

LETTER

It's not just what you have, but how you use it: solar-positional and behavioural effects on hummingbird colour appearance during courtship

Richard K. Simpson*  and Kevin J. McGraw

School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA

*Correspondence: E-mail: rksimpson9@gmail.com

Abstract

Animals exhibit a diversity of colours that can play key roles in mating interactions. However, we presently lack an understanding of the relative importance of the environment, behaviour and natural reflective properties of colourful ornaments in shaping an individual's colour appearance during mating displays. We investigated interactions among structurally based plumage, display environments and courtship shuttle displays of male Costa's hummingbirds (*Calypte costae*) to test how these elements may differentially contribute to colour appearance during shuttles. Male position relative to the sun was the strongest predictor of colour appearance, with shuttle behaviours and feather reflectance playing smaller roles. Furthermore, male solar orientation and shuttling behaviour (e.g. shuttle width) were repeatable among displays, whereas male colour appearance mostly was not. These results emphasise the contributions of behaviour and environment to colour-signalling and suggest that relying on reflectance measurements of colourful ornaments alone provides an incomplete picture of ecologically relevant visual phenotypes of displaying animals.

Keywords

Calypte costae, dynamic coloration, plumage reflectance, sensory drive, shuttle display, structural coloration.

Ecology Letters (2018) 21: 1413–1422

INTRODUCTION

Many animals (e.g. butterflies, birds) exhibit a striking array of colours whose functions include thermoregulation (Stuart-Fox *et al.* 2017), sexual signalling (Bradbury & Vehrencamp 2011) and predator deterrence (Stevens 2015). For colourful traits to evolve as signals, as posited by the sensory drive hypothesis, they must be both discriminable in the environment (Cronin *et al.* 2014) and acted upon by intended receivers (Endler 1992). However, colourful traits are not always statically presented, but can be part of dynamic displays that include changing postures, orientations and movements (e.g. *Anolis* lizards, Fleishman 1992), all of which may affect how the colour appears in space and time (Hutton *et al.* 2015). Thus, to fully understand how colour signals are transmitted, function and evolve, we must holistically study colour ornaments as they are presented and vary in their natural environmental and behavioural contexts.

Per sensory drive model (Endler 1992), many environmental factors can influence colour-signal transmission and evolution, including water depth (Seehausen *et al.* 2008) and vertical location in a forest (Endler 1993; Gomez & Thery 2004). Additionally, animals often interact with the environment by seeking out specific lighting conditions (Endler & Thery 1996; Heindl & Winkler 2003; Seehausen *et al.* 2008; Cronin *et al.* 2014; Simpson & McGraw 2018a) or orienting themselves in specific ways relative to the sun (Hamilton 1965; Rutowski *et al.* 2007; Dakin & Montgomerie 2009; Bortolotti *et al.* 2011) to increase their conspicuousness and/or colour contrast. There are also examples of how animals use behaviour, independent of environment, to increase their colour

conspicuousness, such as animals covering/hiding colour patches and presenting them only in specific situations (Hansen & Rohwer 1986) or using displays to increase colour detection by catching the attention of the receiver (Ord & Stamps 2008). However, environmental and behavioural influences on coloration do not act independently, and recent work on peacocks (*Pavo cristatus*; Dakin & Montgomerie 2013), broad-tailed hummingbirds (*Selasphorus platycercus*; Simpson & McGraw 2018b) and blue moon butterflies (*Hypolimnas bolina*; White *et al.* 2015) demonstrated, by measuring the animal's colourful ornaments as they were used during a display under similar environmental conditions, that colourful ornaments, display behaviours and the environment all interact together to produce colour appearance.

Previous work in *Anolis* lizards examined interactions among male colourful dewlaps, display behaviours and display environment to assess how these components influenced the strength of conspecific responses (i.e. mating, aggression; Fleishman 1992; Persons *et al.* 1999; Macedonia *et al.* 2013). Although this work is important for elucidating receiver behaviour, we do not fully understand the relative importance/strength of the contributions of colourful ornaments, display behaviours and the environment towards colour appearance (i.e. the composite product of sender traits) during signalling events. Other work on colour appearance has either focused on understanding the mechanisms of the interactions between each element (e.g. White *et al.* 2015) or only tested how variation in some (e.g. solar environment; Simpson & McGraw 2018b) but not all three elements predict/influence colour appearance. For example, do males appear more colourful simply because of the natural reflectance properties of their colourful ornament

or because of how they present it behaviourally or relative to the environment?

Angle-dependent structural coloration provides an opportunity to assess the spatiotemporal mechanisms of colour–behaviour–environment dynamics, because the appearance of these structurally coloured ornaments is dependent on both the angles of illumination (i.e. environment) and observation (i.e. receiver; Doucet & Meadows 2009). Additionally, the appearance of angle-dependent structural coloration can be changed over time through behaviour (i.e. as an animal moves and changes illumination/observation angle(s)), and temporal aspects of colour appearance (e.g. flashiness – change in colour/brightness during displays) can be important to receivers and linked to reproductive success (Dakin & Montgomerie 2013). We can also measure the natural reflectance properties (hereafter ‘reflectance’) of a structural colour in a standardized setting (i.e. colour of the ornament without the influence of individual environmental/behavioural variation; e.g. Meadows *et al.* 2011). Altogether, we can comprehensively test whether colour appearance during a display is better predicted by the reflectance properties of a male’s angle-dependent plumage, how individuals behaviourally manipulate their coloration during courtship (e.g. as they move towards the receiver), the environment (e.g. solar position) or a combination of the three (Fig. 1).

We studied the interactions between angle-dependent structural plumage reflectance, display behaviour and the solar environment in Costa’s hummingbirds (*Calypte costae*, Bourcier 1839; Fig. 1) to test the predictive power of each element on male colour appearance during courtship. In Costa’s hummingbirds, as in many hummingbirds within the monophyletic ‘Bee’ tribe (McGuire *et al.* 2014), males, but not females, exhibit a stereotyped, close-range courtship behaviour, called the shuttle display, which is characterised as a male rapidly and repeatedly flying back-and-forth horizontally in front of a perched female, while erecting his colourful throat/crown feathers (Hurly *et al.* 2001; Feo & Clark 2010; Clark 2011; Clark *et al.* 2011, 2012, 2013; Simpson & McGraw 2018b; Fig. 1; Supporting Information videos S1 and S2). These males also possess conspicuous angle-dependent structural plumage patches on their throat and crown (females lack these) that directionally reflect incident lighting (Fig. 1). We video-recorded shuttle displays of free-ranging male Costa’s hummingbirds in the field and later captured these males and plucked their colourful throat feathers. We then used the feathers and spatiotemporally mapped display paths of males to re-create their orientation-and-position-specific shuttles in the field (*sensu* Simpson & McGraw 2018b) to determine the colour appearance of male Costa’s hummingbirds from the

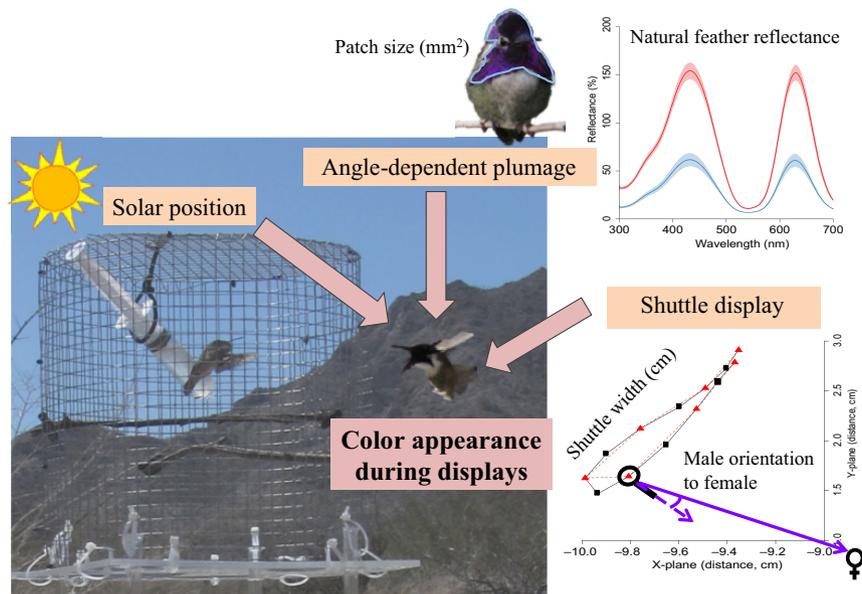


Figure 1 A schematic of the tripartite set of variables that may influence male colour appearance during displays. In the centre of the diagram is a male in mid-shuttle displaying to a female in a cage (centre-left), representing male colour appearance during displays. Illustrated at top-right are the plumage/colour properties of males that may influence colour appearance: male plumage-patch size (crown and gorget; outlined in light blue) and the reflective properties of male angle-dependent structurally coloured feathers (lines indicating average reflectance across males and shaded regions showing standard error; the red line represents plumage reflectance with no feather tilt and blue line represents feather reflectance with a 10° tilt – see text for more details). Situated at top-left are the putative environmental influences on colour appearance: solar elevation and display location relative to the sun. Finally, at bottom-right, we show the behavioural properties of a male shuttle display that may influence colour appearance: male shuttle width, how males orient relative to the female (purple arrows) and the distance between the displaying male and female. The graph depicts a representative average path for a male Costa’s hummingbird shuttle display (black squares and line) by one male. From this average display path, we selected seven representative points (red triangles) to use for our display recreations and photography (see text for details), which closely depict the full average display path (red dashed line). All distances are in centimetres, and the female would be located at the origin (0,0; not shown) and is depicted by the female symbol. Male angle of orientation to the female is measured as the angle between the female’s head (solid purple arrow) and the male’s bill (dashed purple arrow), with both arrows originating near the base of the male’s bill. A cartoon of the male’s head and bill is in black. Error bars are not shown, to improve clarity of visual presentation.

female's point of view (who are always watching the male shuttle with at least one eye; RKS, pers. obs.).

We used an information-theoretic, model-averaging approach to investigate the extent to which feather-reflectance properties, display behaviours and the solar environment explained variation in male colour appearance during the shuttle display. Although there may be significant contributions of plumage reflectance, shuttling behaviours and solar environment on actual colour appearance during courtship displays, we predicted that male behaviour and the position of the sun relative to the male may better predict how a male's colour appears as he displays than the natural plumage reflectance itself, due to the dynamic nature of angle-dependent plumage presentation during shuttling.

Additionally, we tested the repeatability of shuttle behaviours, sun orientations and colour appearances for each male's display, as measuring repeatability of these traits could further explain the interactions between behaviour and the environment and their link to colour appearance (i.e. the result of those interactions). For example, because colour appearance during a display may depend upon behavioural and environmental variation, we would not expect colour appearance to be repeatable if those traits are also not repeatable. We specifically predict that repeatability in male display behaviours, such as angle of orientation to the female, would lead to repeatability in colour flashiness, whereas repeatability in male display position relative to the sun would affect the repeatability of colour appearance, overall, while shuttling repeatability of overall colour appearance while shuttling.

Finally, we tested whether male shuttle locations were positioned (1) to the sun in a consistent way (i.e. environmental effect), (2) to the female in a consistent way (i.e. behavioural effect) or (3) both. We predicted that male Costa's hummingbirds, similar to other avian and non-avian species (including a congener, Anna's hummingbirds, *C. anna*, during dive displays; Hamilton 1965; also see Dakin & Montgomerie 2009; Bortolotti *et al.* 2011), would display with the sun in front of them, to increase their colour conspicuousness, although in a recent study of broad-tailed hummingbird shuttles (Simpson & McGraw 2018b) we showed that males do not shuttle in a specific location relative to the sun. We also predicted that male display location would be determined by the female's location, such that males shuttle as close to the female as possible to best ensure that females can discriminate their coloration and displays (i.e. improve signal efficacy based on distance; How *et al.* 2008) or to prevent females from escaping while males display (Stiles 1982).

MATERIALS AND METHODS

Field-site and capture methods

We studied Costa's hummingbirds during the 2015 breeding season (March) at the University of California, Riverside, Boyd Deep Canyon Desert Research Center (33.648543, -116.376909), in the Colorado Desert (see Supporting Information S1 for ethical approval). We captured female hummingbirds using feeder drop-traps (Russell & Russell 2001) and temporarily housed them in captivity (2–3 days) before

presenting them to males in the field to elicit shuttle displays. We captured males after they were filmed (see more below) at sugar-water feeders on their territories using a combination of drop-traps and mist-net Russell traps (Russell & Russell 2001). Males were consistently found on their same territories before and after filming/capture, and we captured males shortly after their filming (i.e. within 20 days), so we were confident that the males we caught were those who we filmed (Simpson 2017; Simpson & McGraw 2018b). We measured male folded wing chord, bill length and body mass, and plucked feathers ($n = 7–10$ per bird) from their gorget, specifically from the area under their bill, within ~5 mm on either side of the bill (see Supporting Information S2 for justification). Finally, we quantified gorget and crown size (area, in mm²) using photographs of males on their left and right sides following our previously established methods (Simpson & McGraw 2018b). Briefly, using ImageJ (Schneider *et al.* 2012), we measured the pixels in each photo occupied by each half of a male's angle-dependent gorget and crown feathers and summed the two measures to get total plumage-patch area, using each male's bill length to size-calibrate each photo.

Eliciting and filming courtship displays

Following previous methods employed with several hummingbird species (Clark & Feo 2010; Feo & Clark 2010; Clark 2011; Clark *et al.* 2011, 2013; Simpson & McGraw 2018b), we elicited male shuttle displays by presenting one of our two captive females (females were alternated each filming day) in a wire-mesh cylindrical cage with a clear-Plexiglas bottom (30.5 cm tall by 30.5 cm diameter) about 1.3 m off the ground on a male's territory in an open area between his main perches. We placed a high-definition video camera (Sony HDR-CX330; 60 frames/s progressive scan) pointed up, underneath the cage containing the female (Fig. 2), which allowed us to film male movements in the same horizontal plane as the perched female (also the plane in which the male displays; RKS pers. obs.; Supporting Information video S2; Simpson & McGraw 2018b) and film female reactions/positions during the displays (*sensu* Simpson & McGraw 2018b; Supporting Information video 1; see Supporting Information text S2 for additional details).

Quantifying variation in male shuttles

For each recorded shuttle display, we mapped the male's horizontal movement (i.e. display path) frame-by-frame using the open-source video-analysis program Tracker (Brown 2017). Following the methods in Simpson & McGraw (2018b), we measured the x-y coordinates of a male's head through his display movements, as this allowed us to track the position of a male's gorget relative to the female while shuttling (Supporting Information video 1; Fig. 2). Because males exhibit subtle to no vertical movement during their shuttles and display in the same vertical plane of the female (RKS pers. obs.; Supporting Information video S2; Simpson & McGraw 2018b), we did not quantify variation in male vertical positioning. We understand that our method does not perfectly capture every angle and movement of the males as they display, but we are

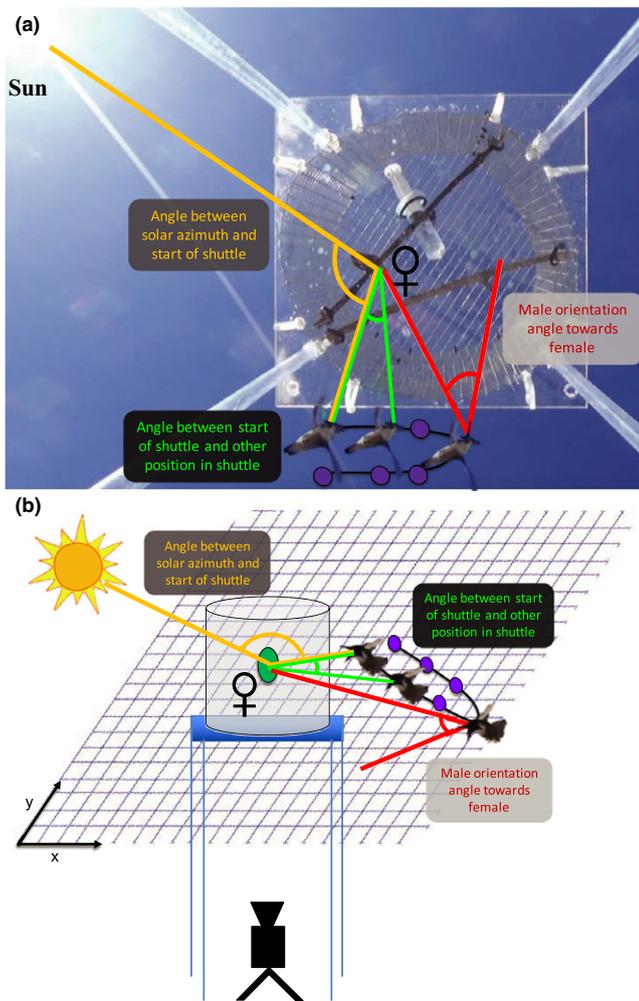


Figure 2 Two-dimensional (2D; a) and three-dimensional (3D; b) visual representation of how we re-created male shuttle displays and the angles we measured. In both panels, the orange text and angles depict how we measured the angle between the solar azimuth and the starting position of the male shuttle path, relative to the female. The green text and angles depict how we measured the angle between the male starting position and all other positions in his display path, relative to the female (see Supporting Information S5 for additional details). The red text and angles depict how we measured male angle of orientation, which is the angle between the male's gorget and female, using the male's bill as a reference since the bill is perpendicular to the part of the gorget from which we plucked feathers. (a) The 2D depiction (taken directly from a shuttle video) of each measured angle. The female is in the centre of the cage and marked with the female symbol; the sun is in the top left-hand corner; and the male is mid-shuttle in the centre bottom (male shown on display positions 1, 2 and 4, while other positions are indicated by purple circles). (b) The 3D depiction (re-created cartoon illustration) of each measured angle. The cage is illustrated by the grey cylinder and is on top of a clear plexiglass bottom (blue square) supported by plastic rods (blue vertical lines); the camera (placed below the cage pointing upwards) is depicted by the black camera cartoon on a tripod; the female is illustrated as the green oval with the female symbol next to it, with the male mid-shuttle on the right side of the cage (male shown on positions 1, 2 and 4 of display, while other positions are indicated by purple ovals). The grid represents the horizontal plane of the perched female, which is the same plane in which the male displays; the x- and y-axes are noted in the bottom left of the grid. All angles depicted in this figure are taken from that horizontal plane. All shuttle display positions in both the 3D and 2D panels are also connected by a black line to illustrate the display path.

confident that the movements we did quantify capture the significant variation in male colour appearance due to shuttle displays (Simpson & McGraw 2018b). For each display, using these coordinates, we calculated the dimensions of an average shuttle cycle (i.e. one back-and-forth movement; in cm; range in number of shuttle cycles = 6–207 cycles across males, with one display only having one complete cycle filmed; Fig. 1). We then calculated the shuttle cycle width (in cm) from this average shuttle cycle, by measuring distance between the turn-around point and the start point of the average shuttle (Fig. 1). We also calculated the number of shuttle cycles per display and the average translational velocity (cm/s) of the average shuttle cycle; however, both measures were significantly correlated with shuttle width ($r > 0.5$, $P < 0.01$), so we removed them from our analyses. For each display bout, we then quantified the angle of the male's plumage orientation towards the female during shuttling, by measuring (from seven representative points from each shuttle cycle, selected based on shuttle shape; Simpson & McGraw 2018b; Fig. 1) the angle between the plane of the centre of the male's gorget (feathers beneath the bill) and the female's head (Fig. 2). These angles were measured from specific frames also using Tracker (Brown 2017). We then calculated an average male-to-female orientation angle for each of the seven positions, and also calculated the average and standard deviation of these seven averaged angles.

We quantified male display location relative to the solar azimuth (and female) and solar elevation during his display using the location of each male's average shuttle cycle relative to compass north, the time and date of the display and a solar calculator (Hoffmann 2017; Fig. 2). We used Rayleigh tests of uniformity from the *circular* R package (Agostinelli & Lund 2013) to test whether a shuttling male exhibited a uniform pattern in display location relative to the sun. To use male display location relative to the sun in mixed linear models, we converted the circular measure of male display location relative to the sun (0 – 360°) to a linear measure – angular deviation in male display location relative to the sun, which ranged from 0° (sun located behind the male as he displayed) to 180° (sun located in front of the male; Simpson & McGraw 2018b).

Finally, we quantified whether the location at which each male shuttled (based on the start point of each shuttle) was the closest possible location to the female, given the presence of the cage. To do this, we identified the closest location on the cage wall to the female, and then calculated the angle between this point and the beginning point in a male's shuttle. Males with an angular distance close to 0° displayed at or near the closest location outside of the cage to the female, while males with a measure close to 180° displayed at the furthest location from the female. We used one-sample *t*-tests to test whether the average male angular distance to the female was significantly different from angular distances of 0° , 45° , 90° , 135° and 180° (similar results were obtained when using circular statistics to calculate the 95% CI of male angular distance to the female).

Plumage reflectance measurements

We followed spectrometric methods of Meadows *et al.* (2011) to quantify angle-dependent reflectance properties of each

male's feathers in a controlled laboratory setting. These methods, tested by Meadows *et al.* (2011) on feathers of the congener Anna's hummingbird, produced highly repeatable results for colour quantification by measuring angle-dependent feathers one at a time (as opposed to in a stacked arrangement). We used an Ocean Optics USB2000 spectrometer and PX-2 pulsed xenon lamp (Dunedin, FL, USA) and set the receiving probe normal to the feathers, while setting the light probe at 41° from the horizontal plane (based on the average solar elevation during male displays; position of light probe relative to receiving probe would be equivalent to a male directly facing the sun while displaying – similar to our results below; Meadows *et al.* 2011). The feathers had their calami facing towards the light probe, mimicking the orientation of male feathers on the hummingbirds. In a dark room, we measured reflectance at ca. 0.4 nm intervals from 300 to 700 nm for six feathers per male, with the feathers flat in the horizontal plane (i.e. males facing the female), and then re-measured them tilted 10° to the right (i.e. an angle representing the maximum male angle of orientation towards the female, exceeded only during three displays to a maximum of 15°), so that we could calculate a measure of angle dependence for each male based on his possible movements (*sensu* Meadows *et al.* 2011; Dakin & Montgomerie 2013; Van Wijk *et al.* 2016). We did not alter the position of the receiver probe while measuring feather reflectance, because we were focused on male behaviours and interactions in this study, rather than female behaviours/responses. The feathers we measured here were the same six feathers that we photographed (see below) to quantify each male's colour appearance during displays. Although our feather-reflectance measurements do not take into account all possible illumination/observation angles (as in Harvey *et al.* 2013; McCoy *et al.* 2018), we did base our spectrometric methods on averaged biologically relevant environmental and behavioural conditions, with the aim of quantifying male feather reflectance in a standardized and comparable way (similar to Dakin & Montgomerie 2013).

We averaged reflectance spectra for six feathers per male (see Fig. 1 for example) and then used avian visual models to assess spectral properties through the eyes of an ultraviolet (UV) sensitive avian visual system in the R package *pavo* (Maia *et al.* 2013), because hummingbirds possess four colour-sensing photoreceptors and can see into the UV spectrum (Herrera *et al.* 2008; but see Odeen & Hästad 2010). We calculated standard tetrachromatic colour variables in avian visual space (Stoddard & Prum 2008) in *pavo* (Maia *et al.* 2013); specifically, hue theta (hereafter: 'red-green-blue' or 'RGB hue'), hue phi (hereafter: 'UV hue'), chroma (r.achieved in *pavo*; Stoddard & Prum 2008; Maia *et al.* 2013) and luminance. We then calculated the angle dependence for each colour variable by taking the absolute difference between each colour variable at no tilt and at 10° tilt. We were unable to neatly compact the four measures of feather reflectance using principal components analysis (PCA), but could collapse angle dependence in luminance, chroma and UV hue into a single principal component (PC; see Supporting Information S3 for details). Higher values of 'feather angle dependence PC' indicated less angle dependence in luminance, chroma and UV hue (Supporting Information Table S1).

Display re-creations and quantifying male colour appearance during displays

We quantified male colour appearance during a display by moving the six gorget feathers we plucked from each male (mounted on black cardstock) through their quantified average shuttle paths in the field and using a full-spectrum DSLR camera (see Supporting Information S4 for photography details) attached to a special lazy-Susan apparatus (described in Simpson & McGraw 2018b) to photograph the feathers from the female's point of view, while also accounting for chromatic adaptation (Stevens *et al.* 2007). This permitted the re-creation of the orientation- and position-specific movements of males during their displays (Simpson & McGraw 2018b; Fig. 2; see Supporting Information S5 for details). We calculated RGB hue, UV hue and chroma (Stoddard & Prum 2008) for each position in each re-created display bout using relative cone stimulation values from multispectral photographs, in the R package *pavo* (Maia *et al.* 2013). Luminance was also calculated from double-cone stimulation from the photos using the *Multispectral Imaging* package in ImageJ (Troschianko & Stevens 2015).

We used average colour and % change in colour during a display as our measures of colour appearance, which were calculated from the tetrachromatic colour variables for each position in a shuttle cycle and highly correlated with our other dynamic colour measurements (i.e. maximum colour, colour standard deviation, colour range; Simpson & McGraw 2018b; see Supporting Information 4 for details). Because of the positive correlations ($r = 0.32\text{--}0.65$) between all % change in colour appearance variables (i.e. RGB hue, UV hue, chroma, luminance), we collapsed them into a single PC (see Supporting Information S3 for details): '% change in coloration PC', with higher values indicating males that had lower % changes in luminance, chroma, RGB hue and UV hue. Additionally, there was a strong negative correlation between average luminance and UV hue appearance during a display ($r = -0.86$, $P < 0.001$), which we collapsed into an 'average luminance and UV Hue PC', with higher values indicating males that were brighter but with less UV reflectance (Supporting Information Table S1; see Supporting Information S3 for details).

Statistical analyses

We used an information-theoretic, model-averaging approach to investigate the extent to which male feather-reflectance properties, display behaviours and solar environment (i.e. solar position as a male displayed) explained variation in male colour appearance during shuttle displays. Briefly, we built global mixed linear models with all our plumage, behavioural and solar environment fixed effects (see Supporting Information S6 for full list) and then used Akaike weights for all subsequent models to calculate the summed weight, or relative importance (RI), for each variable. We also calculated the average beta value for each fixed effect across models. Because summed weights can be misleading in terms of actual importance of a given fixed effect (Galipaud *et al.* 2014), we created a final mixed linear model for each colour-appearance variable, using only fixed effects with an $RI > 0.5$. We only interpreted fixed effects that were significant predictors of a

given colour-appearance variable in these final mixed linear models (see Supporting Information S6 for details).

RESULTS

Effects of male plumage-patch size, feather reflectance, shuttle behaviour and solar environment on male colour appearance during displays

Male display location relative to the solar azimuth was the best and a significant predictor of % change in colour PC

(Relative Importance: RI = 0.89; Fig. 3a) and average chroma appearance during displays (RI = 57; Fig. 3c), with males that shuttled more directly in front of the sun (relative to the female) exhibiting significantly greater changes in their colour appearance (marginal R^2 (mR^2) = 0.29) but also appearing less chromatic (mR^2 = 0.14; Table 1) during their displays. We found the best predictors of average luminance and UV hue PC were male display location relative to the solar azimuth (RI = 0.99), solar elevation (RI = 0.70) and feather UV Hue (RI = 0.72; Fig. 3b), and all three predictors were

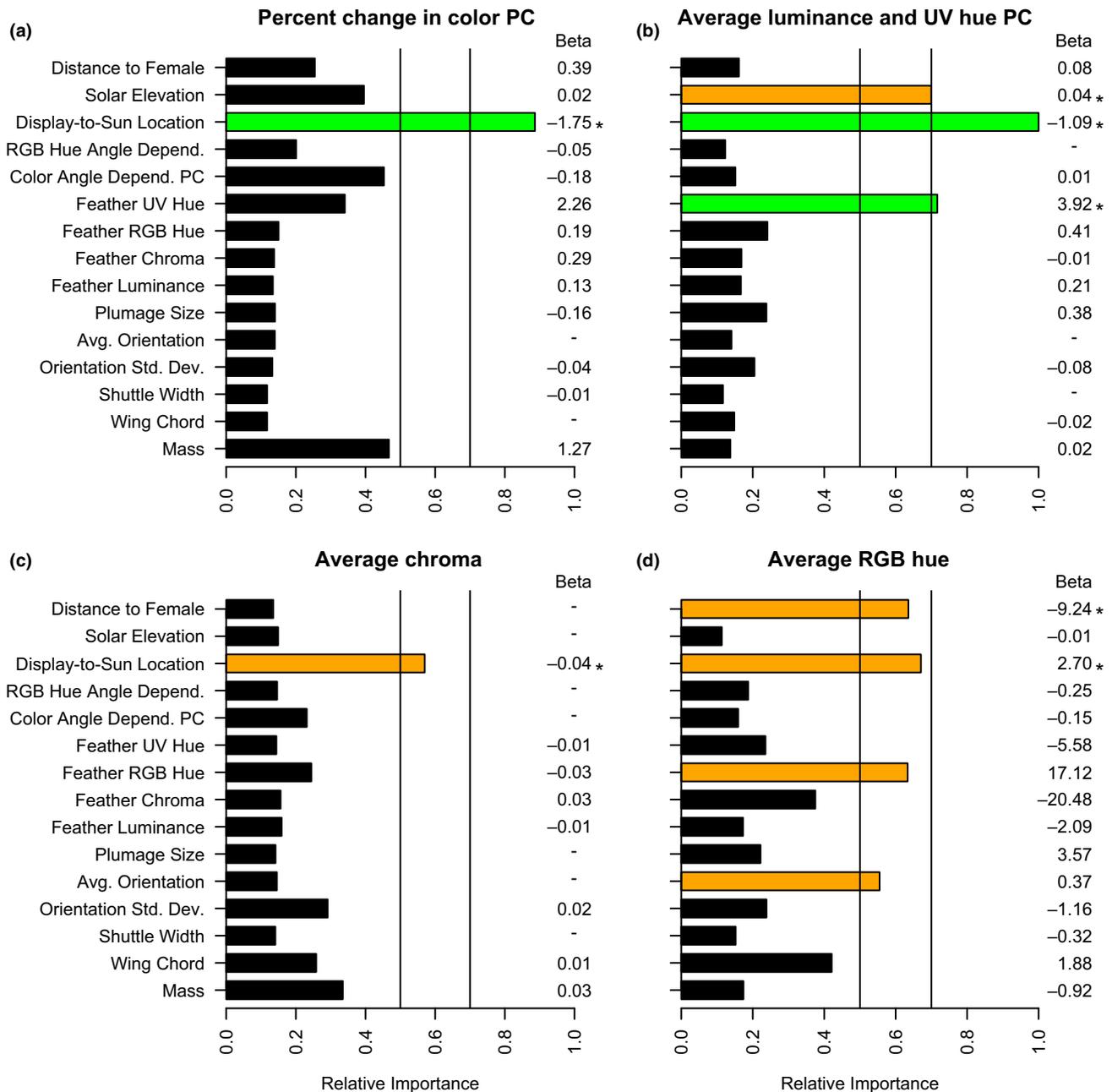


Figure 3 Results from information-theoretic model-averaging analyses, illustrating the relative importance (RI) of each fixed effect (left-hand side) on male colour appearance: (a) Percent change in colour PC during a display; (b) Average luminance and UV Hue PC during a display; (c) Average chroma during a display; and (d) Average RGB hue during a display. Fixed effects with a RI > 0.7 are indicated with green bars, whereas fixed effects with a RI between 0.5 and 0.7 are indicated with orange bars. The average beta for each effect is on the right-hand side of each plot, and asterisks indicate significant effects in the final linear mixed models, which only contained fixed effects with RIs > 0.5.

Table 1 Results from final linear mixed models, containing fixed effects with a relative importance (RI) of 0.5 or greater, in which we tested the influence of male plumage reflectance, shuttling behaviours and solar environment on male colour appearance during courtship displays. Male ID, Julian date and female used to elicit displays were all random effects in these models. Marginal R^2 values are listed below the response variable for each model; they measure the variation explained by the fixed effects in each model. The relative importance of each fixed effect, calculated from model averaging (see methods), is given next to the effect

Response variable	Fixed effects (RI)	Estimate	Std. Err.	<i>t</i> -value	<i>P</i> -value
% Change in Colour PC $mR^2 = 0.29$	Intercept	2.47	0.74	3.32	< 0.01
	Display-to-Sun Location (0.89)	-2.11	0.58	-3.64	< 0.01
Avg. Luminance and UV Hue PC $mR^2 = 0.74$	Intercept	-0.48	0.71	-0.68	0.51
	Solar Elevation (0.70)	0.06	0.02	3.72	< 0.01
	Display-to-Sun Location (0.99)	-1.01	0.13	-7.75	< 0.01
	Feather UV Hue (0.72)	4.80	1.66	2.89	0.02
Avg. Chroma $mR^2 = 0.14$	Intercept	0.53	0.04	13.07	< 0.01
	Display-to-Sun Location (0.57)	-0.07	0.03	-2.27	0.03
Avg. RGB Hue $mR^2 = 0.46$	Intercept	-0.24	14.03	-0.02	0.99
	Distance to Female (0.64)	13.68	5.10	-2.68	0.01
	Display-to-Sun Location (0.67)	3.50	1.34	2.61	0.01
	Feather RGB Hue (0.63)	16.50	8.27	2.00	0.05
	Avg. Orient. Angle (0.56)	0.33	0.24	1.40	0.17

Significant effects are in bold.

significant effects in the final model ($mR^2 = 0.74$; Table 1). Therefore, on average, males that shuttled more directly in front of the sun appeared brighter but with less UV reflectance, while males who shuttled while the sun was higher in the sky and had more UV coloured feathers appeared less bright but with more UV reflectance during displays (Table 1). Finally, the best predictors of average RGB hue appearance were angular distance to the female (RI = 0.64), male display location relative to the solar azimuth (RI = 0.67), feather RGB hue (RI = 0.63) and average orientation angle during a display (RI = 0.56; Fig. 3d), but only angular distance to the female and male display location relative to the solar azimuth were significant effects in the final model ($mR^2 = 0.46$; Table 1). Thus, males that shuttled with the sun more directly in front of them and were closer to the female had plumage that appeared more red-shifted during displays (Table 1).

Repeatability of male display behaviour characteristics and male colour appearance during displays

We found that, across displays, male Costa's hummingbirds had significantly repeatable shuttle widths, display locations relative to the sun and average orientation angles to the female (Table 2). Standard deviation in male orientation angle to the female and angular distance to the female were not significantly repeatable across displays (Table 2). We also found that average male UV hue appearance was significantly repeatable across displays, but that no other average colour appearance or % change in colour appearance variables were significantly repeatable (Table 2).

Environmental and behavioural drivers of male display location

We found that shuttling male Costa's hummingbirds did not display in a uniform spatial pattern relative to the sun (Fig. 4) and instead significantly displayed with the sun in front of them (Avg. \pm SD: $218.0^\circ \pm 62.9^\circ$; Vector Length = 0.55;

Table 2 Repeatability estimates for male display location relative to the sun, shuttle display components and colour appearance variables

Behavioural/colour variable	Repeatability	$F_{8,18}$	<i>P</i> -value
Display-to-Sun Location	0.36	2.67	0.04
Distance to Female	0.00	0.99	0.48
Shuttle Width	0.33	2.45	0.04
Avg. Orientation Angle to Female	0.37	2.73	0.04
Std. Dev. Orientation Angle to Female	-0.11	0.71	0.68
Percent Change in Luminance	-0.28	0.36	0.93
Percent Change in Chroma	0.24	1.93	0.12
Percent Change in RGB Hue	0.17	1.61	0.19
Percent Change in UV Hue	-0.06	0.84	0.58
Avg. Display Luminance	0.29	2.23	0.08
Avg. Display Chroma	-0.21	0.48	0.85
Avg. Display RGB Hue	0.09	1.30	0.30
Avg. Display UV Hue	0.43	3.25	0.02

Significant repeatability scores are marked in bold.

Table S6). We also found that males displayed at a specific angular distance from the female (Avg. \pm SD: $40.0^\circ \pm 29.2^\circ$; Vector Length = 0.88); this location was not the closest location possible outside the cage relative to the female (i.e. 0°), nor the furthest from the female (i.e. 180°), but in between (Table S7).

DISCUSSION

We investigated variation in angle-dependent structurally coloured plumage reflectance, shuttling behaviour and solar environment in male Costa's hummingbirds to understand how these traits and their interactions drive male colour appearance during courtship displays. We found partial support for our prediction that male display location relative to the sun (i.e. the environment) was the strongest predictor of male colour appearance during displays, and that the reflectance properties of male structurally coloured feathers had less predictive power (i.e. only one significant predictor across all colour-appearance

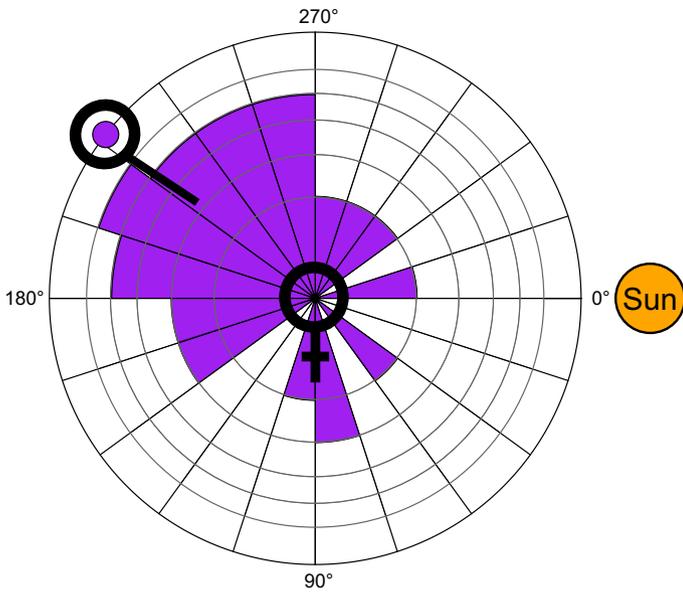


Figure 4 A circular distribution diagram of shuttle displays by male Costa's hummingbirds relative to the sun, which significantly face the sun in a non-uniform pattern. Filled in purple cells in the inner circles represent the number of males in a given male position bin ($n = 1-6$, respectively; bins = 18°). The purple point on the outer circle represents average display location relative to the sun for males. This average was not statistically different from 180° (sun in front of the male) but was significantly different from $0^\circ/360^\circ$ (sun behind the male). Location of sun is at 0° (indicated by the cartoon of the sun), the female (indicated by the female symbol) is located in the centre of the cage/diagram and the males, which would display around the cage, were always roughly facing inward towards the female (indicated by the cartoon of the male head around the average display location relative to the sun).

models). Although we also found male shuttle display behaviours (e.g. shuttle width) to be less predictive of colour appearance, we did find support that male display behaviour (i.e. distance from the female) predicted average RGB hue during displays. Altogether these results provide evidence that elements of behaviour, plumage and the environment interact to produce a male's colour appearance during display, but that the strongest predictor of colour appearance is the environment (i.e. male solar orientation).

Consistent with the sensory drive hypothesis and previous work on peacocks and hummingbirds (Dakin & Montgomerie 2013; Simpson & McGraw 2018b), we found that individuals that display with the sun more directly in front of them appeared brighter, more colourful and flashier (i.e. exhibit greater colour change), but these other studies did not evaluate the relationship between reflectance properties of the colourful ornament and colour appearance during display. We found that males whose feathers were naturally the most colourful/brightest were not necessarily those males that appeared most colourful/bright while displaying. Furthermore, we found that angle dependence of male plumage reflectance was not related to colour flashiness during courtship. The general lack of relationships between feather-reflectance properties and colour appearance during display demonstrate that animal coloration can be both

environmentally and behaviourally manipulated, regardless of what an individual inherently looks like (i.e. while not displaying the trait). Thus, our results support the notions that (1) animal coloration should be studied as a dynamic trait in space and time and not be exclusively measured outside the context of the environment and display (Hutton *et al.* 2015) and (2) behavioural and environmental components of a display can be just as or more important in predicting colour appearance than the ornament's natural reflectance properties (as per the sensory drive hypothesis; Endler 1992).

We also sought to understand the drivers of male display location and found support for our prediction that males shuttled while facing the sun, which is contrary to our recent work on a related North American hummingbird (*Selasphorus platycercus*; Simpson & McGraw 2018b), but consistent with other work on sun-directed displays in birds and butterflies (Rutowski *et al.* 2007; Dakin & Montgomerie 2009; Bortolotti *et al.* 2011). Interestingly, although male display behaviours are often shaped by female behaviours or how close the female is relative to the displaying male (Patricelli *et al.* 2002; How *et al.* 2008; Echeverri *et al.* 2017), we found that male Costa's hummingbirds did not display as close to the female as possible. In fiddler crabs (*Uca perplexa*), males increase their claw-waving display intensity as females approach them (How *et al.* 2008), and jumping spiders (*Habronattus pyrrithrix*) alter their orientations relative to the female's position as they display (Echeverri *et al.* 2017). Other males will alter their behaviours based on female feedback, such as male satin bowerbirds (*Ptilonorhynchus violaceus*), which temper their displays based on how startled females are (Patricelli *et al.* 2002). Males from most 'Bee' hummingbird species chase females into small trees/bushes and then display to them, and we have previously observed males of this and other species shifting their shuttle locations in response to female movement (Simpson & McGraw 2018b; C.J. Clark, pers. comm.). Rather, our results here suggest that displaying male *C. costae* may be more focused on their position relative to the sun, and since we found that male position relative to the sun was a strong predictor of male colour appearance (stronger than male distance to the female as well), we suggest that male display behaviours evolved to maximize colour appearance/presentation in their given display environment.

By measuring the repeatability of male behavioural, environmental and ornamental-plumage traits, we can further understand the interactions (i.e. colour appearance) among colourful ornaments, behaviours and the environment. For example, since male colour appearance is the result of interactions between the reflectance properties of male feathers, shuttle displays and the environment, we would expect the repeatability of male colour appearance to depend upon how repeatably males behave and position themselves relative to the sun across displays. We found that a male's shuttle width, average orientation angle relative to the female and display position relative to the sun were significantly repeatable across his display bouts, but that the variation in male orientation towards, as well as his distance to,

the female were not. We also found that only one – average UV hue colour appearance – out of eight of the male colour-appearance variables was significantly repeatable across displays. The lack of repeatability in some male behaviours, such as variation in male orientation towards the female, could result in colour appearance being less repeatable, especially with regards to flashiness. Additionally, the lack of repeatability of variance in male orientation towards a female (i.e. differences in how males orient their plumage towards females throughout a display) also would generate variation in an individual male's colour appearance among displays. Comparing the repeatability of signalling traits (e.g. behaviour) and the product of their interactions (e.g. colour appearance) is a great way to better understand how interactions between individual traits are linked to the product of their interactions, and we believe this novel idea will be helpful in future studies on interactions and emergent properties of animal signals.

Overall, our study illustrates the importance of considering complex contributions of behavioural and environmental variation in understanding the dynamic properties of ornate animal colours. Our findings that behaviours and the solar environment are equal and better predictors, respectively, of colour appearance during a courtship display than natural feather reflectance are unique and generate interesting questions about how other animals may alter their colour appearance behaviourally or environmentally and how these traits evolved through sensory drive. Finally, we found that the environment (i.e. solar position) predicts courtship display location (in addition to male colour appearance) instead of male distance from the female, further illustrating the importance of the environmental influences on dynamic colour traits. Altogether, our study demonstrates the need to more comprehensively study signalling traits and their interactions, to better understand the mechanisms and functions of signal use in natural environments.

ACKNOWLEDGEMENTS

We thank Russell A. Ligon, Brett M. Seymoure, Ronald L. Rutowski, Stephen C. Pratt, Jimmy A. McGuire, Christopher J. Clark, Meghan E. Duell and the McGraw lab for their support, helpful discussions with the background and methods of this study, and for providing feedback on this manuscript. Further, we thank Allan Muth and the University of California Boyd Deep Canyon Desert Research Center for their help and logistical support in the field. We thank Alysia Apple, Avery Underwood, Bailey Ash, Christina Piarowski and Jade Gates for their help with video and image analysis. Finally, we thank Tim Coulson, Greg Grether and three anonymous reviewers for helpful and insightful feedback on this manuscript. This work was supported by Arizona State University (ASU), the ASU chapter of Sigma Xi Grants-in-Aid of Research, the Animal Behavior Society Student Research Grant, the Society for Integrative and Comparative Biology Grants-in-Aid of Research, and T & E Inc. Grants for Conservation Biology Research.

AUTHORSHIP

Both authors developed the conceptual framework of the study. RKS collected data and conducted the analyses. RKS wrote the first draft of the manuscript, and both authors contributed substantially to revisions.

DATA ACCESSIBILITY STATEMENT

Analyses reported in this article can be reproduced using the data provided by Simpson & McGraw 2018c. <https://doi.org/doi:10.5061/dryad.9095kg7>.

REFERENCES

- Agostinelli, C. & Lund, U. (2013). R package “circular”: Circular Statistics (version 0.4-7).
- Bortolotti, G.R., Stoffel, M.J. & Galva, I. (2011). Wintering Snowy Owls *Bubo scandiacus* integrate plumage colour, behaviour and their environment to maximize efficacy of visual displays. *The Ibis*, 153, 134–142.
- Bradbury, J.W. & Vehrencamp, S.L. (2011). *Principles of Animal Communication*. Sinauer Associates Inc., Sunderland, MA.
- Brown, D. (2017). Tracker: video analysis and modeling tool. Version 4.8.0. Available at: <https://physlets.org/tracker/>. Last accessed 03/17/2017.
- Clark, C.J. (2011). Wing, tail, and vocal contributions to the complex acoustic signals of courting Calliope hummingbirds. *Curr. Zool.*, 57, 187–197.
- Clark, C.J. & Feo, T.J. (2010). Why do *Calypte* hummingbirds “sing” with both their tail and their syrinx? An apparent example of sexual sensory bias. *Am. Nat.*, 175, 27–37.
- Clark, C.J., Feo, T.J. & Escalante, I. (2011). Courtship displays and natural history of scintillant (*Selasphorus scintilla*) and Volcano (*S. flammula*) Hummingbirds. *Wilson J. Ornithol.*, 123, 218–228.
- Clark, C.J., Feo, T.J. & Bryan, K.B. (2012). Courtship displays and sonations of a hybrid male broad-tailed × black-chinned hummingbird. *Condor*, 114, 329–340.
- Clark, C.J., Feo, T.J. & van Dongen, W.F.D. (2013). Sounds and Courtship Displays of the Peruvian Sheartail, Chilean Woodstar, Oasis Hummingbird, and a Hybrid Male Peruvian Sheartail × Chilean Woodstar. *Condor*, 115, 558–575.
- Cronin, T.W., Johnsen, S., Marshall, N.J. & Warrant, E.J. (2014). *Visual Ecology*. Princeton University Press, Princeton, NJ.
- Dakin, R. & Montgomerie, R. (2009). Peacocks orient their courtship displays towards the sun. *Behav. Ecol. Sociobiol.*, 63, 825–834.
- Dakin, R. & Montgomerie, R. (2013). Eye for an eyespot: how iridescent plumage ocelli influence peacock mating success. *Behav. Ecol.*, 24, 1048–1057.
- Doucet, S.M. & Meadows, M.G. (2009). Iridescence: a functional perspective. *J. R. Soc. Interface*, 6(Suppl 2), S115–S132.
- Echeverri, S.A., Morehouse, N.I. & Zurek, D.B. (2017). Control of signaling alignment during the dynamic courtship display of a jumping spider. *Behav. Ecol.*, 28, 1445–1453.
- Endler, J. (1992). Signals, signal conditions, and the direction of evolution. *Am. Nat.*, 139, S125–S153.
- Endler, J. (1993). The color of light in forests and its implications. *Ecol. Monogr.*, 63, 1–27.
- Endler, J. & Thery, M. (1996). Interacting effects of lek placement, display behavior, ambient light, and color patterns in three neotropical forest-dwelling birds. *Am. Nat.*, 148, 421–452.
- Feo, T.J. & Clark, C.J. (2010). The Displays and Sonations of the Black-Chinned Hummingbird (Trochilidae: *Archilochus alexandri*). *Auk*, 127, 787–796.
- Fleishman, L. (1992). The influence of the sensory system and the environment on motion patterns in the visual displays of anoline lizards and other vertebrates. *Am. Nat.*, 139, 536–561.

- Galipaud, M., Gillingham, M.A.F., David, M. & Dechaume-Moncharmont, F.X. (2014). Ecologists overestimate the importance of predictor variables in model averaging: a plea for cautious interpretations. *Methods Ecol. Evol.*, 5, 983–991.
- Gomez, D. & Thery, M. (2004). Influence of ambient light on the evolution of colour signals: comparative analysis of a neotropical rainforest bird community. *Ecol. Lett.*, 7, 279–284.
- Hamilton, W. III (1965). Sun-oriented display of the Anna's Hummingbird. *Wilson Bull.*, 77, 38–44.
- Hansen, A.J. & Rohwer, S. (1986). Coverable badges and resource defence in birds. *Anim. Behav.*, 34, 69–76.
- Harvey, T.A., Bostwick, K.S. & Marschner, S. (2013). Directional reflectance and milli-scale feather morphology of the African Emerald Cuckoo, *Chrysococcyx cupreus*. *J. R. Soc. Interface*, 10, 20130391.
- Heindl, M. & Winkler, H. (2003). Vertical lek placement of forest-dwelling manakin species (Aves, Pipridae) is associated with vertical gradients of ambient light. *Biol. J. Linn. Soc.*, 80, 647–658.
- Herrera, G., Zagal, J.C., Diaz, M., Fernández, M.J., Vielma, A., Cure, M. *et al.* (2008). Spectral sensitivities of photoreceptors and their role in colour discrimination in the green-backed firecrown hummingbird (*Sephanoides sephaniodes*). *J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol.*, 194, 785–794.
- Hoffmann, T. (2017). SunCalc. Available at: <https://www.suncalc.org>. Last accessed 12/29/2017.
- How, M.J., Hemmi, J.M., Zeil, J. & Peters, R. (2008). Claw waving display changes with receiver distance in fiddler crabs, *Uca perplexa*. *Anim. Behav.*, 75, 1015–1022.
- Hurly, T., Scott, R. & Healy, S. (2001). The function of male rufous hummingbirds. *Condor*, 103, 647–651.
- Hutton, P., Ligon, R.A., McGraw, K.J., Seymoure, B.M. & Simpson, R.K. (2015). Dynamic color communication. *Curr. Opin. Behav. Sci.*, 6, 41–49.
- Macedonia, J.M., Clark, D.L., Riley, R.G. & Kemp, D.J. (2013). Species recognition of color and motion signals in *Anolis grahami*: evidence from responses to lizard robots. *Behav. Ecol.*, 24, 846–852.
- Maia, R., Eliason, C.M., Bitton, P.P., Doucet, S.M. & Shawkey, M.D. (2013). pavo: an R package for the analysis, visualization and organization of spectral data. *Methods Ecol. Evol.*, 4, 906–913.
- McCoy, D.E., Feo, T., Harvey, T.A. & Prum, R.O. (2018). Structural absorption by barbule microstructures of super black bird of paradise feathers. *Nat. Commun.*, 9, 1.
- McGuire, J.A., Witt, C.C., Remsen, J.V., Corl, A., Rabosky, D.L., Altshuler, D.L. *et al.* (2014). Molecular phylogenetics and the diversification of hummingbirds. *Curr. Biol.*, 24, 1–7.
- Meadows, M.G., Morehouse, N.I., Rutowski, R.L., Douglas, J.M. & McGraw, K.J. (2011). Quantifying iridescent coloration in animals: a method for improving repeatability. *Behav. Ecol. Sociobiol.*, 65, 1317–1327.
- Odeen, A. & Håstad, O. (2010). Pollinating birds differ in spectral sensitivity. *J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol.*, 196, 91–96.
- Ord, T.J. & Stamps, J.A. (2008). Alert signals enhance animal communication in “noisy” environments. *Proc. Natl Acad. Sci.*, 105, 18830–18835.
- Patricelli, G.L., Uy, J.A., Walsh, G. & Borgia, G. (2002). Male displays adjusted to female's response. *Nature*, 415, 279–280.
- Persons, M.H., Fleishman, L.J., Frye, M.A. & Stimphil, M.E. (1999). Sensory response patterns and the evolution of visual signal design in anoline lizards. *J. Comp. Physiol. - A Sensory, Neural Behav. Physiol.*, 184, 585–607.
- Russell, S.M. & Russell, R.O. (2001). The North American banders' manual for banding hummingbirds.
- Rutowski, R.L., Macedonia, J.M., Merry, J.W., Morehouse, N.I., Yturralde, K., Taylor-Taft, L. *et al.* (2007). Iridescent ultraviolet signal in the orange sulphur butterfly (*Colias eurytheme*): spatial, temporal and spectral properties. *Biol. J. Linn. Soc.*, 90, 349–364.
- Schneider, C.A., Rasband, W.S. & Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods*, 9, 671–675.
- Seehausen, O., Terai, Y., Magalhaes, I.S., Carleton, K.L., Mrosso, H.D.J., Miyagi, R. *et al.* (2008). Speciation through sensory drive in cichlid fish. *Nature*, 455, 620–626.
- Simpson, R.K. (2017). Courtship and territorial behaviors of three hummingbird species in Arizona. *Arizona Birds*, 2017, 1–7.
- Simpson, R.K. & McGraw, K.J. (2018a). Multiple signaling in a variable environment: expression of song and color traits as a function of ambient sound and light. *Biotropica*, 50, 531–540.
- Simpson, R.K. & McGraw, K.J. (2018b). Two ways to display: male hummingbirds show different color-display tactics based on sun orientation. *Behav. Ecol.*, 29, 637–648.
- Simpson, R.K. & McGraw, K.J. (2018c). Data from: It's not just what you have, but how you use it: solar-positional and behavioral effects on hummingbird color appearance during courtship. Dryad Digital Repository. <https://doi.org/doi:10.5061/dryad.9095kg7>
- Stevens, M. (2015). Anti-predator coloration and behaviour: a longstanding topic with many outstanding questions. *Curr. Zool.*, 61, 702–707.
- Stevens, M., Parraga, C.A., Cuthill, I.C., Partridge, J.C. & Troscianko, T.S. (2007). Using digital photography to study animal coloration. *Biol. J. Linn. Soc.*, 90, 211–237.
- Stiles, F.G. (1982). Aggressive and courtship displays of the male Anna's hummingbird. *Condor*, 84, 208–225.
- Stoddard, M.C. & Prum, R.O. (2008). Evolution of avian plumage color in a tetrahedral color space: a phylogenetic analysis of new world buntings. *Am. Nat.*, 171, 755–776.
- Stuart-fox, D., Newton, E. & Clusella-trullas, S. (2017). Thermal consequences of colour and near-infrared reflectance. *Philos. Trans. R. Soc. B Biol. Sci.*, 372, 2016.0345.
- Troscianko, J. & Stevens, M. (2015). Image calibration and analysis toolbox - a free software suite for objectively measuring reflectance, colour and pattern. *Methods Ecol. Evol.*, 6, 1320–1331.
- Van Wijk, S., Bélisle, M., Garant, D. & Pelletier, F. (2016). A reliable technique to quantify the individual variability of iridescent coloration in birds. *J. Avian Biol.*, 47, 227–234.
- White, T.E., Zeil, J. & Kemp, D.J. (2015). Signal design and courtship presentation coincide for highly biased delivery of an iridescent butterfly mating signal. *Evolution*, 69, 14–25.

SUPPORTING INFORMATION

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

Editor, Greg Grether

Manuscript received 16 May 2018

First decision made 6 June 2018

Manuscript accepted 19 June 2018