Two ways to display: male hummingbirds show different color-display tactics based on sun orientation

Running header: Broad-tailed hummingbird coloration and sun orientation

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ABSTRACT

Animals exhibit a diversity of ornaments and courtship behaviors, which often co-occur and are used for communication. The sensory drive hypothesis states that these traits evolved and vary due to interactions with each other, the environment, and signal receiver. However, interactions between colorful ornaments and courtship behaviors, specifically in relation to environmental variation, remain poorly understood. We studied male iridescent plumage (gorgets), display behavior, and sun orientation during courtship flights (shuttle displays) in broad-tailed hummingbirds (*Selasphorus platycercus*), to understand how these traits interact in both space and time to produce the perceived coloration of males. We also tested how gorget coloration varies among males based on their plumage, behavioral, and morphological characteristics. In contrast with previous work on other animals, we found that displaying males did not directionally face the sun, but instead displayed on a continuum of solar orientation angles. The gorgets of males who tended to face the sun during their displays appeared flashier (i.e. exhibited greater color/brightness changes), brighter, and more colorful, whereas the gorgets of males who tended to not face the sun were more consistently reflective (i.e. little color change) and had greater UV reflectance. We found that males who produced consistent colors had larger gorgets, whereas males with flashier gorgets were better able to maintain their angles of orientation towards the female. Our study illustrates how visual traits interact in complex ways with each other and the environment and how males of the same species can use multiple tactics to dynamically display their coloration.

Key words: Broad-tailed hummingbird, courtship, dynamic coloration, iridescence, *Selasphorus platycercus*, sensory drive

LAY SUMMARY

Male broad-tailed hummingbirds use two different courtship-display tactics to show off their iridescent throat coloration based on how they orient to the sun. Some males tended to face the sun while courting females, creating a flashy color-display, while others tended to not face the sun, making them appear consistently colorful. The males with flashier displays were better able to maintain their orientation towards females, while males who appeared more consistently colored had larger throat patches.

INTRODUCTION

Animals exhibit a wide diversity of ornamental traits and courtship behaviors. Many animals possess these traits together, and use them to communicate, such as in mate attraction or competitive interactions (Andersson 1994; Bradbury and Vehrencamp 2011). Regardless of their communicative function, selection will favor signals that can be effectively transmitted through the environment so they are detectable and conspicuous to the intended receivers at the appropriate locations and times (Endler 1992). The sensory drive hypothesis predicts that the diversity of ornamental traits and display behaviors evolved through selection acting upon transmission efficacy across species and environments (Endler 1992; White and Kemp 2015), and has been used to explain ornament diversity across environments in several clades (e.g. manakins: Pipridae, Endler and Thery 1996; Heindl and Winkler 2003a; surfperch: Embiotocidae, Cummings 2007; and African cichlids: Cichlidae, Seehausen et al. 2008). However these studies typically do not address the role of or interactions between multiple, often co-occurring elaborate display features. For example, courtship behaviors can modulate the transmission efficacy and perception of a color patch (Hutton et al. 2015), because animals either manipulate the color patch itself (e.g. cover it; Hansen and Rohwer 1986) or alter the environment in which they display (Uy and Endler 2004). In these dynamic communication systems, the overall presentation and perception of an ornament during a display is the product of the interactions between the morphological ornament (e.g. reflectance, directionality), behavioral display (e.g. posture, orientation), and environment (Dakin and Montgomerie 2013; White et al. 2014; Hutton et al. 2015).

Colorful ornaments provide some of the most interesting systems to study sensory drive, as many colorful animals also perform behavioral displays, and the perception of colorful traits can be greatly affected by the environment (Endler 1992; Endler 1993; Hutton et al. 2015). Previous work has examined the interactions between colorful ornaments and pertinent characteristics of the environment, such as the lighting conditions (Endler and Thery 1996; Johnson 2000; Heindl and Winkler 2003a; Heindl and Winkler 2003b; Chapman et al. 2009) and/or the background against which the color is presented (Uy and Endler 2004; Uetz et al. 2010). Animals may orient their displays toward the sun (Rutowski et al. 2007; Dakin and Montgomerie 2009; Dakin and Montgomerie 2013) or seek out specific light environments (e.g. forest light gaps) to best enhance their coloration and/or contrast (Endler and Thery 1996; Heindl and Winkler 2003a; Heindl and Winkler 2003b). In some cases, behaviors associated with color signaling have also been studied, such as an individual moving between environments (e.g. manakins: Pipridae, Heindl and Winkler 2003a; Heindl and Winkler 2003b). Yet there can be more complex behavioral interactions where coloration dynamically interacts with the environment due to specialized body movements or orientations relative to the environment (Rutowski et al. 2007; Dakin and Montgomerie 2013; White et al. 2014; Hutton et al. 2015).

There are many examples in animals of how colorful ornaments and behavioral displays are presented and interact sequentially (e.g. *Monarcha* flycatchers - song perceived first, then color, Uy and Safran 2013) or simultaneously (e.g. butterflies - color and behavior perceived at same time, Rutowski et al. 2007; White et al. 2014). In some cases, it is thought that colorful traits may increase the detectability or discriminability of some or all of a display behavior (Hebets and Uetz 2000; Uetz et al. 2009; Byers et al. 2010), and this can be especially true when the display behaviors are rapid or complex (e.g. manakins*;* Pipridae*;* Prum 1990; Barske et al. 2011) or viewed at longer distances (suggested in Zanollo et al. 2013). For example, wolf spider (*Schizocosa ocreata* & *S. rovneri*) leg tufts used during a display have been found to increase the likelihood of a male being detected (Uetz et al. 2009). Other work has suggested that behavioral displays increase the detectability or discriminability of a color signal, such as in great bustards (*Otis tarda*) that lift their white tails towards the sun during courtship (Olea et al. 2010) or *Anolis* lizards perform a pushup alert display to increase to detectability of their full display (Ord and Stamps 2008). Behaviors can also change the environment for displaying or the color patch itself, such as in golden-collared manakins (*Manacus vitellinus*) and great bowerbirds (*Ptilonorhynchus nuchalis*) that behaviorally alter their display court by clearing leaf litter and selectively showing colorful decorations, respectively, to improve color (plumage or object) contrast against the background (Uy and Endler 2004; Endler et al. 2014) or in red-winged blackbirds (*Agelaius phoeniceus*) that reveal their hidden colorful epaulets during social encounters (Hansen and Rohwer 1986). In either case, one trait enhances the other to improve overall transmission efficacy, which has important implications for how these traits evolved (Endler 1992; White and Kemp 2015).

Iridescent coloration in animals offers a striking example of how behavioral interactions with a color patch are important for the transmission efficacy of both color and display behaviors. The appearance of iridescent coloration (i.e. hue) depends on the angles of observation and illumination (Doucet and Meadows 2009), and some animals possess highly directional iridescent coloration that is only colorful/detectable at specific and often narrow observation/illumination angles (e.g. *Lacerta schreibeiri* lizards, Pérez i de Lanuza and Font 2014). Thus, iridescent coloration may rapidly change as animals move or change how they orient themselves towards the light source and observer during courtship, and these angle-dependent properties may allow individuals to either optimally/directionally present their coloration in a highly consistent (i.e. always-on) way or to flash on/off to the receiver in a given environment (Doucet and Meadows 2009). Recent work in male peafowl (*Pavo cristatus*) and blue moon butterflies (*Hypolimnas bolina*) has demonstrated how iridescently colored males orient themselves at specific angles relative to the sun and receiver to produce flashier and/or more colorful displays (Dakin and Montgomerie 2009; White et al. 2014), and males that are more colorful and/or flashy obtain greater reproductive success (Kemp 2007; Dakin and Montgomerie 2013). This work laid the foundation for testing if or how more complex courtship behaviors may interact with both iridescent coloration and the environment to produce the colors perceived by the receiver, and how this interaction might shape the evolution of dynamic colors.

We studied the interactions between iridescent coloration and courtship behavior and how both traits interact with the environment (i.e. the sun) in broad-tailed hummingbirds (*Selasphorus platycercus*). Broad-tailed hummingbirds, like many hummingbird species, possess conspicuous iridescent color patches, and in this species their iridescent patch is located on the throat (gorget) in males (females lack this patch) and is highly angle dependent (Supplementary video S1). Broad-tailed hummingbirds also are part of a monophyletic tribe, the bee hummingbirds (McGuire et al. 2014), almost all of which possess a distinct, close-range courtship behavior called the shuttle display (Hurly et al. 2001; Feo and Clark 2010; Clark 2011; Clark et al. 2011, 2012, 2013). Shuttles are characterized by a male repeatedly and rapidly flying back and forth (i.e. in a horizontal plane) in front of a female and erecting his colorful ventral feathers to create a larger, flatter surface (e.g. Clark 2011; Supplementary videos S2 and S3). Acoustic components of shuttle displays have been characterized (e.g. Clark 2011; Clark et al. 2012, 2013), but visual elements of these displays are virtually unstudied and provide an ideal system to investigate how morphologies like coloration may interact dynamically with behavior and the environment (i.e. sources of illumination such as the sun and sky) during courtship.

We video-recorded naturally occurring shuttle displays of male broad-tailed hummingbirds and later collected iridescent throat feathers from captured males, so that we could recreate the orientation- and position-specific displays in the field to measure what male hummingbirds looked like from the female's perspective. We used these data both to evaluate the mechanisms of how male color and behavior interacted with and varied by the environment, because a male’s perceived coloration by a female during a display could be greatly influenced by how he oriented himself relative to the sun and female. One possibility is that males display while facing the sun, similar to Anna's hummingbird dive displays (*Calypte anna*; Hamilton III 1965) and other avian and non-avian species (Dakin and Montgomerie 2009; Olea et al. 2010; Bortolotti et al. 2011; Klomp et al. 2017), and optimize their conspicuousness by reflecting more light with their colorful traits. Alternatively, it is possible that males vary their orientation towards the sun and/or female to produce a more flashy (i.e. on/off) display (White et al. 2014).

All of this, however, ignores the fact that males can change the orientation of their feathers as they move in space, thus potentially creating even more complex dynamics for how reflective/on-off they appear in the eyes of the viewing female. Thus, ultimately the primary goal of this study was to describe the spatiotemporal mechanics of color-display-environment interactions and if/how they result in male color variation during shuttle displays across individuals. By gathering additional data about male phenotype, we were also able to test how male courtship behavior, plumage reflectance, and morphological traits varied with perceived male coloration by females during a display, which allows us to use color-display dynamics to propose possible efficacy-based functions of male coloration during courtship.

METHODS:

Field site and capture methods

We studied broad-tailed hummingbirds during their breeding season in Coconino National Forest, near Elden Springs (35.227336, -111.600045) and Lake Marshall (35.130207, -111.533226), in Northern Arizona, USA in June and July of 2014 and 2017. At both sites we captured female hummingbirds using feeder drop-traps (Russell and Russell 2001), and these females were temporarily housed in captivity (fed with Nektar-plus solution; Nekton, Pfozheim, Germany) and subsequently used to elicit male shuttle displays (see below). Males were captured on their territories using feeders and a combination of drop-traps and mist-net Russell traps (Russell and Russell 2001), after they were filmed (see below). Males were consistently found at their same territories before and after filming/capture, and so we were confident that the males we caught were the males we filmed (Simpson 2017). For males, we measured wing chord (distance between the wrist joint and tip of longest primary feather), bill length, and body mass, and we plucked feathers (n=7-10) from their gorget, specifically from the area under their bill, within ~5 mm on either side of the bill. Finally, we quantified gorget size (area, in mm^2), by photographing males on their left and right sides in a uniform lighting environment before we plucked feathers (Canon PowerShot SX510 HS; no zoom; 4000x3000 pixels). In ImageJ (Schneider et al. 2012), we measured the pixels occupied by each half of the male's gorget and summed the two measures to get total plumage-patch area. To avoid measuring the same feathers across photos, we used the position of the bill to determine the center of the gorget, and only measured the gorget area on one side of the bill/center. We used male bill length to size-calibrate each photo.

Eliciting and filming courtship displays

Following previous methods employed with several hummingbird species (Clark and Feo 2010; Feo and Clark 2010; Clark 2011; Clark et al. 2011, 2013), we elicited male shuttle displays by presenting a caged female (cylindrical cage ca. 1.3 m off the ground and 30.5 cm tall x 30.5 cm diameter) on a male's territory in an open area between his main perches. Males were found in open juniper-piñon pine woodland (i.e. spaced out trees/shrubs, open canopy; ca. 7000 ft. elevation), and male territories were identified as the concentrated areas within which males perched, patrolled, and exhibited space-defense behaviors (Simpson 2017). Cage location on male territories was designed to mimic males displaying naturally to females, which often occurs with females inside bushes or low trees and males displaying to them in the open. Further we used a cylindrical cage to eliminate the effect of corners (i.e. a square cage) on male display movement patterns. We used multiple females (2014: n=3; 2017: n=2) to elicit displays, and alternated which female was used each day. We placed one high-definition video camera (Sony HD progressive video cameras; Sony HDR-CX330; 60 frames/s) beneath the clear- or wire-mesh-bottomed cage holding the female, which allowed us to film male horizontal movements and female reactions/positions during the displays (Supplementary video S2). Males move little in the vertical plane during back-and-forth shuttles (pers. obs.; Supplementary video S3), so we did not film/quantify variation in vertical positioning during displays. The direction of north was marked in each video using either a dry erase marker or placing a stick pointing north, and we noted the time and date of the display for later calculations of solar position in the sky. Video recording took place all day (0730-1900 hrs.) from 3-13 July 2014 and 4-7 July 2017; we filmed 11 males and 14 shuttle displays in total (i.e. multiple displays for 3 males; 1 instance of large change in male solar orientation between his displays). We found no relationship between male sun orientation and solar elevation (Correlation: r=0.25, t=0.9, p=0.4), meaning that males did not orient towards the sun in particular ways at different times of the day (i.e. different solar elevations). Males typically displayed in clear or partly cloudy skies, so the sun was almost always visible.

Quantifying variation in male shuttle displays

For each recorded shuttle display, we mapped the male's movement (i.e. display path) frame-by-frame using the open-source video-analysis program Tracker (Brown 2017). In each video, we set the location of the female as the origin and set the width of the cage as the calibration measurement because the male always displayed in the same plane as the cage and female (e.g. Supplementary video S3). Specifically, we measured the x-y coordinates of a male's head through his display paths, as this allowed us to track the positions of each male's gorget (being presented to the female while shuttling; Supplementary video S2; Figure 1).

We spatially tracked each back-and-forth movement (i.e. a full shuttle cycle; Clark et al. 2012) for shuttle displays and used them to calculate the dimensions of an average shuttle cycle (in cm.) for individual males (e.g. Figure 1). A typical shuttle cycle for a broad-tailed hummingbird male is a figure-eight pattern (Figure 1). We calculated the shuttle cycle width (cm.) from this average shuttle cycle, by measuring distance between the apex (the end of the figure-eight) and the start point of the average shuttle. To measure whether this average shuttle cycle calculation accurately represented each display bout of a male, we randomly selected four males and found that the shuttle cycle width of the average shuttle cycle was not significantly different from five randomly selected shuttle cycles per male (t-test for all: $p>0.05$). We also calculated the translational velocity (cm/s) of the shuttle display from the average shuttle cycle; however shuttle cycle width and speed were highly positively correlated (Correlation Test: $r = 0.91$, $t = 8.39$, $p<0.0001$), so we removed speed from our analyses to avoid redundancy.

From each display bout, we also measured the angle between the plane of the center of the male's gorget (feathers beneath the bill) and the female's head (i.e. the angle of the male's plumage orientation towards the female during the display; Figures 1). To measure male orientation towards the female throughout the shuttle, we selected nine representative points from the average shuttle cycles (including the apex, start, mid, and end points; Figure 1 - red triangles), and for every shuttle cycle in a display, we measured this orientation angle at each of the nine points. We then calculated an average male-to-female orientation angle for each of the nine positions. We also calculated the standard deviation of these nine averaged angles as a measure of how variably a male orients himself towards the female during his display.

Finally, based on the location of each male's average shuttle cycle relative to compass north and the time and date of the display, we used a solar calculator (Hoffmann 2017) to determine a male's orientation towards the sun relative to the female from his head position at the first mid-point of his display for each display bout (i.e. relative to the solar azimuth) and the solar elevation during each male's display. We used Rayleigh tests of uniformity from the *circular* R package (Agostinelli and Lund 2013) to test whether or not males were orienting towards the sun in a uniform pattern and to test if they were facing a specific direction relative to the sun (180°: facing the sun directly; 0/360°: facing away from the sun). We then converted the circular measure of male orientation angle to the sun (0-360°) to a linear measure - angular deviation from facing the sun, which ranged from 0° (directly facing away from the sun) to 180° (directly facing the sun), for our subsequent analyses.

Display re-creations and quantifying male coloration during displays

To quantify perceived male coloration during a display, we moved the feathers we plucked from each male through their quantified average shuttle paths, while using a camera to photograph the feathers from the female's point of view, in order to recreate the orientation- and position-specific movements of males during their displays. Specifically, we calculated the angular distance between each of the nine positions from the average shuttle cycle and the first midpoint of the shuttle (i.e. crossing point of figure eight; Figure 1) - the first midpoint would have an angular distance of 0. We also calculated the angle relative to north for the first midpoint of each average cycle. Thus, we could position the feathers of each male where he displayed in the field, relative to north and the sun, and move those feathers in space through his average shuttle cycle. All positioning of the feathers during a display recreation was conducted using a compass. In addition to moving the feathers through the nine points of a male's average shuttle cycle, we also re-created the orientation of the feathers at each position using the average angle of orientation per position. This method allowed us to move and orient each male's feathers as if he were displaying to a female, using his exact movements and orientations in a controlled and standardized fashion. This method was used over quantifying feather coloration on naturally displaying, rapidly moving males due to the inability to record full-spectrum (ca. 300-700 nm wavelengths) high-speed video, which prevented objective color quantification through the avian visual system (see below). Our method also avoided the difficulties of positioning a video camera at the female's point of view without obstruction of the camera or disturbance of the male or female.

Because hummingbirds possess four color-sensing photoreceptors and can see into the ultraviolet (UV) spectrum (Herrera et al. 2008, but see Odeen and Håstad 2010), we quantified the relative cone simulation values of gorget feathers through the eyes of a bird using a newly developed digital photography technique that works from multispectral color photographs (Stevens et al. 2007; Troscianko and Stevens 2015). We mounted six gorget feathers plucked from each male on individual squares of black matte cardstock that were taped to a wooden block with a 2% and 99% calibrated Spectralon reflectance standard (Labsphere Inc.). This wooden block was then placed on a lazy-Susan rotator, which allowed us to orient the feathers relative to the camera (representing the female), based on the male's average angles of orientation (Supplementary figure S1). We used individual feathers instead of stacking feathers due to the lack of repeatability and measurement errors when stacking iridescent feathers and measuring their color (Meadows et al. 2011). We photographed each male's feathers as we moved them through the position- and orientation- specific display locations using a full-spectrum DSLR camera (Canon 7D with a quartz sensor instead of glass from http://advancedcameraservices.co.uk; 5184 x 3456 pixels) equipped with an El Nikkor 80 mm enlarging lens that can also transmit UV light (Supplementary figure S1). Using Bradaar light filters, we took a UV-light-only photo (ca. 300-400 nm) and a visible-light-only photo (ca. 420-680 nm; Supplementary figure S1). Then, we used the *Multispectral Imaging* package (Troscianko and Stevens 2015) in ImageJ (Schneider et al. 2012) to create the multispectral photos and calculate cone stimulation values for an avian visual UV-vis system (Vorobyev and Osorio 1998; Stevens et al. 2007; Herrera et al. 2008; Troscianko and Stevens 2015; see Supplemental text S1 for additional details).

Using the R package *pavo* (Maia et al. 2013), we calculated the tetrachromatic color variables (Stoddard and Prum 2008) for each position in each recreated display bout using the relative cone stimulation values from the multispectral photographs. We calculated hue theta (i.e. red-green-blue or RGB hue), hue phi (i.e. UV hue), and chroma (i.e. r.achieved in *pavo;* Stoddard and Prum 2008; Maia et al. 2013). We calculated luminance based on the stimulation of the double-cone for each position in a recreated display for each display bout using the *Multispectral Imaging* package in ImageJ (Troscianko and Stevens 2015).

To quantify the dynamics of male coloration during shuttle displays, we took the tetrachromatic color variables for each position in a shuttle cycle and calculated average color, maximum color, and three measures of color variation (standard deviation, range, and absolute % change). We found moderate degrees of collinearity between some of these variables (typically between average and maximum color and between % change in color, color sd, and color range; $r > 0.6$; see Supplementary tables S1-4) and reduced them to % change in color and average coloration per tetrachromatic color variable. We then conducted principal components analyses (PCA) on the % change in color and average coloration variables separately (i.e. RGB hue, UV hue, chroma, luminance; see Supplemental text S2 for details). PCA resulted in two dynamic plumage-color principal components: "% change in coloration PC," with higher values indicating males had higher % changes in chroma and RGB hue and "average coloration PC," with higher values indicating males that were brighter and more chromatic, but with less UV reflectance (Supplementary table S5). Percent change in luminance, % change in UV hue, and average RGB hue during a display were left as their own variables.

Display reconstructions and photography were conducted in Coconino National Forest, AZ from 18-25 July 2017, with one set conducted in Tempe, AZ on 25 July 2017. All display reconstructions occurred when the sun was not obstructed by clouds. Although solar position does not vary much from year to year, there is great variation in the solar position throughout a single year, so we adjusted when the photos were taken to account for temporal variability. When we photographed the feathers during a display reconstruction, the sun was on average 3.0° (standard deviation: ± 4.0°) different for the solar azimuth and 1.9° (± 1.1°) different for the solar elevation compared to the position of the sun during the original display. Thus our re-creations were done with very similar solar positions to when the males actually displayed.

Statistical analyses

To test for covariation between the environment (i.e. solar position and male orientation to the sun) and male perceived coloration during shuttle displays, we conducted mixed linear models using male orientation to the sun and solar elevation as fixed effects predicting our five dynamic plumage-color variables, and with male ID, Julian date, year, and female used to elicit the display as random effects. We did not control for time of day, as this directly influences solar elevation, which is one of our fixed effects. While year only had two levels, which could cause issues with our models, removing year as a random effect did not change our results qualitatively, therefore we left it in. To understand links between male morphological/behavioral traits and gorget coloration during courtship, we also conducted mixed linear models using male body mass, wing chord, shuttle width, plumage patch size, and variation in angles of orientation towards the female as fixed effects predicting our five dynamic color-display variables and using the same random effects as our previous models. We kept these analyses separate both because they were testing different hypotheses and due to the low sample size per fixed effect in the combined model. We used the Benjamini and Hochberg (1995) method to control the false discovery rate for each set of mix-linear models, due to the multiple comparison. The results after this p-value adjustment were overall similar, and so we present the results without the adjustment, but note which effects are lost with the adjustment (Tables 2 and 3). All statistical analyses were conducted in the statistical platform R (R Development Core Team 2012). We created and tested each multiple mixed linear model using the R packages *lme4* (Bates et al. 2015), *lmerTest* (Kuznetsova et al. 2016), and *MuMIn* (Barton 2016). For each model we also calculated marginal R^2 values, which illustrate the amount of variance explained by the fixed factors in the model. Finally, we tested the assumptions of normality for each model by evaluating the residuals plotted in a qq-normal plot, and if this assumption was violated, we transformed the data using either natural-log, square-root, square, or quartic transformations. These transformations successfully restored normality in each case.

Ethical Approval

All applicable national and institutional guidelines for the care and use of animals were followed. All work on this project was conducted with the approval of the Arizona State University Institutional Animal Care and Use Committee (17-1545R). Permission and permits to study broad-tailed hummingbirds in Coconino National Forest were granted by the United States Fish and Wildlife Services (MB088806-3), Arizona Game and Fish Department (SP772725), and Coconino National Forest (PEA0943).

RESULTS

Male orientation towards the sun during displays

We found that, on average, shuttling male broad-tailed hummingbirds did not significantly orient themselves towards or away from the sun, but instead displayed in a uniform spatial pattern with no specific mean angle towards the sun (Figure 2; Table 1).

Effects of solar position and orientation on variation in male perceived coloration during displays

We found that a male's degree of orientation towards the sun during shuttles was significantly positively related to % change in gorget luminance and % change in gorget color PC (Figure 3a-b, 4a-d; Table 2, S6), such that the iridescent feathers of males who tended faced the sun during their displays changed more in perceived brightness, chroma, and RGB hue relative to those who tended to not face the sun during their displays. Solar position and orientation in these models explained 47% of variation in % change in gorget luminance and 27% of variation in % change in gorget PC (marginal R^2 values; Table 2). Additionally, we found that degree of male orientation towards the sun during shuttling was significantly positively related to average color PC and average perceived RGB hue of iridescent plumage (Figure 3c-d, 4a-d; Table 2, S6), meaning that the gorget feathers of males who faced the sun during shuttles appeared brighter, more chromatic, and more red-shifted, but had less UV coloration. Finally, we found that solar elevation during male shuttles was significantly positively related to perceived gorget RGB hue (Table 2, S6), meaning that iridescent feathers of males who shuttle displayed when the sun was higher in the sky appeared more red-shifted. Solar position and orientation explained 44% of variation in average color PC and 70% of variation in average RGB hue (marginal R^2 values; Table 2) in these models. We found no other significant relationships between gorget coloration and solar elevation and orientation (Table 2, S6).

Male trait effects on variation in male perceived coloration during displays

We found that % change in gorget luminance during shuttles was significantly positively related to male wing chord and significantly negatively related to male body mass, gorget size, and variation in angle of shuttle orientation towards the female (marginal $R^2 = 0.84$; Figure 5; Table 3, S7); thus, males whose gorgets changed most in brightness (i.e. flashing on and off more) had longer wings, weighed less, had smaller gorgets, and kept a more persistent angle of shuttle orientation towards the female. We also found that % change in gorget color PC was significantly negatively related to male gorget size, shuttle width, and variation in angle of orientation towards the female (marginal $R^2 = 0.17$; Figure 5; Table 3, S7), such that males who changed more in chroma and RGB hue (i.e. were flashier) during shuttles had smaller gorgets, narrower shuttle displays, and more persistent orientation angles towards the female. Additionally, we found that % change in gorget UV coloration of males was significantly negatively related to male wing chord, shuttle display width, and variation in angle of orientation towards the female (marginal $R^2 = 0.50$; Figure 5; Table 3, S7), meaning that males whose gorgets changed more in UV reflectance during shuttling had shorter wings, narrower shuttle displays, and more persistent angles of orientation towards the female. We found no other relationships between % change in color and male traits (Figure 5; Table 3, S7).

Considering average perceived gorget color parameters, we found that average gorget color PC was significantly positively related to male shuttle display width and wing chord (marginal $R^2 = 0.30$; Figure 5; Table 3, S7), such that males whose gorgets appeared brighter, more chromatic, and reflected less UV light had wider shuttle displays and longer wings. Further, average RGB hue of gorgets was significantly positively related to male shuttle display width (marginal $R^2 = 0.29$; Figure 5; Table 3, S7), meaning that males with wider shuttle displays appeared to have more red-shifted iridescent plumage. No other relationships between average coloration and male traits were detected (Figure 5; Table 3, S7).

DISCUSSION

We characterized spatial and temporal dynamics of colorful male plumage, courtship displays, and the lighting environment in broad-tailed hummingbirds to understand how both sun orientation and male behavioral and morphological traits explained variation in dynamic perceived male coloration. Contrary to our original predictions, we found that males did not significantly orient themselves towards the sun during shuttle displays. Instead we found that males displayed along a continuum between facing the sun and facing away from the sun. Further, we detected two different dynamic color-display tactics along this sun-orientation continuum: 1) males who tended to face the sun while shuttling appeared brighter, more colorful, and flashier (i.e. higher % change in color), and 2) males who tended to not face the sun while shuttling had more consistent gorget coloration (i.e. little change in coloration) and greater UV reflectance during their displays. This result demonstrates light-environment specific color expression during behavioral displays and is consistent with the notion that animal colors, especially iridescent ornaments, are not just static features, even during behavioral displays, but can be dynamically modulated (Hutton et al. 2015).

In prior work, environmental (e.g. acoustic, lighting) features have been shown to modify an animal signal like song or coloration, but our findings are unique in that we considered the dynamics of two co-occurring male traits (plumage color and courtship behavior). For example, several studies have found that colorful males prefer to behaviorally display in specific light environments (Endler and Thery 1996; Heindl and Winkler 2003a; Heindl and Winkler 2003b) or will more completely display when the sun is out (Sicsú et al. 2013) or more visible (Chapman et al. 2009). However, many animals possess complex display behaviors, which can continuously modify or alter how a color patch interacts with the environment (Hutton et al. 2015; Patricelli and Hebets 2016). Our findings that males who tended to face the sun appeared more colorful, brighter, and flashier are consistent with previous work on color-display-environment dynamics in peacocks and butterflies (Dakin and Montgomerie 2009; White et al. 2014; Klomp et al. 2017), although, unlike these other species, male broad-tailed hummingbirds do not all specifically orient towards the sun. This growing body of work examining color-display-environment interactions illustrates the importance of both the environment and behavior on animal coloration.

In this study, we found that males who tended to not face the sun during their shuttle displays appeared less colorful and bright but had very consistent coloration while displaying. The reduction in chroma and brightness is most likely due to the differences between illumination from a powerful point source (i.e. the sun) versus a diffuse and less radiant source (i.e. the sky; Cronin et al. 2014). And because the sky is a non-directional light source, we do not expect dramatic effects of shifts in angles of illumination on iridescent feather reflectance, leading to a consistent color display. Further, although males who tended to not face the sun while displaying varied more in their angles of orientation towards the female, these departures would have less of an effect on perceived color, due to the non-directional light source.

On the other hand, we found that males who tended to face the sun during shuttles appeared brighter, more colorful, and flashier. When iridescent structures are illuminated by the sun at specific angles, they are highly reflective (Rutowski et al. 2007; Doucet and Meadows 2009; Meadows et al. 2011; White et al. 2014), due to the ordered arrangement of feather micro- and nano-structures (Prum 2006; Bradbury and Vehrencamp 2011). The high specificity of directional reflection from iridescent feathers therefore makes it much easier for males to produce a flashy display by altering their solar orientation. When males maintain persistent angles of orientation towards a fixed point other than the sun, such as a female, then their angles of orientation relative to the sun will vary as they display. This would explain the unexpected result that flashier males had more persistent angles of orientation towards the female during their display, because the orientation towards the female was relatively fixed, while the angle towards the sun was variable. These variable angles of orientation towards the sun would produce a flashy display, due to the differences in how the iridescent gorget was illuminated by the sun.

Our results raise the question of why males exhibit so much variation in color-display tactics. One potential explanation is that males transition between the two different display strategies to present females with a novel/different stimuli (i.e. negative frequency-dependent mating advantage (Hughes et al. 2013). These dynamic color displays could then allow males to adapt their displays given the population of other males and female preference, however understanding how these different color-display stimulate females and how their frequencies change across males/breeding seasons. Another hypothesis is that females might be spatially directing where males display in order to evaluate how males can flexibly adapt and display in less optimal environments ("receiver-imposed handicap hypothesis;" proposed in Hutton et al. 2015). We occasionally, both in natural courtship events and during our observations of males displaying to caged females, did observe males shifting their shuttle location in response to female movement, providing some anecdotal support for this hypothesis. Alternatively, males of several bee hummingbird species have been observed to chase females into bushes or small trees and display to them from outside the foliage (pers. comm. CJC), which suggests that males can govern where they display to females and are attempting to getting as close to the female as possible when displaying. Thus, a more thorough manipulation experiment would be needed to determine the extent to which our observed variation in perceived male coloration was due to actions by the male, female, both, or other unmeasured features of the environment (see more below).

We also found that males with consistent color-displays during shuttles had larger gorgets. Larger color patches/ornaments are preferred by females in several other bird species (e.g. Zuk et al. 1990; Qvarnström et al. 2000; Qvarnström et al. 2003; Chaine and Lyon 2008; Griggio et al. 2010), so we propose that males in this species who have larger gorgets may be favored to show this trait off more consistently. On the other hand, the flashy color-displays of other males may be used to emphasize and/or amplify those males' behavioral (shuttle) displays (Prum 1990; Byers et al. 2010; Barske et al. 2011), as in *Schizocosa* wolf spiders (Hebets and Uetz 2000; Uetz et al. 2009). Male broad-tailed hummingbirds who better maintained their angles of orientation to the female produced flashier color-displays, and thus the flashiness could be emphasizing and/or amplifying the ability of these males to maintain their orientation angles to the female during shuttles (i.e. male skill or the ability to perform difficult tasks well; Byers et al. 2010). We also found that flashier and more colorful males weighed less, and it has been suggested that male broad-tailed hummingbirds minimize their feeding throughout the day to maintain a low weight, which aids in flight performance and displays (Calder et al. 1990). Therefore, smaller males might be better able to perform these flashy displays while not experiencing the negative effects of reduced food intake. Future manipulations changing/limiting where males can display relative to the female and female choice experiments are needed to test and untangle these proposed efficacy- and quality-based functions of these color-displays.

In this study, we focused on the contribution of the sun, as an environmental factor, to variation in male color-displays, but there are other aspects of the environment (e.g. wind speed, likelihood of nearby predators, etc.) that could influence spatial positioning of shuttling males. However, based on our findings and observations, males are intensely focused on the female as they display, so they may not pay much attention to other environmental factors; more work is needed to test this. Male display position could also be partially explained by males minimizing the distance between them and the female, but our females often perched nearer the center of the cage and did not move once the males started displaying, preventing us from testing this explicitly. It is also possible that male coloration is not used during courtship and may function as a signal during aggressive interactions, which would suggest that males are not selecting display locations based on their color at all; though our observations indicate that males are showing off their gorgets during courtship while aggressive interactions are mostly chases. We also did not quantify the micro- or nanostructures of these hummingbird feathers to assess how variation in these structures might further explain the color-behavior-environment relationships. Future work should incorporate these structural components of feathers to understand how they affect both behavior and color appearance.

Our work here focused on the visual components of the shuttle display, which are also accompanied by a mechanical sound (Clark et al. 2012) produced by rapid wing-beating (Feo and Clark 2010). These mechanical sounds could be related to male flashiness during a display, as wing-beat frequency might influence or limit variation in the kinematics of male display paths, which could then affect the colors males can produce during a display. Thus there could be additional mechanistic and functional interactions/dynamics between the color-displays tactics and sounds. Further, these males all exhibited exaggerated dive displays in addition to shuttles while courting females, and these dive displays might also play a role in where males shuttle relative to the female and sun. Hummingbird dive displays produce additional mechanical sounds (Clark and Feo 2008; Christopher J Clark et al. 2011) and push these males to extreme performance limits (Clark 2009), and a male's ability to deal with these limits could further dictate the dynamics of male shuttle displays through physiological tradeoffs between the musculature/coordination needed for each type of display. Future work should incorporate acoustic and visual components of shuttles and dives to more fully understand these complex, multi-modal courtship displays.

Our study provides a unique look into how multiple visual traits interact and are influenced by the environment during courtship. A recent review has emphasized the idea that colors can be considered and studied like behaviors as dynamic traits (Hutton et al. 2015), and our work illustrates this. The perceived coloration of these broad-tailed hummingbird males during their displays by females varied greatly based on how males oriented relative to the sun (i.e. a continuum between towards and away from). Further, we hypothesized that male traits - plumage patch size, behavioral performance - would be better emphasized through one of the two different color-display tactics, and together this suggests that these tactics could be in part driven by variation in the individual traits. Altogether, our study adds to the growing body of work illustrating how both trait-trait and trait-environment interactions are vital to the understanding of both the function and evolution of male coloration and behavioral displays.

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DATA ACCESSIBILITY

Analyses reported in this article can be reproduced using the data provided by Simpson and McGraw 2018.

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FIGURE LEGENDS

Figure 1. Representative mean path for a male broad-tailed hummingbird's shuttle display. This display path was taken by averaging all shuttle cycles (one back-and-forth movement) from a single display bout (black squares and line) by one male. From this average display path, we selected nine representative points (depicted as 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 centimeters, and the female would be located at the origin (0,0) and is depicted by the female icon. We also measured male angle of orientation is relative to the female which is depicted for one point in the average shuttle path by the purple arrows. Male angle of orientation 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 for a clearer presentation.

Figure 2. Distribution of shuttle displays by male broad-tailed hummingbirds relative to the sun, which was statistically indistinguishable from uniform. Inner circles represent the number of males in a given sun-orientation bin (n = 1, 2, 3 respectively; bins=18°). The magenta point on the outer circle represents average sun orientation for males. This average was not statistically different from 180° (facing towards the sun) and 0°/360° (facing away from the sun). Location of sun is at 0° (indicated by the cartoon of the sun), the female (indicated by the female symbol) is located in the center of the cage/diagram, and the males, which would display around the cage, where always roughly facing

inward towards the female (indicated by the cartoon of the male head around the average sun orientation point).

Figure 3. Male sun orientation was significantly positively related to (a) % change in gorget luminance during a display, (b) % change in gorget color PC (chroma & RBG hue) during a display, (c) average gorget color PC (luminance, chroma, & UV hue) during a display, and (d) average gorget RGB hue during a display. Orientation angle to the sun was transformed from a circular 0°-360° variable to a linear 0-180° measure of male angular deviance from directly facing away from the sun (which is at 0°), as illustrated by the head of the broad-tailed hummingbird under 0 on the x-axis, and 180° indicating a male is directly facing the sun, as also illustrated by the head of the hummingbird under 180 on the x-axis. Percent change in luminance and % change in color PC were log transformed, while average RGB hue was quartic transformed. Trend-lines represent the relationships between each plumage color variable and male orientation angle to the sun.

Figure 4. Males who tended to face the sun while shuttling (red) appeared (a) brighter, (b) more chromatic, (c) more red-shifted, (d) had less UV coloration, and were (a-c) flashier (greater % color change) in terms of luminance, chroma, and RGB hue than males who tended to not face the sun (black). In statistical analyses, male sun orientation was a continuous variable, but this figure helps illustrate the two display tactics along the sun-orientation continuum. Males who tended to face away from the sun had orientations from 0°-90° and 270°-360°, while males who tended to face the sun had orientations

from 90°-270°. Further breaking down sun orientation into multiple bins resulted in too little data per group, which is why we chose two groups. Flashiness is illustrated by the non-flatness of the line across points. Error bars represent standard errors, and in some cases (a & c), males who did not face the sun had standard errors that were too small to be fully plotted. The X-axis shows male shuttle display movement in angular distances from the first midpoint of the shuttle path.

Figure 5. Standardized coefficients plot of multiple mixed linear models demonstrating how male morphological (wing chord and body mass), plumage (gorget size), and display (shuttle width and standard deviation in male angles of orientation during display) traits explain variation in dynamic color expression in male broad-tailed hummingbirds. The fixed effects are plotted on the left, and the response variables are indicated by the different colored points/error bars. The points represent the standardized regression estimates from the mixed-linear models and the error bars represent 95% confidence intervals of the standardized regression estimate. Asterisks and dashes beside the names of the fixed effects represent significant or non-significant effects on the response variable, respectively.

TABLES

Table 1. Circular average solar orientation (± circular standard deviation) and vector length (measure of dispersion; 0 = dispersed; 1 = highly concentrated) and the results from both Rayleigh tests of uniformity and Rayleigh tests with a specified alternative mean direction.

Group (n)	**Avg. Solar Orientation ± Standard Deviation**	**Solar Orientation Vector Length**	**Rayleigh Test of Uniformity p-value[a]**	**Rayleigh Test with alternative mean direction (180°) p-value[a]**	**Rayleigh Test with alternative mean direction (0°) p-value[a]**
All displays (14)	322.1° ± 86.2°	0.32	0.24	0.93	0.07

[a] P-values greater than 0.05 indicate that the null hypothesis of uniformity or a lack of specified mean direction (respectively) are not rejected.

Table 2. Results from linear mixed-model analyses testing the effects of male orientation to the sun and solar elevation on iridescent plumage color appearance during shuttle displays.

Response Variable	Fixed Effects	Beta Estimate	Std. Error	t-value	P-value
% Change in Luminance	**Orientation to Sun**	**0.011**	**0.002**	**4.39**	**<0.01**
$R^2_m = 0.47$	Solar Elevation	-0.011	0.010	-1.20	0.26
% Change in Color PC	**Orientation to Sun**	**0.011**	**0.003**	**3.52**	**<0.01**
$R^2_m = 0.27$	Solar Elevation	-0.002	0.013	-0.18	0.86
% Change in UV Hue	Orientation to Sun	-0.004	0.006	-0.72	0.49
$R^2_m = 0.07$	Solar Elevation	-0.007	0.019	-0.37	0.72
Avg. Color PC	**Orientation to Sun**	**0.017**	**0.006**	**3.07**	**0.01**
$R^2_m = 0.44$	Solar Elevation	0.032	0.022	1.45	0.18
Avg. RGB Hue	**Orientation to Sun***	**0.001**	**<0.001**	**2.45**	**0.03**
$R^2_m = 0.70$	**Solar Elevation**	**0.005**	**0.001**	**3.70**	**<0.01**

Male ID, Julian date, year, and female used to elicit displays were all random effects in these models.

Marginal R^2 values are below the response variable for each model, which explain the variation explained by the fixed effects in each model. See supplementary table S6 for conditional R^2 values and intercept results.

Significant effects are in bold.

Asterisks indicate effects that are lost when controlling for the false discovery rate (Benjamini and Hochberg 1995).

Table 3. Results from linear mixed model analyses testing the effects of male shuttle width, gorget size, and morphological traits on changes in iridescent plumage color appearance during shuttle displays.

Response Variable	Fixed Effects	Beta Estimate	Std. Error	t-value	p-value
% Change in Luminance	**Gorget Size***	**-0.01**	**0.005**	**-2.61**	**0.03**
$R^2_m = 0.84$	Shuttle Width	0.02	0.015	1.52	0.18
	Orientation Angle Std. Dev.	**-1.77**	**0.209**	**-8.45**	**<0.01**
	Mass	**-1.29**	**0.258**	**-5.02**	**<0.01**
	Wing Chord*	**0.28**	**0.098**	**2.83**	**0.02**
% Change in Color PC	**Gorget Size**	**-0.02**	**0.001**	**-31.13**	**<0.01**
$R^2_m = 0.17$	**Shuttle Width**	**-0.12**	**<0.001**	**-289.78**	**<0.01**
	Orientation Angle Std. Dev.	**-1.82**	**0.011**	**-167.46**	**<0.01**
	Mass	-0.38	1.025	-0.37	0.72
	Wing Chord	-0.54	0.349	-1.56	0.16
% Change in UV Hue	Gorget Size	0.02	0.007	3.00	0.07
$R^2_m = 0.50$	**Shuttle Width**	-0.21	0.008	-27.16	<0.01
	Orientation Angle Std. Dev.	-1.00	0.140	-7.11	0.01
	Mass	0.32	0.387	0.83	0.47
	Wing Chord	**-1.78**	**0.134**	**-13.25**	**<0.01**
Avg. Color PC	Gorget Size	-0.02	0.019	-1.37	0.22
$R^2_m = 0.30$	**Shuttle Width***	**0.19**	**0.029**	**6.60**	**0.03**
	Orientation Angle Std. Dev.	-0.45	0.457	-0.99	0.41
	Mass	0.59	0.975	0.61	0.57
	Wing Chord	**1.53**	**0.352**	**4.34**	**<0.01**

Avg. RGB Hue	Gorget Size	<0.01	0.002	0.27	0.80
$R^2_m = 0.29$	**Shuttle Width***	**0.02**	**0.006**	**3.09**	**0.05**
	Orientation Angle Std. Dev.	-0.01	0.085	-0.15	0.89
	Mass	0.12	0.111	1.07	0.32
	Wing Chord	0.09	0.045	1.90	0.11

Male ID, Julian date, year, and female used to elicit displays were all random effects in these models.

Marginal R^2 values are below the response variable for each model, which explain the variation explained by the fixed effects in each model. See supplementary table S7 for conditional R^2 values and intercept results.

Significant effects are in bold.

Asterisks indicate effects that are lost when controlling for the false discovery rate.