any male colouration pattern population differences caused by one or more mechanisms (i.e. phenotypic plasticity and selection), as may occur in a natural population responding to light environmental change.

We tested the hypothesis that male colour pattern components will change within populations and diverge among different light environments. We predict phenotypic divergence in male colour patterns because (1) guppy male colouration pattern components are heritable (Winge, 1927; Endler, 1978, 1980, 1983; Brooks & Endler, 2001), (2) female preferences change under different light environments (Long & Houde, 1989; Gamble *et al.*, 2003; Cole & Endler, 2015), and (3) sexual selection can drive the evolution of colour patterns in the absence of predation with divergent effects in populations living in different visual conditions (Endler, 1980). Additionally, common plastic responses to the

different light environments may also play a role in male colour pattern phenotypic divergence. Hence, our predictions are based on the functions of male colour pattern components, rather than causal mechanisms. More specifically, we predict that, under each experimental light environment, colour patches (as guppies see them) that increase chroma and luminance, and change hue to match the light, as well as those which stimulate the most sensitive guppy cones, will increase in populations over time.

In summary, the logic of our experiment is divergent light environments lead to diverged conspicuousness of males, leading to divergent female choice, leading to phenotypic divergence in the colour pattern components affecting male conspicuousness. Overall, understanding the interaction between colour and light and how each signal component may phenotypically change under each light environment is one of the first critical steps in elucidating their function and how these signal components may work together.

Materials and methods

Animal collection, care and treatment

We collected the foundation of our experimental guppy (*Poecilia reticulata*) population in September 2010 from Alligator Creek in Bowling Green Bay National Park (Queensland Permit WITK07655010) Queensland, Australia (19°26.79′ S 146°58.65 E′), where they were introduced between 50 and 100 years ago. The foundation population consisted of about 300 adults and 50 juveniles but the effective population size was probably larger because female guppies store sperm (Kobayashi & Iwamatsu, 2002; López-Sepulcre *et al.*, 2013), leading to a mean of 3.5 sires per brood in nature (Neff *et al.*, 2008). Guppies from the foundation population were distributed into 12 tanks (each 3 × 1.5 × 0.5 m deep; approximately 2220 L), as this reflects a typical natural pool size. Distribution into the 12 tanks was carried out by starting the foundation in one tank, allowing it to grow to a few thousand, dividing it into two tanks, allowing the two to grow, dividing both into four tanks, then all four into eight and then all eight into 12. During each distribution, males, females and juveniles were exhaustively collected from the existing tanks and divided equally into the target tanks. This ensured that all 12 tanks started with very similar genetic variation, equalized population mixing, multiple paternity and large population sizes to minimize founder effects and subsequent genetic drift.

Throughout the experiment, all tanks were maintained in a constant temperature of 24 ± 1 °C and were illuminated from above using four low flicker (200 Hz) daylight fluorescent tubes under a 12:12 hour light–dark cycle. To monitor population density, we counted male and female adults (and estimated juvenile numbers) in each population every 12 months. Population density (~1000 adults/tank) was stable throughout the experiment. Guppies were fed a combination of brine shrimp nauplii (*Artemia salina*; INVE Aquaculture Inc.), algae discs (Wardley) and flakes (Fish Breeders Choice) daily. All care and treatment of these guppies were in compliance with and approved by the Deakin University Animal Welfare Committee (A21-2010 and G01-2012).

Light environments

We exposed the twelve guppy populations to three different ambient light treatments, each with four replicates. From September 2011, the overhead fluorescent lighting was filtered through a 'Moss Green' filter (Roscolux, filter number 89), a 'Lilac' filter (Roscolux, filter number 55) or a clear film filter (functioning as a neutral density filter to equalize total irradiance among treatments), to yield each of the three light environment treatments, Green-F89, Lilac-F55 and Clear-CF, respectively. The irradiance spectra of these three light treatments were recorded by an Ocean Optics USB2000+ spectrometer calibrated for photon flux (visually relevant units, μmol photons m^{-1} s^{-1} nm^{-1}) with a Li-Cor LI-1800–02 optical radiation calibrator. These three filter treatments were selected to be similar to different natural light environments (Endler, 1993b; Kranz *et al.*, 2018). Green-F89 are more similar to forest shade natural light environments (Endler, 1993b). In contrast, Lilac-F55 and Clear-CF are more similar to small gap natural light environments (Endler, 1993b). Each light environment stimulates guppy cone types differently (Fig. S1).

Experimental design and sampling

We sampled a total of 800 male guppies in our experiment. Initially, 200 foundation population males were sampled between October 2011 and February 2012; we will refer to this as sample S1. Then, after approximately 2 years in our laboratory evolution experiment, 600 male guppies were sampled between August and October 2013; this is sample S2. Sample S2 consisted of 50 male guppies from each of the 12 experimental mesocosms (three light treatments, each with four replicate populations) and is approximately between four and eight (Reznick *et al.*, 1997; Endler *et al.*, 2001) overlapping generations on from S1.

Between the S1 and the S2 samples, the effective overlapping generation time was probably closer to 6–8 for five reasons. First, large mature females were removed from each population every 6 months to reduce the numbers of older females contributing to the next generation (female guppies have indeterminate growth, and hence age is proportional to size; Houde, 1997). Second, guppies sexually mature at

approximately 3 months and females give birth every 20–30 days, meaning that, after 2 years there will be some 8th generation fish present. Third, males live for approximately 1.5 years in the laboratory (AMK personal observations), and similarly, Reznick *et al.* (2004) found the age at last reproduction of laboratory-reared fish from two low predation natural populations and equivalent food levels to be 1.5–1.7 years. Forth, ageing in guppies leads to reduced performance such as swimming acceleration (Reznick *et al.*, 2004) suggesting that older males would be less successful in courtship and offspring production, reducing the effects of previous generation fish on the current population, in addition to mortality. Fifth, to insure, we sampled from the most recent generation in sample S2, in May 2013, approximately 150 fry from each tank were placed in an isolating chamber (94 L) with fine mesh sides located within the parental tank, providing the same conditions as the other fish in the same tank. These fish were allowed to grow to sexual maturity before sampling. Consequently, our populations could potentially evolve over several generations. We allowed overlapping generations to keep our experimental system as close to natural conditions as possible.

Colour pattern measurements

We recorded guppy male colour patch areas using a Nikon D5100 digital camera with a 60 mm Nikkor lens. Prior to all photography, the digital camera was set at a standard height and situated at 90 °C to the lateral plane of the specimen. Lighting conditions were standardized with the area illuminated using two FSL fluorescent tubes and a Ziess (CL 1550 ECO) light source with two illuminating fibres positioned at 45 °C to the guppy's dorsum. The digital camera was manually set at f13, shutter speed of 1/50 and an ISO setting of 100. Prior to each batch of digital photography, the white balance was measured and set and colour standards recorded. Each male guppy was then anaesthetized in a 0.2% aqueous solution of ethyl 3-aminobenzoate methane sulphonic acid salt (MS-222) for approximately 20 s and then immediately photographed on its right lateral side. A scale bar was also included in each photograph. A total of 800 male guppies (sample S1 $n = 200$ and sample S2 $n = 600$) were photographed using this apparatus.

To quantify male guppy colouration in each sample, each digital image was imported into Adobe Photoshop CS5 and the total area (excluding the dorsal fin and the gonopodium), body area (excluding the dorsal fin, gonopodium and tail) and each present colour class were manually selected and saved as its own layer. There are 13 colour classes in total: black, fuzzy black (shape and size modified during courtship, see Baerends *et al.*, 1955), orange, silver, gold, green, green-blue, violet, silver reticulation, black reticulation, yellow reticulation and yellow black (YB) reticulation (for further details see Table S1). Each photoshop psd file was then saved and exported into MATLAB software that extracted all the layers and the scale, measured the relative area and number of patches present for each colour class in each male.

We also obtained mean reflectance measurements of each colour class, as well as body colouration, from 120 anesthetized males. The males used for reflectance spectra measurements were a random subset ($n = 10$) of the 50 male guppies photographed from each of the 12 experimental mesocosms in late 2013 (sample S2). All males were kept under laboratory light conditions (see Fig. S1 for details) for approximately 3 weeks prior to being measured. As previously described in Cole & Endler (2015), we measured guppy patch reflectance spectra over the 300–700 nm guppy visible range (Archer *et al.*, 1987) using an Ocean Optics 2000+ spectrometer coupled to a Li-Cor LI-1800-06 quartz microscope/telescope through a ultraviolet-visible (UV-VIS)-rated fibre optic cable. A black curtain to minimize stray light shielded the entire apparatus. Using its adjustable diaphragm, we set the LI-1800-06 to measure 0.9-mm-diameter areas on the guppy surface by facing down perpendicular to the guppy's side. A movable mirror in the optical axis allowed us to know exactly what the spectrometer would sense when the mirror was switched from the viewport to the fibre optic. The guppy or Spectralon white standard was illuminated by an Ocean Optics PX-2 xenon flash lamp set to illuminate at a 45° angle, simulating illumination of a guppy by Snell's window and the LI-1800-02 simulating a female viewing the male from his side (Cole & Endler, 2015). Reflectance was calibrated with white and black standards.

Cone captures

We used the Vorobyev & Osorio (1998) photopic receptor noise model to estimate the cone (photoreceptor) responses to each guppy colour patch within each light environment. This model requires information on the peak spectral sensitivity (λ_{max}) and relative abundance of each cone class. Guppy eyes express multiple photoreceptor opsins (e.g. Sandkam *et al.*, 2015; Kranz *et al.*, 2018), and, based upon microspectrophotometry (MSP), the long-wavelength sensitive (LWS) cone λ_{max} vary among individuals (Archer *et al.*, 1987; Archer & Lythgoe, 1990). On the basis of MSP data (Archer *et al.*, 1987; Archer & Lythgoe, 1990; Watson *et al.*, 2011) opsin gene spatial expression data (Rennison *et al.*, 2011) and opsin gene expression levels in our laboratory environment (Kranz *et al.*, 2018), we used the following λ_{max} for our guppy eye model: 359 nm (ultraviolet sensitive [UVS]; SWS1), 408 nm (short-wavelength sensitive [SWS]; SWS2b), 465 nm (medium-wavelength sensitive [MWS]; Rh2-2) and 560 nm

(long-wavelength sensitive [LWS]; LWS-1), for the single cones, and the sum of the 543 nm (MSW) and 560 nm (LWS) spectra for the double cones. (We chose LWS 560 nm based on the observation that no LWS Serine variants have yet been cloned and sequenced from our population [AMK, personal observations]). We included the guppy cornea and lens transmission spectrum (data from Archer & Lythgoe, 1990; R. Douglas, personal communication, 10 October 2000) in the calculations. We used the von Kries correction (Vorobyev & Osorio, 1998) to allow for chromatic adaptation to each light environment because correction for chromatic adaptation is necessary whenever the viewer is in a given light environment for more than about a minute (Endler *et al.*, 2014). Aside from these guppy-specific parameters, our calculations followed the methods, described in Endler & Mielke (2005).

Calculation of guppy contrast measures

To explore the effect of light environment on the appearance of male colour signal components, we calculated guppy eye-based measures of luminance, chroma, and hue for each male colour class under each light environment. These three measures depend on the cone photon captures for each guppy colour in each environment (Endler, 1990; Endler & Mielke, 2005; Cole & Endler, 2015). We calculated luminance (*Lm* or light/dark) as the degree of stimulation of the double-cone photoreceptors by a given colour patch in a given light environment. The relative simulations of different cones code the perception of colour. As a result, each set of four cone relative stimulations can be plotted in a tetrahedron of height 1 after using the von Kries correction (Endler & Mielke, 2005). We calculated chroma (*Cr*) of a particular colour in a particular light environment as the distance between the tetrahedral grey (achromatic) point (all cones equally stimulated) to the tetrahedral point for that colour and environment, *Cr* has a maximum of 0.75—the distance from the grey point to any vertex (tetrahedral tip). For hue, a tetrahedral plot would require two measures equivalent to latitude and longitude. Consequently, we used hue (*Hu*) derived from the alternative 4-cone colour space, LSMU (Endler & Mielke, 2005) as follows: Let *a,b,c,d* be the von Kries corrected relative cone stimulations ($a + b + c + d = 1$). Then, the two axes are (a–c) and (b–d), and the hue angle (*Hu*) is obtained by converting these Cartesian coordinates to polar coordinates. *Hu* increases from red (LWS only at 0°) through UV (pure UVS at 270°) through UV-purple (short+long) and back to red at 360°. In summary, *Cr* (chroma) is a measure of how differently the photoreceptors are stimulated, *Hu* (hue) depends on which particular cone combinations are differentially stimulated and *Lm* (luminance) is the degree of stimulation of the double-cone photoreceptors (Endler & Mielke, 2005; Cole & Endler, 2015).

Calculations and statistical methods

All analyses were performed using MATLAB version R2016a, except for statistical modelling and testing, and the multivariate analysis, which was performed in R (R Core Team, 2016). Using the protocols suggested in Zuur *et al.* (2010), we found that our data violates all of the assumptions of parametric tests such as GLMM. This is typical of colour pattern data (Endler & Mielke, 2005). Consequently, we performed all statistical analysis using permutation tests that are largely free of assumptions (Mielke & Berry, 2001; Endler & Mielke, 2005). Our permutation tests were performed using the adonis function in the R package vegan (Okansen *et al.*, 2016), which allows nested data structures similar to GLMMs and ANOVAS. Following the guidance of Mielke & Berry (2001), we used 10 000 permutations and the AltGower method to preserve beneficial geometric and error properties (Mielke & Berry, 2001; Endler & Mielke, 2005; Okansen *et al.* 2016). This method has the desirable properties of our LAD-MRPP method (least sum of absolute deviations multiresponse permutation procedure) (Endler & Mielke, 2005) but allows a greater diversity of data structures (Okansen *et al.* 2016).

In order to perform statistical comparisons of reflectance spectra shapes, we first standardized each spectrum so that it summed to 100; this allows comparison of spectral shape (colour) independent of total reflectance. Data at different wavelengths are not independent, violating the assumptions of tests working directly on spectra (Kemp *et al.*, 2015). We used the discrete cosine transform (DCT) to convert each standardized spectrum to a series of 20 statistically independent coefficients (Jain, 1989), implemented by the MATLAB signal processing toolbox function dct. The DCT is similar to the Fourier transform but works on discrete non-cyclic data such as spectra.

Using these above statistical methods, we examined both the change and the divergence of male guppy colour signal components under the three different light environments. The differentiation tests examine whether there has been any change between S1 and S2 within each light environment. The divergence tests examine whether there has been any divergence among the three light environments within S2.

Results

Colour patch changes

The foundation populations (sample S1) showed no significant differences among future light treatments replicate populations for any colour relative area (RA) (all $P < 0.05$ in permutation tests), with the exception of the green colour class ($P = 0.029$) (Table S2). No permutation test *P* values were significant for future light

treatments after sequential Bonferroni tests (Table S2). In addition, the only colour classes that showed heterogeneity among the 12 different foundation populations (i.e. tanks) were black ($P = 0.028$), green ($P = 0.00015$), violet ($P = 0.031$) and silver ($P = 0.019$) (Table S2). Only the green colour class remained significant for tank after sequential Bonferroni tests (Table S2). Taken together, these results indicate that all 12 populations started out with similar colour pattern RA distributions (with all future treatments populations being homogeneous for all colour patch classes, but some tanks being variable with respect to the green colour class).

The relative areas of six of the colour class components (black, fuzzy black, orange, yellow reticulation, green and violet) changed between the S1 and S2 samples (Fig. 1, Table 1). Of these, only the four chromatic colours changed in each light environment (Fig. 1). The relative area (RA) of black and fuzzy black increased over time for all three light environments. The orange RA decreased in Lilac-F55 populations, whereas yellow RA decreased in Clear-CF populations. Green RA increased in Clear-CF populations and decreased in Green-F89 populations. Violet RA increased in Green-F89 populations. In addition, for Lilac-F55 populations, the number of fuzzy black, orange and violet patches also decreased (Table S3a). A summary of the changes in colour pattern elements is shown in Fig. 4a.

Colour patch area divergence

The relative areas of four colour class components (fuzzy black, orange, green and violet) diverged between the Clear-CF, Green-F89 and Lilac-F55 population S2 samples (Fig. 2, Table 2). Again, three of these colour class components are chromatic (orange, green and violet; Fig. 2). Fuzzy black, orange and green colour patches RAs were comparatively larger in males from the Clear-CF light populations (i.e. CF > F89 and F55). In contrast, violet RA significantly diverged among all three light environments (i.e. F89 > CF > F55). In many cases, there was also significant variation among tanks within light environments, indicating potential evolutionary contingency (Table 2). There was no significant divergence in patch numbers among the different light environments (Table S3b).

Guppy patch size spatial resolution

Although relative patch area (RA) predicts female choice in guppies better than absolute patch size (Long & Rosenqvist, 1998), it is relevant to ask whether or not guppies can see the differences in patch size that we observed among the light treatments. Table S4a and b shows the differences in mean patch length and height for each colour class, respectively. The spatial resolution of guppies is 0.24–0.25 cycles per degree calculated from both female lens mean focal length and cone spacing as well as behavioural measurements (Long & Rosenqvist, 1998; Fleishman & Endler, 2000). At the light intensities found in our experimental tanks (2–5 μMol m^{-2} s), male guppies court at 2–3 cm from females with the sigmoid display occurring somewhat closer (Long, 1993; Long & Rosenqvist, 1998). Taking the 3 cm courtship distance in conjunction with the spatial resolution of female guppy eyes, results in the estimate that females can resolve patches as small as 0.15 mm. In sum, even if we take the more conservative acuity disc (Endler, 1978) as 0.2 mm, this is sufficient for female guppies to see the differences we observe in male colour pattern components (Table S4a and b).

Colour patch spectral divergence

The light environment was also a significant predictor of differences in the reflectance spectra of green, orange, violet and silver signal components (Figs 3 and S2, Table 2). For brevity, we will refer to UV to blue (300–450 nm) as short wavelengths or SW, green to yellow (450–550 nm) as middle wavelengths (MW) and orange to red (550–700 nm) as long wavelengths (LW). These terms are only for convenience in referring to different parts of spectra.

Green patches diverged to become more SW, and less MW and LW, reflective in Lilac-F55 light populations, compared to Clear-CF light populations (Fig. 3, Table 2). This results in green patches with lower *Cr* for Lilac-F55 males (Table S5). Green patches also diverged to become more SW and less MW reflective in Green-F89 light populations but did not change in LW, compared to Clear-CF light populations. Again, the effect is green patches with lower *Cr* for Green-F89 males (Table S5). In addition, when compared to Clear-CF guppies, *Hu* for green patches' was SW shifted in guppies that lived under the Green-F89 or Lilac-F55 light conditions (Fig. S3).

Orange patches diverged from the Clear-CF populations with an increase in SW and a decrease in LW for both Green-F89 and Lilac-F55 populations (Table 2, Fig. 3). This results in orange patches with a lower *Cr* and decreased *Lm* for both F89 and F55 males (Table S5). Again, compared to Clear-CF guppies *Hu* for orange patches is UV/SW shifted in guppies that have lived under the Green-F89 or Lilac-F55 light conditions (Fig. S3).

Violet patches diverged from the Clear-CF populations with an increase in SW for both Green-F89 and Lilac-F55 populations (Table 2, Fig. 3). The effect is violet patches with higher *Cr* for both F89 and F55 males (Table S5). In addition, violet patch reflectance increased more in the shorter wavelengths (i.e. more UV) in Lilac-F55 populations,

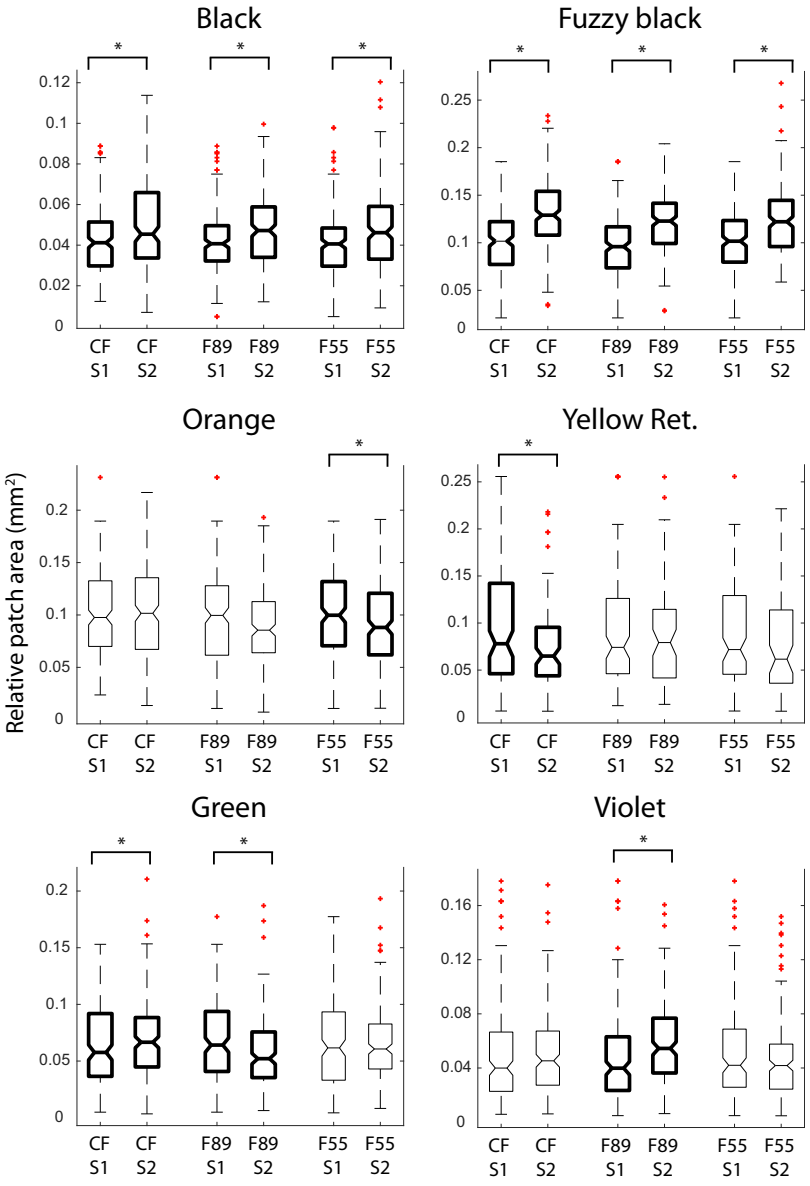

Fig. 1 Box plots comparing the relative patch areas between samples S1 (2011) and S2 (2013). Bold box plots and asterisks show where time was a significant predictor for relative area colour patch changes, based on the permutation test results shown in Table 1. CF, Clear-CF filter; F89, Green-F89 filter; F55, Lilac-F55 filter. S1, foundation samples taken in 2011. S2, experimental samples taken in 2013. Only colour patch classes with statistically significant changes are shown. Permutation tests were carried out with the four replicate populations nested within the three treatments (see Table 1 and Materials and Methods for details).

whereas it increased in longer wavelengths (i.e. more blue) in Green-F89 populations (Fig. 3). For the Lilac-F55 populations, the reflectance spectra of silver patches also shifted to become more SW reflective and less LW reflective. This results in silver patches with higher *Cr* for Lilac-F55 males (Table S5). Violet and silver patches do not diverge much in *Hu* after evolving in the Green-F89, Lilac-F55 or Clear-CF populations (Fig. S3).

A summary of the divergence in both RAs and spectral shape for each colour pattern component is shown in Fig. 4b.

Colour patch measures

Chroma (*Cr*), hue (*Hu*) and luminance (*Lm*) estimate the visual effects of the interaction among light, reflectance spectra and guppy eyes. The three light environments

Table 1 Probabilities resulting from permutation tests for differences between colour patch areas between samples S1 (2011) and S2 (2013).

	Clear-CF				Green-F89				Lilac-F55			
Patch relative area (RA)	Sign	Time	Tank	Time*Tank	Sign	Time	Tank	Time*Tank	Sign	Time	Tank	Time*Tank
Black	+	**0.0003** (0.028)	0.051 (0.018)	**< 10^{-5}** (0.08)	+	**0.0018** (0.026)	0.2 (0.011)	**0.036** (0.021)	+	**< 10^{-5}** (0.045)	0.26 (0.009)	0.063 (0.017)
Fuzzy Black	+	**< 10^{-5}** (0.162)	**0.002** (0.03)	0.068 (0.014)	+	**< 10^{-5}** (0.14)	**0.014** (0.022)	0.68 (0.003)	+	**< 10^{-5}** (0.087)	0.36 (0.007)	0.23 (0.009)
Orange		0.79 (0.0001)	**0.042** (0.02)	0.66 (0.004)		0.074 (0.007)	**0.0024** (0.034)	0.11 (0.014)	–	**0.017** (0.015)	0.95 (0.0009)	**0.032** (0.021)
Yellow	–	**0.0089 (0.031)**	0.11 (0.028)	0.77 (0.005)		0.51 (0.002)	0.1 (0.034)	0.1 (0.035)		0.39 (0.004)	0.81 (0.005)	0.62 (0.01)
Gold		0.62 (0.006)	0.75 (0.031)	**0.01** (0.371)		0.66 (0.005)	0.51 (0.058)	0.35 (0.051)		0.62 (0.007)	0.25 (0.12)	0.87 (0.02)
Green	+	**0.031** (0.012)	**0.026** (0.023)	**0.0012** (0.04)	–	**0.0053** (0.021)	0.65 (0.004)	0.52 (0.006)		0.59 (0.0008)	**0.022** (0.026)	0.059 (0.019)
Violet		0.56 (0.0009)	0.24 (0.012)	0.81 (0.003)	+	**0.015** (0.017)	0.3 (0.011)	0.4 (0.008)		0.091 (0.008)	0.055 (0.023)	**0.032** (0.026)
Silver		0.098 (0.013)	0.51 (0.012)	0.81 (0.004)		0.12 (0.011)	0.66 (0.007)	0.11 (0.028)		0.65 (0.0009)	0.19 (0.02)	0.57 (0.008)

Bold values are statistically significant Pr (>*F*) values. Sign only shows if change is statistically positive or negative over time. All R^2 values are reported in brackets. Permutations = 10 000. See Materials and Methods for details.

differentially affect *Cr*, *Hu* and *Lm* of most of the colour patches (Table S5). A male guppy moving from the clear environment to one of the other two will experience both increases and decreases in *Cr*, *Hu* and *Lm* among its coloured patches. This arises from (1) the various degrees of match and mismatch between the ambient light spectra of each environment and the reflectance spectra, (2) the reflectance spectra of each colour class and (3) both light matching and spectral shape relative to the cone absorption spectra (Endler, 1993a,b; Endler & Houde, 1995; Endler & Mielke, 2005). As such, the different *Cr*, *Hu* and *Lm* values for each colour class component under each light condition may predict their changes in relative area between samples S1 and S2. Table 3 shows the relationship between the percentage change in *Cr*, *Hu* or *Lm* for a male guppy going from the Clear-CF to either Green-F89 or Lilac-F55 light environments and the divergence results presented above. Figure 5 shows the relationships between the percentage change in *Cr*, *Hu* or *Lm* for a male guppy going from the Clear-CF to either Green-F89 or Lilac-F55 light environments vs. the percentage increase or decrease in patch area between samples S1 and S2. There is a significant positive relationship for *Cr* in Green-F89 (Fig. 5a; $t_6 = 3.05$, $r^2 = 0.61$, $P = 0.022$) and for luminance in Lilac-F55 (Fig. 5f; $t_6 = 2.84$, $r^2 = 0.57$, $P = 0.03$). In the Green-F89 light environment, patches with positive *Cr* changes increase, whereas colours with negative changes decrease in patch area (Fig 5a). In the Lilac-F55 light environment, patches with positive luminance changes increase, whereas patches with negative changes decrease (Fig. 5f).

Discussion

The colour patches of male guppies living in the 12 populations exposure to three different light environments in our multigenerational experiment changed and diverged in their relative area (RA), number of patches and colour (reflectance spectral shape). Our visual modelling demonstrates that the three different light environments directly and divergently change the efficiency of colour patch signal components. For each colour patch under each light environment, we estimated three visual statistics – chroma (*Cr*), hue (*Hu*) and luminance (*Lm*) – based upon the ambient light treatments, the colour patch reflectance spectra and known neural processing in the retina and brain of guppies (see also Cole & Endler, 2015). We find that the majority of colour patch changes in our guppy populations (see Fig. 4) could be predicted and understood from how these *Cr*, *Hu* and *Lm* statistics change with light spectrum (Fig. 5 and Table 3).

Colour patch changes over time

We experimentally demonstrated that the light environment is a significant predictor of the phenotypic

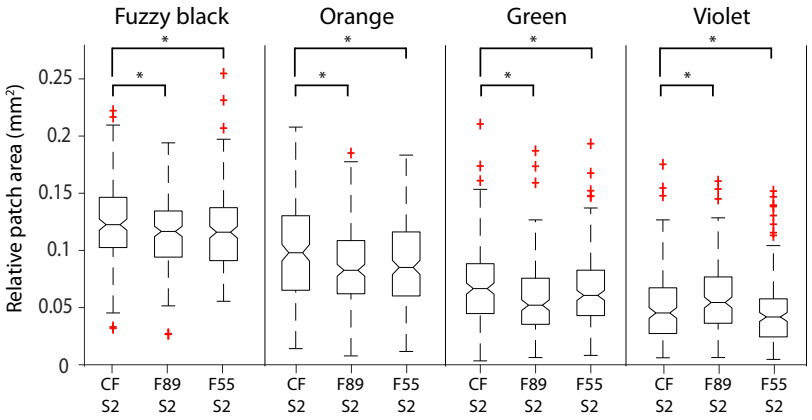

Fig. 2 Box plots comparing the relative patch areas of CF-S2 populations to the F89-S2 or F55-S2 populations. Only colour patch classes where the light environment was a significant predictor for relative area colour patch divergence are shown. Asterisks indicate that all the comparisons were statistically significant based on the permutation test results shown in Table 2. CF, Clear-CF filter; F89, Green-F89 filter; F55, Lilac-F55 filter. S2, experimental samples taken in 2013. Permutation tests were carried out with the four replicate populations nested within the three treatments (see Table 2 and Materials and Methods for details).

Table 2 Probabilities resulting from permutation tests for patch area and spectral shape divergence among S2 (2013) samples.

	Relative patch area				Spectral shape			
			Env. difference sign				Env. difference	
Patch component	Light Env.	Tank in Light Env.	F89-CF	F55-CF	Light Env.	Tank in Light Env.	F89-CF	F55-CF
Black	0.83 (0.0006)	**< 10^{-5}** (0.08)			**0.013*** (0.095)	**0.016*** (0.331)		
Fuzzy Black	**0.031** (0.011)	**0.0004** (0.05)	–	–	0.91 (0.041)	0.98 (0.242)		
Orange	**0.011** (0.015)	**0.0093** (0.036)	–	–	**< 10^{-5}** (0.062)	**0.0002** (0.104)	**Y**	**Y**
Yellow	0.4 (0.008)	**0.031** (0.082)		–	0.34 (0.097)	0.45 (0.4)		
Gold	0.57 (0.025)	0.2 (0.273)			0.13 (0.063)	0.25 (0.201)		
Green	**0.0012** (0.022)	**< 10^{-5}** (0.073)	–	–	**0.0007** (0.055)	**0.01** (0.098)	**Y**	**Y**
Violet	**0.0004** (0.028)	**0.012** (0.039)	**+**	–	**0.0006** (0.069)	0.26 (0.068)	**Y**	**Y**
Silver	0.063 (0.017)	0.31 (0.032)			**0.025** (0.051)	0.31 (0.101)		**Y**

Bold values are statistically significant Pr (>*F*) values. Env. difference sign only shows if the change is statistically positive or negative between the different light environments. Env. difference only shows if the change is statistically different between the different light environments (Y, yes). All R^2 values are reported in brackets. Permutations = 10 000. See Materials and Methods for details.
*Differences may be an artefact, due to black patches being so dark that they result in noisy spectral reflectance measurements (JAE, personal observations).

changes in both colour patch relative areas (RA) and total patch numbers in our large guppy populations over a 2-year period (summarized in Fig. 4a).

We observed two different trends in colour patch relative area (RA) changes. First, we found that black and fuzzy black increased their RAs over time, irrespective of the light environment. This indicates a more generalized function for these two achromatic signal components. For example, black and fuzzy black patches may function in (1) species recognition (Cole & Endler, 2015), (2) modifying the perception of other colour patches (Brooks & Caithness, 1995; Brooks, 1996) or (3) enhancing within guppy visual contrast to increase overall signal conspicuousness (Endler, 1990). Highly light-absorbent black and fuzzy black patches will increase overall guppy pattern visual contrast in terms *Cr*, *Hu* and *Lu* no matter what other colour patches they are near or touching because the very low reflectance and chroma of black will contrast with the much higher values of all the other colours. The idea that black and fuzzy black components can modify the perception of within guppy visual contrast is also consistent with nonlinear selection analysis that shows that black and fuzzy black are selected together with orange and iridescence (Blows *et al.*, 2003; Cole & Endler, 2015). Black and fuzzy black component changes may also be due to phenotypic plasticity (e.g. Ruell *et al.*, 2013). This is especially apparent for fuzzy black

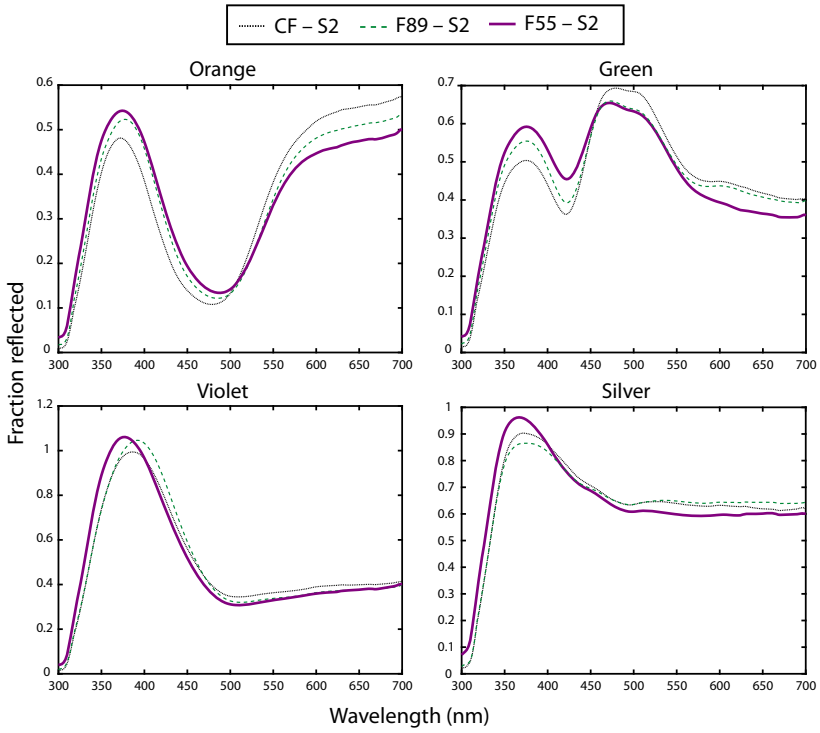

Fig. 3 Mean reflectance spectra of colours that significantly diverged in S2 populations. Only colour classes where the light environment was a significant predictor for spectral shape divergence are shown (see Table 2). CF, Clear-CF filter; F89, Green-F89 filter; F55, Lilac-F55 filter. S2, experimental samples taken in 2013. Permutation tests were carried out with the four replicate populations nested within the three treatments (see also Fig. S2 and Materials and Methods for details).

patches, because their facultative expression (i.e. how 'light' or 'dark' the patch appears) is determined by melanosome dispersal that is regulated by molecular changes that occur in the endocrine and/or nervous systems (reviewed in Fujii, 2000).

Second, we found that the chromatic violet, green, yellow reticulation and orange colour RAs changed in different directions under the three different light environments. We discuss the relationship between RA changes and colour component properties separately for each light environment below.

RA changes under the Green-F89 light environment

Under the Green-F89 light environment, violet RAs increase over time and green RAs decrease over time (Figs. 1 and 4, Table 1). These RA changes are consistent with their respective *Cr* (chroma) changes because violet patches increase in *Cr*, whereas green patches decrease in *Cr* when moved from the Green-F89 to the Clear-CF environment (see Tables 3 and S4). Based on *Cr* calculations, we also expected orange RA to decrease over time. However, while orange RAs decrease between S1 and S2 in the Green-F89 populations, tank was the statistically significant predictive factor in our permutation tests, rather than time (Table 1). The differential and plastic expression of some RH2 and LWS opsin genes in Green-F89 populations over multiple generations (Kranz *et al.*, 2018) also raises the interesting possibility that other components of the visual system may at least partially compensate for any reduced efficiency in long-wave signal elements (e.g. orange patches). Overall, under the Green-F89 conditions, colour patch relative areas change in ways predicted from how the light environment influences the efficiency of their colour patch *Cr* (i.e. colours with positive *Cr* changes increase, whereas colours with negative *Cr* changes decrease; Fig. 5). This suggests that increasing colour patch *Cr* plays an important role in improving male guppy signal efficiency but is also dependent upon the colour of the patch component relative to the light environment.

RA changes under the Lilac-F55 light environment

Under the Lilac-F55 light conditions, we find that only orange RAs and patch numbers decrease over time (Tables 1 and S3a). This is associated with a decrease in orange *Cr*, *Hu* and *Lm* under the Lilac-F55 compared to the Clear-CF light environment (Table 3). However, when changes in RA are examined together, the direction of change correlates most with changes in luminance (Fig. 5f). This suggests that colour patch luminance plays an important role in guppy

Table 3 Predictions for relative area and spectral shape divergence, based on the percentage difference in chroma, luminance and hue for the Green-F89 and Lilac-F55 environments, when compared to the Clear-CF environment.

	Light environment				Divergence results	
	Chroma					
	CF	F89	F55	Predictions based on Chroma	RA divergence	Spectral divergence
Silver	0.0	0.0	**39.3**	F55 ≠ F89 and CF	✗ no divergence	✓ F55 ≠ CF and F89
Violet	0.0	**33.5**	**18.3**	F89 and F55 ≠ CF	✓ F89 > CF ✗ CF > F55	✓ F89 and F55 ≠ CF
Green	0.0	−**22.9**	−**67.9**	F89 and F55 ≠ CF	✓ CF > F89 and F55	✓ F89 and F55 ≠ CF
Orange	0.0	−**14.6**	−**21.4**	F89 and F55 ≠ CF	✓ CF > F89 and F55	✓ F89 and F55 ≠ CF

	Light environment				Divergence results	
	Hue					
Colour patch	CF	F89	F55	Predictions based on Hue	RA divergence	Spectral divergence
Silver	0.0	0.0	**5.2**	No divergence	No relative area divergence predictions based on Hu	✓ no divergence
Violet	0.0	0.3	4.9	No divergence		✓ no divergence
Green	0.0	**31.9**	**52.3**	CF ≠ F89 and F55		✓ F89 and F55 ≠ CF
Orange	0.0	−**16.3**	−**10.5**	CF ≠ F89 and F55		✓ F89 and F55 ≠ CF

	Light environment				Divergence results	
	Luminance					
Colour patch	CF	F89	F55	Predictions based on Luminance	RA divergence	Spectral divergence
Silver	0.0	0.7	−4.1	No divergence	✓ no divergence	✗ F55 ≠ CF and F89
Violet	0.0	−**11.5**	−4.6	F89 ≠ CF and F55	✓F89 > CF ✗ CF > F55	✓F89 ≠ CF ✗ CF ≠ F55
Green	0.0	−0.8	−3.1	No divergence	✗ CF > F89 and F55	✗ F89 and F55 ≠ CF
Orange	0.0	−**17.1**	−**18.3**	F89 and F55 ≠ CF	✓ CF > F89 and F55	✓ F89 and F55 ≠ CF

Chroma, hue and luminance values that are above 5% different from the Clear-CF environment are in bold. All divergence results are from S2 experimental samples, taken in 2013. Ticks indicate consistency between the predictions and the results reported, while crosses indicate inconsistency.

whole-pattern visual contrast efficacy, at least under the Lilac-F55 light conditions.

RA changes under the Clear-CF light environment

Under the Clear-CF populations, we did not predict any temporal changes in colour patch RAs due to changes in signal component efficiency because the light conditions for S1 and S2 remained the same (i.e. Clear-CF condition, which was the control with only a neutral density filter to control for light intensity). Therefore, one possibility is that the increase in green RA and decrease in yellow reticulation RA may be influenced directly by a female preference and results in sexual selection favouring these colour pattern components in the absence of predation (Endler, 1980). Alternatively, founder effects or genetic drift may also play a role (although our initial distribution among the tanks, multiple paternity and large effective population sizes should minimize the effects of drift). Typically in the guppy literature, silver, violet and green are pooled together and referred to

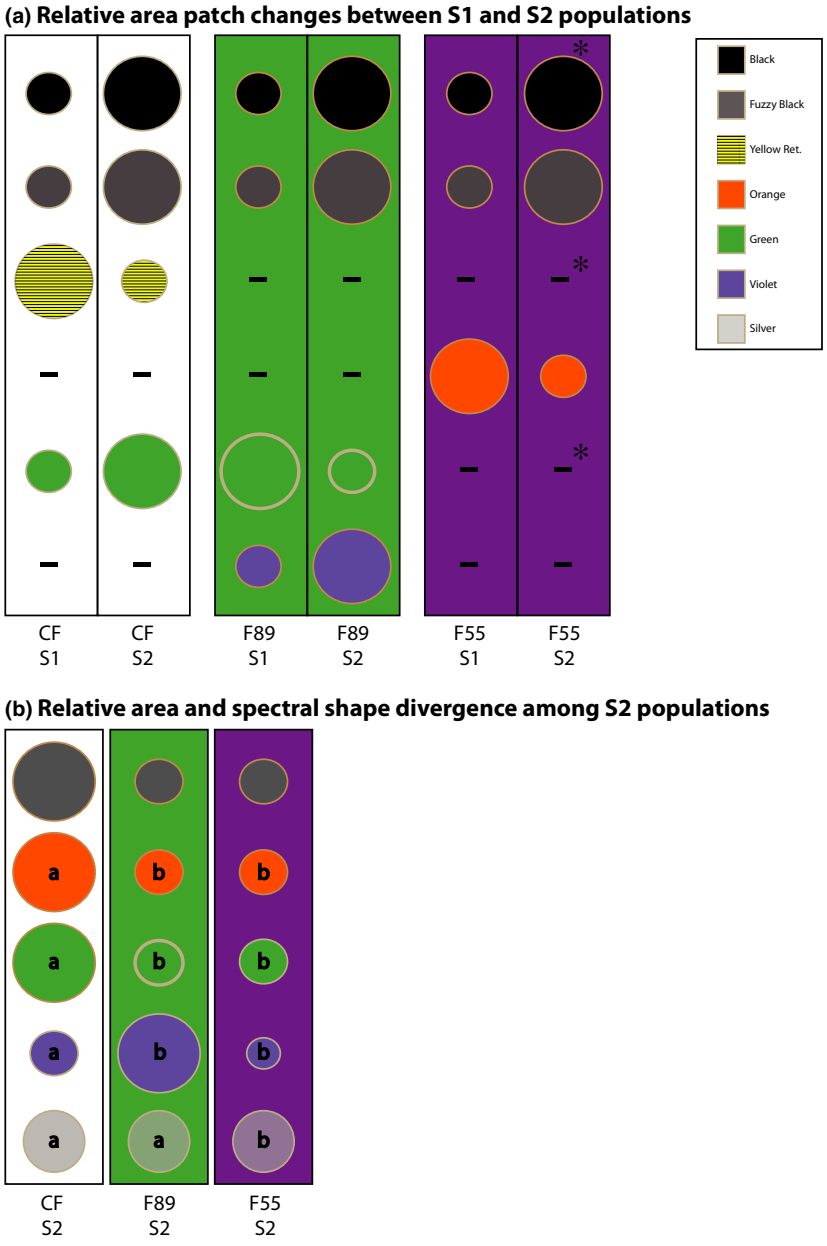

Fig. 4 Summary of colour patch changes in our guppy populations in three different light environments. CF, Clear-CF filter; F89, Green-F89 filter; F55, Lilac-F55 filter. (a) In the top figure, the size of the circles represents statistically significant increases and decreases in each colour patch relative area between the S1 (2011) and S2 (2013) populations (see Materials and Methods for details). Lines indicate no statistically significant changes in relative area. Asterisks indicate statistically significant changes in colour patch numbers (see Table S2 for further details). (b) In the bottom figure, the size of the circles represents statistically significant increases and decreases in each colour patch relative area among the CF, F89 and F55 S2 populations. Letters indicate statistically significant changes in colour patch mean reflectance spectra (see Table 2).

as 'iridescence' and yellow reticulation is often either pooled with orange or ignored completely (for an exception see Cole & Endler, 2015). However, our above observations are consistent with the importance of both 'iridescence' and 'orange' in male guppy attractiveness (Blows *et al.*, 2003; Cole & Endler, 2015).

Colour patch divergence

The light environment is a significant predictor for the phenotypic divergence of colour patches in male guppies, for both relative area (RA) and colour (spectral shape) as shown by significant differences among

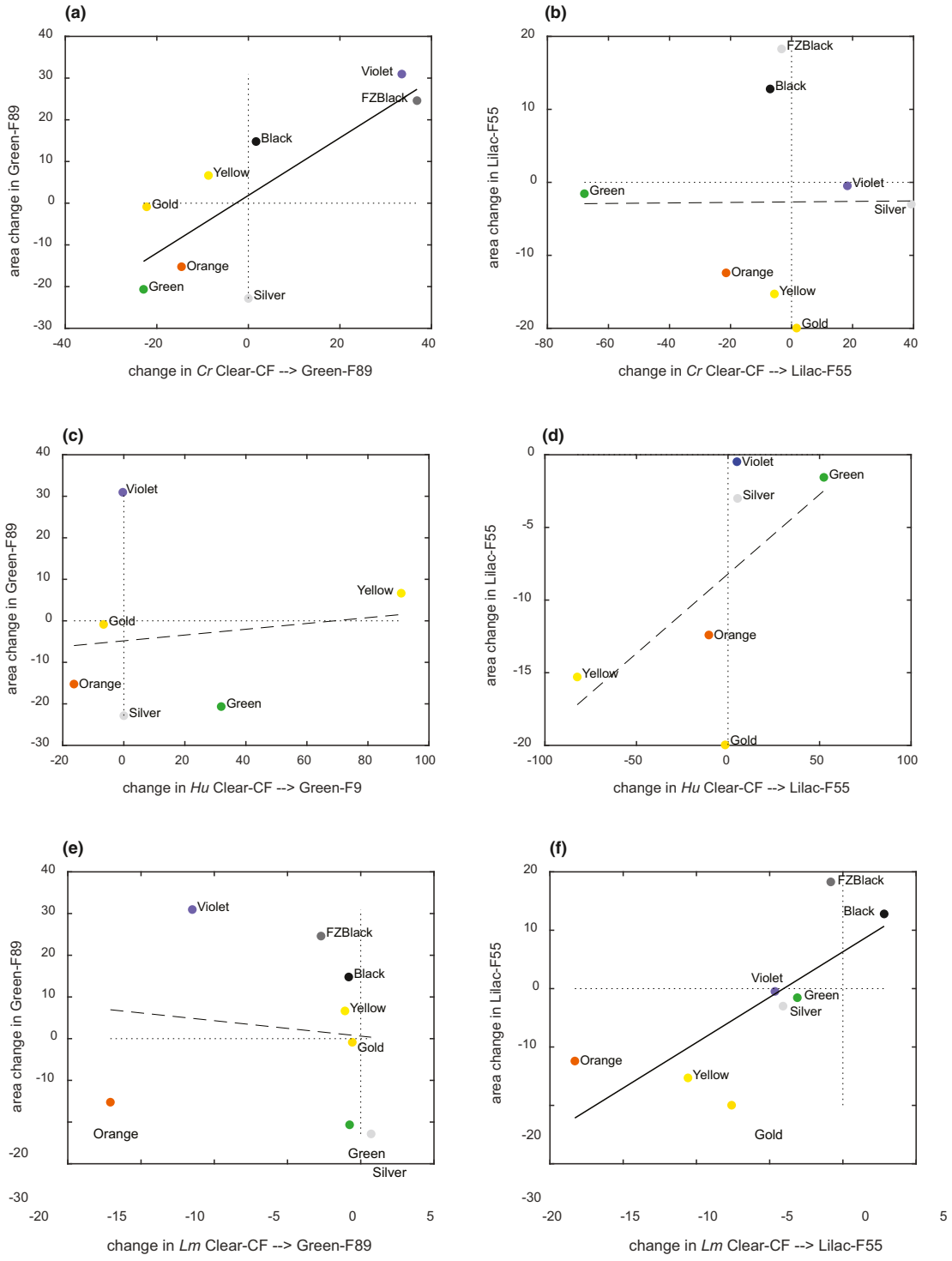

Fig. 5 Relationships between observed changes in calculated visual effects and observed changes in the colours between samples S1 and S2. Visual effects (a-b, c-d, e-f) are chroma (*Cr*), hue (*Hu*) and luminance (*Lm*) and for each colour on a guppy moving from the CF (control) to either the F89 (a, c, e) or F55 (b, d, f) light environment. Percentage change is calculated from median values as 100 * 2 * (A−B)/(A+B), where B is either *Cr*, *Hu* or *Lm* in clear or area in S1 and A is F89 or F55 or area in S2. Solid regression lines are significant and dashed regression lines are not. CF, Clear-CF filter; F89, Green-F89 filter; F55, Lilac-F55 filter. S1, foundation samples taken in 2011. S2, experimental samples taken in 2013 (see Materials and Methods for details).

populations from the three different light environments (sample S2; Table 2). As expected, RA divergence followed similar trends to RA changes with orange, green and violet all diverging among the three different light environments (Table 2, Fig. 4b). In addition, we found that fuzzy black RAs also diverged among the three different light environments (Table 2, Fig. 4b), whereas black RAs did not. This raises the interesting possibility that similar to other chromatophores, guppy black (melanin-based) colour signals may be good candidates to investigate honest signalling as found in birds (e.g. Galván *et al.*, 2015).

For the chromatic colours, we find both divergence in RAs and spectral shape (Fig. 4b) with the majority of differences predicted by one or more of the visual contrast measures (Table 3). This indicates that guppy colour patch differences can occur, not only across the skin (i.e. smaller or larger patches of pigment cells), but also in how different types of pigment cells are organized within the skin itself. In guppies, four major pigment cell classes have been reported in the skin, namely black melanophores, yellow to red xanthophores, violet to green iridophores and (potentially) white leucophores (reviewed in Kottler *et al.*, 2014). Moreover, each different colour patch has been shown to contain multiple pigment types (reviewed in Kottler *et al.*, 2014). Hence, each colour patch is a signal that consists of a mix of different chromatophores resulting in colour patches with different spectral shapes.

Spectral shape divergence

Among the three different light environments, silver, violet, green and orange patches all diverged in their spectral shapes. Below, we discuss the potential consequences that spectral shape changes can have on guppy cone stimulations and chroma and hue visual contrast measures under the different light environments.

In guppies from the Lilac-F55 populations, we observed that both silver and violet colour patches diverged to become more reflective in the short wavelength (300–450 nm) part of their spectrums (Fig. 3, Table 2). In turn, these two colour patches are calculated to be more chromatic when guppies view them under Lilac-F55 light (Table S5). From a calculated physiological perspective, we suggest that silver and violet chroma (*Cr*) increase with spectral shape divergence because under the Lilac-F55 light environment, guppies may have less responsive SWS 'violet – 408 '(λ_{max} 408 nm) and 'blue – 465 '(λ_{max} 465 nm) cones. This is because the Lilac-F55 light more strongly stimulates these cones than the other two light environments (see Fig. S1). Chromatic adaptation to the Lilac-F55 environment means that these cones are less sensitive; this is what the von Kries correction accounts for (Fig. S1, for discussions see Vorobyev & Osorio, 1998; Gamble *et al.*, 2003; and Endler & Mielke, 2005). The UV – 359 (λ_{max} 359 nm) cones will be more sensitive because there is relatively little UV light and the violet and silver colours reflect what little UV there is. Based on our overall visual contrast measures, we predicted that the presence of silver and violet patches would enhance a male's visual contrast under the Lilac-F55 light conditions (Table 3). In turn, we initially hypothesized that their RAs would increase. Yet we did not observe a divergence in their RAs over time or between the Lilac-F55 and Clear-CF light environments. Instead, our spectral shape results indicate that with a divergence in colour patch spectral shape, a male guppy will exploit the ambient light conditions to signal effectively. The above chromatic adaptation explanation is also consistent with the observation of violet patch reflectance increases in longer wavelengths (i.e. more blue) in Green-F89 populations (Fig. 3) because under the Green-F89 light environment, guppies may have more responsive SWS 'violet/408 '(λ_{max} 408 nm) cones (see Fig. S1).

Because the spectral shape of four different colour components diverged among the three different light environments (Fig. 3), it is also logical to predict that their hue (*Hu*) could diverge in addition to their *Cr*. This is because *Cr* is a measure of how differently the photoreceptors are stimulated and *Hu* depends on which particular cone combinations are differentially stimulated (Endler & Mielke, 2005). Indeed, we observe this to be the case for both green and orange patches; they both decrease in *Cr* and diverge in hue (Table S3). More specifically, the hue of both green and orange patches is more SW or UV shifted, respectively, in guppies that have lived under the Green-F89 or Lilac-F55 light conditions (Fig. S3) when compared to Clear-CF guppies. These hue shifts were also predicted based on the percentage difference in a given patches hue under either Green-F89 or Lilac-F55 when compared to the Clear-CF light environment (Table 3). We hypothesis these colour changes are again linked to chromatic adaptation and the importance of stimulating more sensitive guppy photoreceptors at a higher ratio than their less sensitive counterparts in the Green-F89 and Lilac-F55 light environments.

In summary, spectral shapes (colours) diverge in ways that can be predicted from how the light environment influences the efficiency of their colour patch chroma and hue (Fig. 3 and Table S5). We infer that colours diverge in S2 populations to both match their given light conditions and stimulate their most sensitive photoreceptors, resulting in colour signals that enhance visual contrast in different ways under different light conditions.

Possible mechanisms for spectral changes in chromatic colour patches

In guppies, silver, violet and green are all primarily produced by thin film interference and refraction of

incident light waves, via stacked guanine crystals or 'reflecting platelets' found in organelles within iridophores (Kottler *et al.*, 2014). The specific colour produced by the iridophores depends on numerous factors including the orientation of the reflecting platelets relative to each other and the epidermis (e.g. regularity), the number and the distance between the reflecting platelets, the thickness of the cytoplasm and the presence or absence of melanophores (Gundersen & Rivera, 1982; Fujii, 1993, 2000; Grether *et al.*, 2004). Therefore, guppy silver, violet and green signal components may be different in their hue, chroma or luminance as a result of any of these ultrastructure changes.

Additionally, any relationship between the spatial distribution of iridophores and structural patch colour raises the possibility that there could be a trade-off between patch relative area and spectral shape. For example, in both neon tetras (*Paracherodon innesi*) and panther chameleons (*Furcifer pardalis*), increasing the mean distance among guanine nanocrystals causes S-iridophores to shift their selective reflectivity from shorter to longer wavelengths, causing their skin to change from UV to blue or to green to yellow/orange, respectively (Gundersen & Rivera, 1982; Fujii, 1993, 2000; Grether *et al.*, 2004). Hence, we can speculate that a decrease in the mean distance among guanine crystals could shift a guppies' violet's patch reflectivity to shorter wavelengths and the shrinkage might simultaneously decrease its RA (as observed for guppies under the Lilac-F55 environment; Table 3).

In contrast to the structural colours, the ultrastructure of guppy orange patches mainly consists of xanthophores in the stratum spongiosum of the dermis and both xanthophores and iridophores in the hypodermis (Kottler *et al.*, 2014). As a consequence, orange patches may vary in hue, chroma or luminance with an increase or change in iridophores (Grether *et al.*, 2004), as well as due to changes in xanthophore pigment composition (San-Jose *et al.*, 2013).

In guppies, xanthophore pigment composition consists of different ratios of 'yellow' carotenoids (tunaxanthins; λ_{max} near 440 nm) and 'red' pteridines (drosopterins; λ_{max} near 480 nm) (Grether *et al.*, 2001; Hudon *et al.*, 2003). Because carotenoids and drosopterins have different spectral properties, the ratio of two types of pigments affects the shape of the orange spot reflectance spectrum (Grether *et al.*, 2005). Furthermore, drosopterin production is largely heritable (Grether *et al.*, 2005) and synthesized *de novo* from carbohydrates and amino acids, whereas tunaxanthins are obtained by metabolic conversion of dietary carotenoids (Hudon *et al.*, 2003). Our experimental populations were unlikely to be carotenoid-limited because all had access to dietary *Artemia* carotenoids. Given that we experimentally demonstrated that the hue, chroma and luminance of orange colour patches diverge in population under different light environments, we propose that the presence of xanthophore pigments with different spectral properties may allow for greater spectral fine-tuning than could be accomplished with a single pigment type under different signalling conditions.

Taken together, we suggest that divergence in patch components among the three different light environments not only increases a guppy's signal efficacy using different combinations of colours and areas, but might also be achieved by organizing iridophores, xanthophores and melanophores differentially within the skin. Indeed, the hypothesis that colour patch signal adaptation (via either plasticity, selection or a combination of both) can occur by differentially organizing the same toolbox of pigment-expressing cells is well worth future examination.

To date, over 190 vertebrate pigment genes have been classified (e.g. Lorin *et al.*, 2018), including those involved in the most extensively studied of the vertebrate chromatophores, the melanophores (also referred to as melanocytes) (Schartl *et al.*, 2015). For example, genes involved in the melanocortin system play an important role in fish pigmentation (reviewed in Cal *et al.*, 2017). It has also been established that fish chromatophores can be influenced by complex molecular changes that occur in the endocrine and/or nervous systems in response to environmental cues (Fujii, 2000). Furthermore, genes involved in the melanin synthesis pathway appear to be evolutionary conserved in vertebrates (Hoekstra, 2006; Braasch *et al.*, 2008; Lorin *et al.*, 2018), along with other pigment pathways (Lorin *et al.*, 2018). Yet, how many of these genes function, especially in response to environmental changes, remains largely unexplored (for an exception see Galván *et al.*, 2017). Indeed, with so many potential chromatophore pigments types, numbers and shapes capable of generating colour patch diversity, guppies, and teleost fish more generally, are excellent species to further investigate both the complex genetic architecture and the cellular changes that generate complex colouration patterns among both populations and species living in different environments.

Overall, the importance of the complex interplay between structural and pigment colour-producing cells on signal efficacy is beginning to be appreciated in the colouration literature (e.g. Grether *et al.*, 2004). Future examinations of how multiple pigment types differentiate and interact at both the level of the phenotype and genotype under different local environmental conditions (and over different time scales) will be central to understanding the adaptation of sexually selected ornaments in nature.

Conclusions and future directions

In summary, we have provided novel direct experimental evidence that animal colour pattern components are phenotypically different after exposure to different light

environment conditions after a 2-year period. We report that guppy male colour signal components change within populations and diverge among populations living in different light environments in ways predicted from how some of the colour patch statistics vary with light environment. Overall, we found that colour signal components change to become more efficient visual stimuli within their respective light environments. Moreover, the divergence of the chromatic spectral shapes among the different light environments, in addition to relative area (RA) changes, provides further experimental evidence that the colour patches themselves are multicomponent signals that can respond to environmental changes. Indeed, both patch areas and spectral shape (colour + luminance) may change independently or together, through a variety of putative mechanisms. In addition, our work also highlights the importance of not grouping structural colours together because each colour class changes differently in the guppy eye stimulation estimates chroma (*Cr*), hue (*Hu*) and luminance (*Lm*) under different ambient light conditions. While further work is needed to examine the role that both phenotypic plasticity and sexual selection play in colour pattern changes, we believe our results provide validation for the third tenet of the sensory drive hypothesis, that is, changed environmental conditions during signal reception (e.g. ambient light) can select for changes in male visual traits via female perception. Finally, our research has also provided a framework for future experiments that will examine the how these signal component changes directly influence the entire guppy colour pattern. In turn, this will allow us to examine how different signal components work together to form a functional multiple-component signal in a single species living under different light environments.

Acknowledgments

We thank R. Douglas for providing the guppy cornea and lens transmission spectrum; Deakin University for financial support; and Bob Wong, Beata Ujvari and several anonymous reviewers for excellent comments on the manuscript. Guppies (*Poecilia reticulata*) were collected in 2010 with a Queensland Wildlife Parks permit (WITK07655010) and Deakin University ethics approval (A21-2010 and G01-2012). This work was supported by Australian Research Council grants DP110101421 and DP150102817 to J.A.E. The authors declare no conflict of interest.

Author contributions

J.A.E and A.M.K conceived the study and developed and designed the experiments; A.M.K, G.L.C and P.S gathered all experimental data. Data analysis was performed by A.M.K and J.A.E.; A.M.K and J.A.E interpreted the data and wrote the manuscript.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Irradiance and relative cone stimulations for each ambient light condition.

Figure S2 Reflectance spectra for each of the 4 replicate populations from the CF, F89 and F55 light environments for colours that significantly diverged on the basis of permutation tests from Table 2.

Figure S3 Rose diagrams showing orange, green, violet and silver hue values under different light conditions.

Table S1 Colour class patch descriptions

Table S2 Probabilities resulting from permutation tests for differences between colour patch areas within the foundation populations (sample S1).

Table S3 (a) Probabilities resulting from permutation tests for differences between colour patch numbers between samples S1 (2011) and S2 (2013). (b) Probabilities resulting from permutation tests for patch number divergence among S2 (2013) samples.

Table S4 (a) Geometric mean patch length and the probability that females can resolve the size differences. (b) Geometric mean patch height and the probability that females can resolve the size differences

Table S5 Values of hue, chroma and luminance in each light environment in relation to which environment the fish lived in and was tested in.

Received 23 February 2018; revised 14 June 2018; accepted 22 June 2018