

The Evolution of Hummingbird Coloration and Courtship Displays

by

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ABSTRACT

Animals have evolved a diversity of signaling traits, and in some species, they co-occur and are used simultaneously to communicate. Although much work has been done to understand why animals possess multiple signals, studies do not typically address the role of *inter-signal interactions*, which may vary intra- and inter-specifically and help drive the evolutionary diversity in signals. For my dissertation, I tested how angle-dependent structural coloration, courtship displays, and the display environment interact and co-evolved in hummingbird species from the “bee” tribe (Mellisugini). Most “bee” hummingbird species possess an angle-dependent structurally colored throat patch and stereotyped courtship (shuttle) display. For 6 U.S. “bee” hummingbird species, I filmed male shuttle displays and mapped out the orientation- and position-specific movements during the displays. With such display paths, I was able to then recreate each shuttle display in the field by moving plucked feathers from each male in space and time, as if they were naturally displaying, in order to measure each male’s color appearance during their display (i.e. the interactions between male hummingbird plumage, shuttle displays, and environment) from full-spectrum photographs. I tested how these interactions varied intra- and inter-specifically, and which of these originating traits might explain that variation. I first found that the solar-positional environment played a significant role in explaining variation in male color appearance within two species (*Selasphorus platycercus* and *Calypte costae*), and that different combinations of color-behavior-environment interactions made some males (in both species) appear bright, colorful, and flashy (i.e. their color appearance changes throughout a display), while other males maintained a consistent

(non-flashing) color display. Among species, I found that plumage flashiness positively co-varied with male display behaviors, while another measure of male color appearance (average brightness/colorfulness) co-varied with the feather reflectance characteristics themselves. Additionally, species that had more exaggerated plumage features had less exaggerated shuttle displays. Altogether, my dissertation work illustrates the complexity of multiple signal evolution and how color-behavior-environment interactions are vital to understanding the evolution of colorful and behavioral display traits in animals.

DEDICATION

*To my wife and best friend for all her love and support. I could not have done this
without you.*

To my sister and brother for inspiring my creativity as we grew up together.

To my father for helping introduce me to the natural world.

To my mother for raising me to be the man I am today.

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PREFACE

Animals exhibit a wide diversity of ornamental traits and display behaviors, such as colors, horns, and dances. Many animals possess these traits together and use them to communicate (Bradbury and Vehrencamp 2011); a good example of this is the elaborate train that peacocks (*Pavo cristatus*) show off while dancing to females. Why animals possess multiple signals and how they evolved has long puzzled biologists, leading to multiple hypotheses to explain this phenomenon (Møller and Pomiankowski 1993). One of the best-supported hypotheses, the sensory drive hypothesis, predicts that signal diversity evolved through selection favoring traits that can be effectively transmitted through the environment and are detectable to intended receivers (Endler 1992; White and Kemp 2015). The sensory drive hypothesis has been used to explain the diversity of colorful ornaments or display behaviors intra-specifically (e.g. guppies; Endler 1992) and inter-specifically (e.g. manakins; Endler and Thery 1996; Heindl and Winkler 2003a; Heindl and Winkler 2003b). *However, these signals often interact with each other and with the environment, and to date, little work has been done to try to understand how ornaments, display behaviors, and the environment interact and how these interactions shape intra- and inter-specific diversity in multiple signals.*

Interactions between ornaments, behavior, and the environment could be as simple as an animal moving into an environment that enhances its ornament's conspicuousness (Endler and Thery 1996; Heindl and Winkler 2003b), or as complicated as an ornament being perceived differently due to complex body movements relative to the signal receiver and the environment (Rutowski et al. 2007; Dakin and Montgomerie 2013; Hutton et al.

2015; White et al. 2015; White and Kemp 2015). In these dynamic communication systems, the overall appearance of an ornament during a display is the complex product of the interactions between the morphological ornament (e.g. reflectance, directionality), behavioral display (e.g. posture, orientation), and environment (Hutton et al. 2015; Simpson and McGraw 2018a).

Colorful ornaments provide some of the most interesting systems to study signal interactions, as many colorful animals also have behavioral displays, and the appearance of these colorful traits can be greatly affected by the environment (Endler 1992; Endler 1993; Hutton et al. 2015). Previous work has examined how individuals behaviorally interact with the environment, such as through orienting their colorful ornaments towards the sun to optimize visibility (Rutowski et al. 2007; Dakin and Montgomerie 2009; Dakin and Montgomerie 2013) or maximizing detectability of their colorful ornament by seeking out light environments (e.g. forest light gaps) that best enhance their coloration and/or contrast against the background (Endler and Thery 1996; Heindl and Winkler 2003b; Heindl and Winkler 2003a). Behaviors of animals can also modify the display environment, such as in golden-collared manakins (*Manacus vitellinus*) and great bowerbirds (*Ptilonorhynchus nuchalis*) that 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). Yet there can be more complex interactions between behaviors and colorful ornaments, such as the intricate dances of birds-of-paradise (Laman and Scholes 2012) or the wing beats and flight patterns of butterflies (White et al. 2015), where coloration dynamically interacts with the

environment due to specialized body movements or orientations relative to the environment.

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). The interaction between colorful ornaments and complex display behaviors can lead to increased overall detectability or discriminability (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* and *S. rovnerei*) leg tufts used during a display have been found to increase the likelihood of a male being detected (Uetz et al. 2009), and *Anolis* lizards perform a pushup alert display to increase detectability of their dewlap extensions (Ord and Stamps 2008). Behaviors can also manipulate the color patch itself, such as in red-winged blackbirds (*Agelaius phoeniceus*) that reveal their hidden colorful epaulets during social encounters (Hansen and Rohwer 1986). In all of these cases, the colorful ornament and display behavior are interacting with each other and the environment to improve overall transmission efficacy, by producing a potentially unique color appearance that is not possible through a static presentation of a colorful ornament (Hutton et al. 2015), and elucidating how these color-behavior-environment interactions produce variation in color

appearance would aid in our understanding of how these multiple signaling traits co-evolved.

For my dissertation, I aimed to understand the interactions between angle-dependent color ornaments, display behaviors, and the environment and how these interaction properties shaped intra- and interspecific diversity in signaling traits. The appearance of angle-dependent structural coloration is determined by both the angles of illumination and observation, and it has been hypothesized that angle-dependent coloration can interact with display behaviors, body positioning, and color-patch orientation (Doucet and Meadows 2009). For instance, a structurally colored patch can be rapidly turned on and off through behavioral manipulations (e.g. colored wings flapping in butterflies; Rutowski et al. 2007), creating a flashy display, which could increase conspicuousness (White et al. 2015) due to its rapid sequential changes and sharp temporal contrast with itself. In general, however, the interactions between angle-dependent structural coloration and behavioral displays are poorly understood in animals, especially in terms of how the interactions between the color patch, display behavior, and environment create a dynamic color appearance from a static physical ornament (Hutton et al. 2015) and how this dynamic color appearance varies within and among species.

Specifically, I investigated how the interactions between angle-dependent structural coloration, display behaviors, and display environment have led to the diversity in these traits and their interactions (which I term “color appearance during a display”) across multiple species of hummingbirds from the “bee” tribe (tribe Mellisugini; McGuire et al. 2008, 2014). “Bee” hummingbirds are a monophyletic group in which most species

possess both angle-dependent structural coloration and two stereotyped courtship displays (the dive and shuttle displays). The dive display is characterized by the male flying high above the female and diving down near her, often making various sounds with his wings and/or tail (Clark et al. 2018). The shuttle display is typically characterized by a male flying rapidly back and forth horizontally in front of a female, while facing her and erecting his colorful throat patch into a near-flat plane (Feo and Clark 2010; Clark et al. 2011; Clark et al. 2012). As the dive display is seemingly more of an acoustic display, whereas the shuttle display has obvious short-distance visual components to it, I focused on the shuttle display for my dissertation. “Bee” hummingbird species vary in aspects of shuttle display, such as the two-dimensional shape and length of the flight pattern. Males also possess colorful angle-dependent throat patches (gorgets), which vary in color (e.g. orange, purple), size, and angle-dependence (Figure 1,2). Although most species display in open environments (CJC pers. comm.), how males orient towards the sun during displays appears to vary both intra- and interspecifically as well (Figure 2n). Like most birds, hummingbirds have tetrachromatic color vision (i.e. four color photoreceptors; Herrera et al. 2008; Odeen and Håstad 2010), which allows me to model the appearance of a colorful male hummingbird during his display to a female bird using avian visual systems (Vorobyev et al. 1998; Goldsmith and Butler 2003; Endler and Mielke 2005; Hart and Vorobyev 2005; Stoddard and Prum 2008). Additionally, hummingbirds have two areas of high visual acuity in their retinas, so that if a female is watching the male out of one eye or both, she will be able to see him displaying at high resolution (Lisney et al. 2015). Finally, hummingbirds have a high flicker-fusion rate (80 Hz; i.e. the ability to perceive fast-moving objects – faster than

humans can perceive; Fellows 2015), meaning that the color appearances or changes in color appearance that I measured during displays are perceivable to a female as long as she has at least one eye pointed towards the male.

To understand the impact of interactions between angle-dependent structural coloration, courtship displays, and the environment on male color appearance and how all of these traits and their interactions co-evolved, I first tested the mechanisms of how these signaling traits interact with each other and the environment to produce color appearance in one of the study species, broad-tailed hummingbirds, *Selasphorus platycercus* (Appendix C). Next, I tested how plumage color, courtship behavior, and the lighting environment each contribute to intra-specific variation in these interactions in a different species, again chosen for logistical ease and population size (Costa's hummingbirds, *Calypte costae*; Appendix D) to understand if one signal plays a stronger role in the production of color appearance (i.e. do males with more colorful feathers appear more colorful during a shuttle display?). I chose these species for each study due to logistical reasons (i.e. close to ASU, inexpensive), and because I found large populations, which was ideal for amassing a large sample size of males.

Next, I examined how male color appearance varied across 6 breeding North American “bee” hummingbird species and how color-behavior-environment interactions varied as a function of the individual color, behavioral, and environmental features themselves, to test whether signal interactions co-evolved with the signals that interact to produce them (Chapter 1). Finally, to understand the strength of the relationships between a species' angle-dependent structural coloration, display behaviors, and color appearance,

I experimentally tested if and how signal mismatches between coloration and behavior (e.g. the colorful feathers of Costa's hummingbirds when viewed during a black-chinned hummingbird's shuttle display) influenced color appearance for each species (Chapter 2). Overall, while I found that variation in the display environment predicts intra-specific color appearance, variation in male plumage/coloration and behavior predicts inter-specific color appearance, illustrating the complex evolutionary dynamics of signals interactions and their importance in understanding diversity in multiple signals.

CHAPTER 1

THE EVOLUTION OF COMPLEX COURTSHIP SIGNALS: COVARIATION AND INTERACTIONS BETWEEN HUMMINGBIRD DISPLAYS, PLUMAGE, AND APPEARANCE

ABSTRACT

Many animals communicate using multiple signals (e.g. colors, songs, odors). Historically, most attention has been paid to how these traits evolve and function in isolation, but recent work has focused on how signals may interact with one another and produce unique signal-interaction properties. These interaction properties are known to vary within species, but less is known about how they vary among species and especially how such properties may co-evolve with the signals themselves. I studied the evolutionary relationships between angle-dependent structural plumage, courtship (shuttle) displays, solar environment, and male color appearance during a display (i.e. the result of the interactions between the three aforementioned traits) among six species of North American “bee” hummingbirds (Tribe Mellisugini). I found an antagonistic evolutionary relationship between exaggeration in shuttle displays and plumage properties. Further, I found that hummingbird color appearances do vary, dramatically in some cases, among species and that shuttle and plumage properties were strongly correlated with male color appearance among species, such that species with more exaggerated shuttles, but less exaggerated plumage, appeared flashier (i.e. exhibiting greater changes in coloration) but less bright/colorful during courtship. These results highlight the importance of studying the properties of signals and

their interactions both within and among species and reveal that the originating signals do co-evolve with the signal interactions, albeit in different ways.

INTRODUCTION

Animals communicate using a wide diversity of signaling traits, and many animals use multiple signals (e.g. songs, colors, vibrations; Bradbury and Vehrencamp 2011). The question of why animals evolved diverse multiple-signal systems has generated many different hypotheses, most of which fall into one of two main categories of explanations: information-content hypotheses (e.g. multiple messages vs. redundant signals) and signal-efficacy hypotheses (e.g. how aspects of the environment influence signal transmission – sensory drive: Endler 1992; Hebets and Papaj 2005). However, multiple signals are often used simultaneously (e.g. butterfly flight displays; Rutowski et al. 2007; or spider courtship dances; Hebets and Uetz 2000) and interact during use, which can create unique signal interactions (Simpson and McGraw 2018a; Simpson and McGraw 2018c) and/or enhance the efficacy of the interacting signals (Hebets and Papaj 2005). These interactions can influence the evolution of multiple signals through one signal altering/amplifying another signal differently across habitat or signal types (Hebets and Uetz 2000; Hebets 2004), or through signal interactions producing diverse phenotypic properties, which could covary with properties of the interacting signals and/or further optimize signal efficacy based on species/habitat diversity. Thus, to better understand the evolution of multiple signals, it seems critical to investigate how these signals are interacting and how the signal-interaction properties vary among species.

Although signals can interact across modalities (i.e. behavior and odor; Pruett et al. 2016) and within modalities (e.g. visual: leg ornamentation and movement; Hebets and Uetz 2000), colorful ornaments and behavioral displays provide a great system to test the

function and evolution of signal interactions. Many colorful ornaments can be manipulated by behaviors during a display to produce unique color appearances (i.e. the signal-interaction properties; Hutton et al. 2015) and these color appearances are not solely driven by the coloration of the ornament (i.e. brighter/more colorful ornaments do not necessarily produce brighter/more colorful appearances during an actual display; Simpson and McGraw 2018c). Interactions between color and behavior are especially conspicuous for angle-dependent structural coloration, as the appearance of these color patches is dependent upon the angles of illumination and observation (Doucet and Meadows 2009; Meadows et al. 2011). In particular, recent work in peacocks (Dakin and Montgomerie 2013), hummingbirds (Simpson and McGraw 2018a,c), and butterflies (Rutowski et al. 2007; White et al. 2015) has elucidated the complex interactions between colorful ornaments (e.g. wing spots, feathers), display behaviors (e.g. flight patterns, tail rustles), and the sun, which create changing color appearances throughout a display (i.e. flashiness, where the color varies in brightness/chroma/hue over space and time). Importantly, these signal interactions have been found to influence mating success – male peacocks that exhibited greater color change (produced by interactions between tail shaking and their colorful tail eyespots) have more matings (Dakin and Montgomerie 2013). Although this previous work has demonstrated the mechanisms (e.g. Simpson and McGraw 2018c) and functions (e.g. Dakin and Montgomerie 2013) of signal interactions, questions still remain about how signal interactions vary among species or are driven by the expression of particular signaling traits.

The evolution of multiple signals has often been found to be driven by variation in how animals can behaviorally alter themselves relative to their display environment. For example, signals can interact, through behaviors, postures, or gestures, with the environment to increase the transmission efficacy and detectability of those signals (Endler 1992). Evidence supporting how animals alter themselves or the environment to improve signal efficacy has been found among many animals, such as in how several bird, butterfly, and lizard species orient themselves towards the sun to increase their conspicuousness (Dakin and Montgomerie 2009; Olea et al. 2010; White et al. 2015; Klomp et al. 2017) or how animals will seek out specific light environments for courtship (Heindl and Winkler 2003a; Gordon and Uetz 2011). Thus, it is also possible that the properties of signal interactions be influenced by and covary with how males position/orient themselves relative to pertinent characteristics of the environment (e.g. sun, background) during signal use. To this effect, my recent work on hummingbirds found that the male display location relative to the sun was the primary driver of intra-specific variation in male color appearance during a display (Simpson and McGraw 2018a,c), suggesting that male display location could also influence variation in color appearance among species.

The evolution of multiple signals can also be influenced by the relationships between the signaling traits themselves. For example, selection can drive the elaboration of multiple signals at the same time (concerted, or positively correlated, co-evolution), such as in wood warbler song and coloration (Shutler and Weatherhead 1990), which leads to greater signal complexity and diversity. Alternatively, selection can drive the elaboration of one signal at the expense of another (antagonistic/compensatory, or negatively

correlated, co-evolution), due to tradeoffs in trait production (Badyaev et al. 2002), compensations based on environmental changes (Martins et al. 2015; Pruett et al. 2016), or redundancy in signal efficacy (Galván 2008). However, the question remains of how signal-interaction properties covary with properties of the individual signals (e.g. speed of display, orientation of male during display, plumage brightness/chroma/hue). It is possible that signal-interaction properties evolve completely independently from signaling-trait properties, though this seems unlikely based on recent signal-interaction research. For example, *Schizocosa* wolf spiders, colorful leg tufts help increase female receptivity to male behavioral displays (Hebets and Uetz 2000). There could also be production costs or signal efficacy trade-offs between signal-interaction properties and the signals themselves. For example, signal co-elaboration could make it more difficult to present them simultaneously, as they become unwieldy or require too much energy to use individually (e.g. difficulty of flying with large tail feathers; Andersson et al. 2002), which would reduce or negate the effect of the interaction between them (e.g. for color appearance – less bright/chromatic appearance).

In this study, I aimed to evaluate inter-specific variation in the properties of colorful ornaments, behavioral displays, solar-positional environment, and the resulting signal interactions (i.e. male color appearance during a display), to test if and how signal interactions may have co-evolved with the interacting signals and/or the display environment. Specifically, I studied the co-evolution of multiple visual signals and their interactions in hummingbirds from the monophyletic “bee” tribe (Mellisugini; McGuire et al. 2014), because most of the species in this group possess and vary in angle-dependent

structurally colored head coloration (e.g. throat, crown; Figure 1, 2) and a stereotyped, rapid back-and-forth courtship flight display (shuttle display), which are presented simultaneously to the female during courtship (Feo and Clark 2010; Clark 2011; Clark et al. 2011, 2013; Simpson and McGraw 2018a,c; Figure 1, 2) Additionally both of these signaling traits interact with each other and the environment to produce male color appearance during a display (Simpson and McGraw 2018a,c). Further, the species vary in how they orient themselves relative to the sun as they display (Figure 2).

I video-recorded shuttle displays from six North American “bee” hummingbird species (Figure 1) and spatiotemporally mapped each individual’s display movements and orientations towards the female during displays. Then, following my previously established methods (Simpson and McGraw 2018a,c), I moved individual structurally colored feathers plucked from these birds through their shuttle paths/orientations (in the field) to quantify dynamic male color appearance during a display, which varied among species (Figure 3). I estimated covariance in the properties of the signals and their interactions among species (Goolsby et al. 2017) to assess if and how male hummingbird signals and signal interactions co-evolved. I predicted that properties of male shuttle displays, structurally colored plumage, and the solar environment co-evolved, either antagonistically or concertedly, and do not exhibit independent (Ornelas et al. 2009) or de-coupled evolutionary relationships (Wiens 2000). I then predicted that inter-specific differences in male color appearance during a display, as with intra-specific differences (Simpson and McGraw 2018a,c), covary with display position relative to the sun. Additionally or alternatively, I predicted that variation in male shuttle and/or plumage properties will

covary with male color appearance, as these traits vary more among species than they do within species, which should cause them to play a bigger role in the variance of signal interactions.

METHODS

Field sites and capture methods

I studied broad-tailed (*Selasphorus platycercus*; June-July 2014, 2017), black-chinned (*Archilochus alexandri*; May-June 2015, 2016), and Anna's (*Calypte anna*; March 2016) hummingbirds in Arizona, and Costa's (*Calypte costae*; March 2015), Calliope (*Selasphorus calliope*; July 2016), and Allen's (*Selasphorus sasin*; April 2017) hummingbirds in California during their breeding seasons (see Table 1 for location coordinates). All applicable national and institutional guidelines for the care and use of animals were followed, and all work on this project was conducted with the approval of the Arizona State University Institutional Animal Care and Use Committee (protocol # 17-1545R). Permission and permits to study hummingbirds were granted by the United States Fish and Wildlife Services (permit # MB087454-1; MB088806-03), Arizona Game and Fish Department (SP772725), California Fish and Wildlife Services (SC-6598), Boyd Deep Canyon Preserve, Sagehen Creek Field Station, University of California-Riverside, Arizona State University, Appleton-Whittell Audubon Research Ranch, Patagonia-Sonoita Creek Preserve, and Coconino National Forest (PEA0943). I captured female hummingbirds (broad-tailed: $n = 5$, black-chinned = 5, Anna's = 1, Costa's = 2, Calliope = 2, Allen's = 2) from each site using feeder drop-traps (Russell and Russell 2001) and

temporarily housed them in captivity before presenting them to males (of their own species) in the field to elicit male shuttle displays. After male shuttles were filmed (see below), I captured those males (broad-tailed: $n = 11$, black-chinned = 4, Anna's = 6, Costa's = 15, Calliope = 2, Allen's = 2) on their territories using a combination of feeder drop-traps and mist-net Russell traps (Russell and Russell 2001). Males were consistently found on their same territories before and after filming, so I was confident that the males I caught were those that I filmed (Simpson 2017). Unfortunately, I was unable to capture the Anna's hummingbird males that I filmed, so I took spectral measurements and plucked feathers from preserved specimens ($n=5$) that were previously caught on Arizona State University's campus and used in earlier studies (2005-2011; Meadows 2012). For each male, I plucked feathers ($n = 7-10/\text{bird}$) from its colorful throat patch (gorget), specifically from the area underneath the bill (Simpson and McGraw 2018a,c), and photographed each side of each male's colorful gorget (and crown patch for Anna's and Costa's hummingbirds) to quantify size (area, in mm^2) of the structurally colored plumage patch (Simpson and McGraw 2018a,c).

Eliciting and filming male shuttle displays

To elicit male shuttle displays, I presented a female in a wire-mesh cylindrical cage (30.5 cm tall by 30.5 cm diameter), with a clear plexiglass bottom, approximately 1.3 m off the ground to males on their territories, following previous methods employed with these and related hummingbird species (Feo and Clark 2010; Clark and Feo 2010; Clark 2011; Clark et al. 2011, 2013; Simpson and McGraw 2018a,c). I placed a high-definition

video camera (Sony HDR-CX330; 60 frames per second progressive scan) on a tripod underneath the caged female, pointing up, which allowed me to film both male shuttle movements/orientations and female position in the horizontal plane (Simpson and McGraw 2018a,c). Because males display in the same plane as the female and do not move much vertically while shuttling (Simpson and McGraw 2018a,c), I did not measure vertical movement and focused on the horizontal component of the shuttle display. Broad-tailed, Costa's, Allen's, and Calliope hummingbirds were all filmed using a similar set up, with the only difference being the type of cage stand (tripod for broad-tailed, thin plastic rods for Costa's, and thick, clear PVC pipe for Allen's and Calliope; my cage-stand set-up evolved as I progressed and worked under different environmental conditions). However, due to the extreme width of black-chinned hummingbird shuttles, I used two cameras positioned on either side of the caged female, pointing up, to ensure that I could capture whole displays from those males. Also, because Anna's hummingbirds do not exhibit traditional shuttle displays but instead perch and sing to females (Clark and Russell 2012), I fitted a wire ring around the cage, at the same level of the female, for male *C. anna* to perch on and sing from. Some Anna's hummingbird males perched directly on the cage instead of the ring ($n = 3$), but I only quantified the displays of those that perched on the ring ($n = 6$), to ensure that vertical positioning and orientation of males were comparable to other species.

Quantifying male shuttle displays

For each recorded display, I mapped the male's horizontal movement (i.e. display path) following the methods of Simpson and McGraw (2018a,c), using the open-source video-analysis program Tracker (Brown 2017). Briefly, I measured the specific x-y coordinates of each male's head throughout his display, to track the position of his gorget relative to the female. I used these coordinates to calculate an average shuttle cycle (i.e. one back-and-forth movement, in cm), or for Anna's hummingbirds (which do not shuttle) an average singing position, for each display bout. From these average shuttle cycles, I calculated the shuttle width (the distance between the turn-around point and start point) and average translational velocity (cm/s; both 0 for *C. anna*; Clark and Russell 2012).

I also quantified male orientation towards the female during his shuttles by measuring the angle between the center of the male's gorget and the female's head at seven (Allen's, Calliope, and Costa's), nine (broad-tailed), or thirteen (black-chinned) representative points (selected based on shuttle shape and width; Figure 1; Simpson and McGraw 2018a,c). From these angles, I calculated an average male orientation angle for each position and then calculated an overall average and standard deviation in angle of orientation for each display. To quantify male angles of orientation towards the female in Anna's hummingbirds, I measured the male orientation towards the female from every ten frames during each singing bout and used these to calculate the average and standard deviation in male angle of orientation towards the female. Because male shuttle width, shuttle velocity, and standard deviation in male angle of orientation were all highly, positively correlated among species ($r > 0.65$), I collapsed them, using principal

components analysis (PCA) on individual-level data ($n = 40$), into a single principal component (PC): “shuttle behavior PC,” with higher values indicating males with wider, faster shuttles and having more variation in their angles of orientation towards the female (Table 2). For this and all subsequent PCAs (I did separate analyses for different signaling variables – i.e. behavior, color appearance), I only interpreted PCs with an eigen value greater than 1.0, and only interpreted variables represented with a loading of $|0.4|$ or greater. Finally, all PC axes were rotated so that they had a positive correlation with the variables they represented.

I also quantified male display orientation relative to the solar azimuth using the location of each male’s average shuttle cycle relative to compass north and the female, the time and date of each display bout, and a solar calculator (Hoffmann 2017). I then converted the circular measure of male display location to the sun (0 - 360°) to a linear measure – angular deviation in male display location relative to the sun – which ranged from 0° (sun directly behind male as he displayed) to 180° (sun directly in front of male as he displayed) for linear statistics (Simpson and McGraw 2018a,c). Additionally, I quantified the angular distance between the male display location and the closest location to the female given the presence of the cage, such that an angular distance of 0° represents a male displaying at the closest location outside of the cage to the female, while an angular distance of 180° represents a male displaying at the furthest location from the female (Simpson and McGraw 2018c). Finally, I calculated the average of each shuttle display and environmental property per individual (i.e. for individuals with multiple shuttle displays).

Plumage reflectance and angle-dependence measurements

I followed the spectrometric methods of Meadows et al. (2011) to quantify the reflectance properties of each male's feathers in a controlled laboratory setting. I used an Ocean Optics USB2000 spectrometer and PX-2 pulsed xenon lamp (Dunedin, FL) and set the receiving probe normal to the feathers, while setting the light probe based on the average solar elevation during male displays for each species (Table 2). I measured reflectance at ca. 0.4 nm intervals from 300-700 nm for 5-6 feathers per male, with the feathers tilted 0°, 5°, 10°, 15°, and 20° to the side (multiple angles for angle-dependence quantification). Following my previous methods, I did not alter the location of the receiver probe, as I was focused on male signals and interactions in this study, rather than the female (Simpson and McGraw 2018c). The feathers I measured here were the same feathers that I had plucked and photographed (see below) to quantify each male's color appearance during displays.

I averaged reflectance spectra for the feathers per male and used the average ultraviolet (UV) sensitive avian visual model (Herrera et al. 2008) in the R package *pavo* (Maia et al. 2013) to calculate standard tetrachromatic color variables (Stoddard and Prum 2008); specifically, hue theta (hereafter, “red-green-blue” or “RGB hue”), hue phi (hereafter, “UV hue”), chroma (i.e. r.achieved; Stoddard and Prum 2008; Maia et al. 2013), and luminance. I then calculated the angle-dependence of each color variable by measuring the slope between all feather tilt angles. I compressed brightness, chroma, and UV hue feather reflectance into a single PC: “feather reflectance PC,” with higher values indicating brighter, more chromatic, and more UV-reflecting feathers, and I also collapsed the slopes

of luminance, chroma, and UV hue into a single PC: “feather angle-dependence PC,” with higher values indicating higher slopes for luminance, chroma, and UV hue (Table 2).

Display re-creations and quantifying male color appearance

I quantified male color appearance during a display following the methods of Simpson and McGraw (2018a,c). Briefly, I moved the six gorget feathers that I plucked from each male through their quantified average shuttle cycle and photographed them from the female’s point of view, using a full-spectrum DSLR camera (Canon 7D with a quartz sensor) equipped with an El Nikkor 80 mm enlarging lens and two Bradaar light filters (Troscianko and Stevens 2015) attached to a special lazy-Susan apparatus (Simpson and McGraw 2018a,c). I calculated RGB hue, UV hue, and chroma (Stoddard and Prum 2008) for each position in each re-created display using the relative cone stimulation values from the multispectral photographs through *pavo* (Maia et al. 2013). Luminance was calculated from the double-cone stimulation from the photos using the *Multispectral Imaging* package in ImageJ (Troscianko and Stevens 2015). Display re-creations for broad-tailed, black-chinned, and Calliope hummingbirds were conducted near Flagstaff, Arizona, and the times/dates of these reconstructions were adjusted so that the position of the sun closely matched the solar position when these males originally shuttled at their respective field sites. Re-creations for Costa’s, Anna’s, and Allen’s hummingbirds were conducted in Tempe, Arizona, and I also adjusted the times/dates of these reconstructions to match the original solar positions during male shuttle displays.

I calculated the average color and % change in color during a display from the tetrachromatic color variables for each position in a shuttle cycle (Simpson and McGraw 2018a,c) for my measures of color appearance, and then averaged each color appearance variable per individual. I then collapsed the four % change in color appearance variables (i.e. RGB hue, UV hue, chroma, luminance) into a single PC: "% change in color PC," with higher values indicating males with higher percentage changes in luminance, chroma, RGB hue, and UV hue (Table 2). Additionally, I collapsed average luminance, chroma, RGB hue, and UV hue appearance into "average color PC," with higher values indicating males that appeared brighter, more chromatic, and more red-shifted, and exhibited lower UV reflectance (Table 2).

Comparative analyses

All statistical analyses were conducted in R (R Development Core Team 2012). For my analyses, I used a time-calibrated hummingbird phylogeny (McGuire et al. 2014) and removed all other hummingbird species not included in my study using the R package *ape* (Paradis et al. 2004). I calculated and accounted for intra-specific variation in properties of signals and signal interactions (i.e. multiple individuals per species) while estimating the inter-specific correlations between properties of male signals and signal interactions (Goolsby et al. 2017) for my two color-appearance PCs, shuttle display behaviors, plumage/feather variables, and male display location relative to the sun (for full list, see Figure 4). Specifically, I estimated the evolutionary variance-covariance matrices using a Brownian motion model using Pagel's λ , a univariate Ornstein-Uhlenbeck model,

and a multivariate Ornstein-Uhlenbeck model (Eliason et al. 2014; Goolsby et al. 2017), and compared models using Akaike (AIC) and Bayesian information criteria (BIC) to determine the most supported evolutionary model. None of the three evolutionary models (Brownian motion, univariate Ornstein-Uhlenbeck, multivariate Ornstein-Uhlenbeck) were strongly supported over the others (Table 3), so I interpreted the variance-covariance matrix of the Brownian motion model as the default, most parsimonious model, though all models produced qualitatively similar results. The intra- and inter-specific signal/interaction property correlation matrices were calculated using the R package *Rphylopars* (Goolsby et al. 2017), and then I converted each covariance measure into a Pearson's correlation coefficient. I only interpreted correlation coefficients greater than $|0.7|$, based on qualitatively similar statistical results obtained using standard phylogenetic generalized least squared analyses (Revell 2010; see below). While my methods also calculated intra-specific covariation in the properties of hummingbird signals and signal interactions, I found no intra-specific correlations greater than $|0.7|$ (Figure 4).

Because phylogenetic generalized least squares (PGLS; Revell 2010) uses a single data point per species, I averaged each hummingbird plumage, behavior, environmental, and color appearance variable per species. Then, I separately conducted PCAs on shuttle behaviors, feather reflectance, feather angle-dependence, % change in color appearance, and average color appearance variables (Table 4). Using the new PCs (similar representation of variables to PCs above) and non-compressed data, I conducted independent PGLS analyses on how species' plumage, behavioral, and environmental variables predicted color appearance during a display. All correlations from the variance-

covariance estimates with a coefficient greater than $|0.7|$, except one, had statistically significant PGLS models (Table 5). I also conducted PGLS analysis on each relationship between species' plumage, behavioral, and/or environmental variables that had a correlation coefficient greater than $|0.7|$, and again all of these models were statistically significant except two (Table 5), though the relationship between plumage patch size and the individual three components of the Shuttle PC (shuttle width, shuttle velocity, and variation in male orientation towards the female) were all significant (Table 6). I used the R packages *ape* (Paradis et al. 2004), *phytools* (Revell 2012), *caper* (Orme et al. 2013), and *nlme* (Pinheiro et al. 2013) for PGLS analyses.

RESULTS

Covariance among hummingbird signal properties

I evaluated the evolutionary relationships among the male signaling traits (i.e. shuttle display, plumage, and display orientation relative to the sun), by calculating their inter-specific covariances and correlation coefficients while taking into account the intra-specific variation in male signal properties (so all principal components (PC) below were created using individual-level data). Among species, I found a strong positive correlation between shuttle behavior PC (higher values indicate males with wider and faster shuttles, with more variation in angles of orientation relative to the female – i.e. more exaggerated shuttle displays) and plumage patch size ($r = 0.72$, Figure 4, 5d,h), indicating that species with more exaggerated shuttles had smaller plumage patches. I also found that there was a strong positive interspecific correlation between male display distance from the female and

male display position relative to the sun ($r = 0.83$, Figure 4), meaning that species that displayed with the sun in front of them also displayed further from the female. Finally, I found a strong negative correlation between feather reflectance PC (higher values indicate males with feathers that were brighter, more chromatic, and exhibited more ultra-violet (UV) reflectance – i.e. more exaggerated feather reflectance) and feather red-green-blue (RGB) hue slope (higher hue slopes indicate greater angle-dependence in hue; $r = -0.79$; Figure 4) and a strong positive correlation between feather reflectance PC and male display position relative to the sun ($r = 0.81$; Figure 4). In other words, males with more reflective ornamental feathers had feathers that were less angle dependent and tended to face the sun while shuttling. I found no other strong inter-specific correlations between shuttle, plumage, or solar traits (Figure 4).

Covariance between hummingbird signals and their interactions

To understand the evolutionary relationships between properties of 1) male hummingbird shuttle displays (i.e. shuttle width, speed, angles of orientation, distance from female), 2) colorful plumage (i.e. feather reflectance, size, angle-dependence), 3) display location relative to the sun, and 4) the interaction of these three signaling traits (quantified as % change in male color appearance and average color appearance during a display) among species, I calculated their evolutionary covariances and correlation coefficients, while accounting for within-species variance. I found that, among species, the % change in color PC (higher values indicate males that had greater changes in luminance, chroma, RGB hue, and UV hue appearance during displays – i.e. flashier color appearance) was

strongly, positively correlated with shuttle behavior PC (i.e. shuttle exaggeration; $r = 0.95$; Figure 4, 5a,e) and negatively correlated with plumage patch size ($r = -0.82$; Figure 4, 5b,f). In other words, species with more exaggerated shuttles but smaller plumage patches appeared flashier during the display. I also found strong positive inter-specific correlations between average color appearance PC (higher values indicating males that appeared brighter, more chromatic, more red-shifted, but less UV reflecting during displays – i.e. brighter and more colorful appearance), feather reflectance PC (i.e. feather reflectance exaggeration; $r = 0.79$; Figure 4, 5c,g) and feather RGB hue ($r = 0.88$; Figure 4). Specifically, species that had more reflective feathers appeared brighter and more colorful during shuttle displays. Finally, among species, I found that average color appearance PC was positively correlated with male display distance from the female ($r = 0.73$; Figure 4), indicating that species that appeared brighter and more colorful during displays also displayed further from the female. I did not find any other strong inter-specific correlations between color appearance and other shuttle, plumage, or solar traits (Figure 4).

DISCUSSION

I investigated evolutionary covariation between male hummingbird angle-dependent structural plumage, shuttling behavior, display orientation relative to the sun, and male color appearance during courtship displays, which is a property of the interactions between the three aforementioned signaling traits. I found evidence for an antagonistic co-evolutionary relationship between exaggeration in male angle-dependent structural plumage and shuttle displays. Additionally, I found that male shuttle and plumage

properties, but not how males oriented their displays relative to the sun, explained inter-specific variation in color appearance during courtship. Specifically, I found that exaggeration in flashy color appearance positively covaried with exaggeration in shuttle displays, while exaggeration in average color appearance (brightness/colorfulness) covaried with exaggeration in plumage properties. Altogether, these results support my predictions that visual signals within this group of hummingbirds did not evolve independently and that signal-interaction properties do co-evolve with the properties of the separate signals themselves, both in a positive and negative way, suggesting two divergent evolutionary signal complexes and/or potential trade-offs between the signals and their interactions.

The antagonistic relationship that I uncovered between male shuttles and colorful plumage mirrors recent work on the acoustic signals of these hummingbirds, whereby a similar antagonistic co-evolutionary relationship was uncovered between vocal and mechanical sounds produced during courtship (Clark et al. 2018). Clark et al. (2018) suggested that the antagonistic co-evolution between male “bee” hummingbird wing trills and songs is due to the redundant function of these traits during male courtship. Work in bowerbirds (Endler et al. 2014), *Sceloporus* lizards (Martins et al. 2015), and Pelecaniformes (Galván 2008) also found antagonistic evolutionary relationships between coloration and male courtship behaviors, which they suggest is due either to redundancy in signal efficacy (i.e. both signals are not needed to attract or effectively communicate with the receiver, or both stimulate the receiver in comparable ways) or increased animal crypsis overall by relying on signals like behavioral displays that do not always broadcast their

visual effects like colorful ornaments (i.e. compensation based on the environment – predation pressure; Martins et al. 2015). It is possible that hummingbird visual traits follow a similar pattern, with exaggeration in plumage traits serving similar efficacy functions (i.e. unnecessary or similar receiver stimulation) to exaggeration in display behaviors, especially given that exaggeration in each signal is tied to different aspects of male color appearance, which could cause the non-exaggerated signal to become redundant (i.e. large/colorful plumage is redundant with the flashy appearance and exaggerated shuttles display pairing). However, because behaviors and colors are produced by different mechanisms (McGraw 2006; Prum 2006; Clark and Russell 2012; Barske and Fusani 2014) and often relate to different morphological, physiological, or reproductive traits (Kemp and Rutowski 2007; Byers et al. 2010; Taylor et al. 2011), I suggest that these traits do not share a redundant function in terms of information content. Future work is needed to test these predictions.

Variation in the solar-positional environment is a strong driver of intra-specific variation in color appearance and coloration in general in animals (Klomp et al. 2017; Simpson and McGraw 2018a,c), and previous work in lizards and birds has demonstrated how an animal's position relative to the sun can also predict inter-specific variation in coloration (Fleishman 1988; Persons et al. 1999; Heindl and Winkler 2003a). However, within my focal clade of “bee” hummingbirds, I found that male display position relative to the sun was not interspecifically associated with variation in color appearance while shuttling. It is possible that the solar environment has a stronger evolutionary influence on non-changing, non-angle dependent colors (as the aforementioned work focused on

pigment-based coloration) than the structural colors that these hummingbirds possess, as animals with pigment-based colors often seek out specific light environments (e.g. sun spots in a forest) to make their generally-less-reflective coloration more conspicuous (Heindl and Winkler 2003a; Gordon and Uetz 2011). However, the color-environment results from prior studies might be interpreted differently if they measured, as I did here, the properties of interactions between colorful ornaments, display behaviors, and the solar environment, instead of (or in addition to) just the reflectance properties of colorful ornaments. Based on my results here, it seems that covariation between the display behaviors and colorful ornaments has a stronger effect on the evolution of male color appearance during a display than the solar environment.

I did find that inter-specific variation in both male shuttle behaviors and colorful plumage significantly explained species differences in color appearance during courtship. Specifically, I found that species with more exaggerated shuttle displays (i.e. wider, faster, more variation in angles of orientation towards the female) appeared flashier while they displayed (i.e. exhibited greater color change during displays), but species with more exaggerated plumage patches (i.e. brighter and more colorful feather reflectance) appeared more consistently colored, brighter, and more colorful during displays. Because I also found that male shuttle display and plumage traits share an antagonistic co-evolutionary relationship, I suggest that appearing both flashy and maximally bright/colorful during a display is potentially not feasible, due to the mechanistic relationship between flashiness and appearing bright/colorful, or to trade-offs in the production costs of plumage and behavior, which might be similar to the trade-offs found between colorful ornaments and

exaggerated morphological features (i.e. long tails) in widowbirds (*Euplectes ardens*; Andersson et al. 2002). Additionally, while acoustic or olfactory signals are not always combined with color signals in the same way as behavioral displays and coloration (e.g. *Monarcha* flycatchers; Uy and Safran 2013), my results suggest a similar trade-off like those found for song and coloration in cardueline finches (Fringillidae: Carduelinae; Badyaev et al. 2002) and odor and coloration in *Sceloporus* lizards (Pruett et al. 2016). It could also be unnecessary to appear both flashy and bright/colorful if these two color-appearance tactics serve similar signal-efficacy functions. For example, work in Pelecaniformes suggests that species with more elaborate plumage do not need more exaggerated behavioral displays to achieve high levels of conspicuousness (Galván 2008). While this suggests that, over evolutionary time, selection might ultimately reduce the two redundant signals to one, if selection is also acting on signal interactions then the second signal could not be lost, as it is needed to produce the interaction. Altogether, my results support the idea that properties of hummingbird plumage patches, shuttle displays, and their interaction evolved not as one selective unit but two: 1) flashy color appearance with more exaggerated shuttles and less exaggerated plumage, and 2) consistent color appearance with less exaggerated shuttles and more exaggerated plumage, and I hypothesize that this bimodal selection is due to a combination of production trade-offs and limiting redundancy in signal efficacy.

Interestingly, I found that, within this clade of “bee” hummingbirds, species that tended to display with the sun in front of them also displayed further from the female and had brighter feathers. Although I did not find any direct relationship between male display

position relative to the sun and male color appearance, species with naturally brighter and more colorful feathers appeared brighter and more colorful during the display itself. Thus, hummingbird species with brighter and more colorful feathers likely focus more on adjusting their display location relative to the sun, rather than getting as close to the female as possible, in order to optimize their appearance. If the males in these species only focused on getting as close to the female as possible, they may not orient themselves relative to the sun in the best ways to present their plumage (Simpson and McGraw 2018c) and would thus appear darker and less colorful. There are many examples of male animals adjusting their displays based on sun orientation (Hamilton III 1965; Olea et al. 2010; Bortolotti et al. 2011; Klomp et al. 2017; Simpson and McGraw 2018a), and some cases, such as in peacocks, males will attempt to relocate females so that male's colorful ornaments are viewed in the best position relative to the sun (Dakin and Montgomerie 2009). However, it is still puzzling that those “bee” hummingbird species that shuttle with the sun more in front of them do not necessarily appear brighter and more colorful *during* displays.

Altogether, I have demonstrated that signal interaction properties do vary among species and exhibit complex evolutionary relationships with the properties of the individual signals themselves. However, it not clear how these signals are evaluated by receivers. It is possible that male shuttle displays, plumage patches, and color appearance are all evaluated independently, which would then suggest that male color appearance is an emergent signal property (Partan and Marler 1999; Hebets and Papaj 2005), since this aspect of the phenotype only exists as the signals are co-expressed and interact. On the other hand, if all signals are evaluated together, then they may represent a composite signal

(Hebets and Papaj 2005; Clark 2011; Gumm and Mendelson 2011), similar to the diverse plumage ornaments and coloration in California quail (*Callipepla californica*; Calkins and Burley 2003). Based on the evolutionary relationships between the signaling traits and their interactions in this hummingbird clade (flashy-exaggerated shuttle vs. colorful appearance-exaggerated plumage), I suspect that male color appearance is one component within a composite display involving color, plumage size, and shuttle displays, especially since it seems like these signals are being selected as two different units. Future work on receiver behavior is needed to determine if these signals and interactions function independently or synergistically.

Animal signal evolution can be complex, especially when it involves multiple signals that can interact concurrently to generate composite traits or emergent properties. Many studies have tested how multiple signals co-evolved, but little is known about how the properties created by the interactions between these signals also evolved. My work on the correlated evolution of hummingbird courtship shuttling, plumage coloration, and color appearance during a display demonstrated that the signal interaction properties (i.e. color appearance) do covary with properties of the signaling traits themselves (i.e. shuttle displays and colorful plumage). Further, I found that, unlike intra-specific variation in color appearance (Simpson and McGraw 2018a,c), inter-specific variation in color appearance was not driven by variation in how males oriented their displays relative to the sun, but instead was driven by variation in male shuttle and plumage traits. Finally, my results suggest that different aspects of male color appearance (flashiness vs. average color appearance) co-evolved divergently with male plumage and shuttle properties. Altogether

these results demonstrate the multi-layered dynamics between multiple signaling traits and their interactions, and how these complex dynamics may have led to greater diversity in colorful ornaments, display behaviors, and their interactions in animals.

CHAPTER 2

EXPERIMENTAL TRAIT MIS-MATCHES UNCOVER SPECIFICITY OF EVOLUTIONARY LINKS BETWEEN MULTIPLE SIGNALING TRAITS AND THEIR INTERACTIONS IN HUMMINGBIRDS

ABSTRACT

Many signaling traits in animals co-occur (e.g. peacocks behaviorally displaying with their colorful trains), and these traits may co-evolve due to their independent and interactive properties. Previous work has demonstrated ecological drivers of specific evolutionary relationships between signaling traits and the environment (e.g. acoustic properties of forest and song), which then leads to questions about why specific pairs or combinations of multiple signals evolved. Here I asked whether the particular color displays of different species are optimized for presentation with its species-specific courtship behavior. I investigated this in a tribe (Mellisugini) of “bee” hummingbird species, where males exhibit striking angle-dependent ornamental plumage and a stereotyped courtship (shuttle) display, by experimentally creating mis-matches between the behavior and plumage of males from five different species and quantifying how these signal mis-matches influenced male color appearance during a display. I found that plumage/behavior mis-matches significantly altered display flashiness (i.e. % change in coloration during a display) compared to the natural plumage/behavior pairings of the different species, and that such departures in flashiness were most strongly predicted by differences in shuttle-display behaviors compared to other plumage and environmental traits. These results illustrate a

tight (i.e. species-specific) evolutionary relationship between the forms of male shuttle display and color flashiness paired in these “bee” hummingbird species. Further, I found that interspecific variation in male plumage, behavior, and natural color appearance predicted species-averages in deviations between natural and mis-matched flashy color appearance. Altogether, my work provides a new method for testing the coevolution of signals and their interactions and highlights the complex evolutionary relationships between multiple signals in animals.

INTRODUCTION

Many animals possess multiple signals that they use in communication (e.g. peacock jumping spider dances and colorful ornaments; Girard et al. 2011, 2015). There are two sets of hypotheses that are typically invoked to explain diversity in multiple signals. The first set are hypotheses focused on the information contained within the signals (Hebets and Papaj 2005), which can shed light how multiple signals co-evolved with other, non-signaling traits, such as the relationship between Northern Cardinal (*Cardinalis cardinalis*) bill color and body condition and breast color and offspring feeding rate (e.g. Jawor and Breitwisch, 2004; Jawor, Gray, Beall, and Breitwisch, 2004). The second set of hypotheses focuses on signal efficacy and how signals are optimized for transmission and perception in their particular environments (Endler 1992; Hebets and Papaj 2005). For example, various neotropical bird species tend to display or hold territories in environments that make them more detectable or better contrast with the background (Heindl and Winkler 2003a; Gomez and Thery 2004; Endler et al. 2014; Simpson and McGraw 2018b). Although these studies have revealed important evolutionary links between environmental/non-signaling traits and animal signals, questions remain about how and why certain signals co-evolve together (or antagonistically). Multiple signals are often co-expressed, and this can lead to signal interactions, such as one signal making the other more detectable/discriminable or two signals interacting to produce a new, emergent property (Hebets and Papaj 2005). By studying the mechanisms that lead to variation in these signal interactions, I can better understand the specific co-evolutionary coupling between signals and their interactions.

Signaling traits can interact with each other and/or the environment to produce certain, quantifiable, interactive properties (e.g. emergent or composite signals; Hutton et al. 2015). For example, a butterfly's wing beats, angle-dependent colorful scales, and position relative to the sun can produce a strobe-like, flashy color-display that is not possible without trait interactions (White et al. 2015; also see peacocks, *Pavo cristatus*; Dakin and Montgomerie 2013). Other animals, like some hummingbirds, position and orient their colorful ornaments during a display relative to the sun in a way that produces a consistent color-display (Simpson and McGraw 2018a), and the consistent color appearances are not solely the result of the ornament being a specific brightness/coloration but are the result of the interactions between colorful plumage, behaviors, and the environment (Simpson and McGraw 2018c). Signal interactions do not just vary among distantly related species (e.g. butterflies vs. hummingbirds) but can also vary among closely related species, and these signal interactions can co-evolve with the original signals themselves. For example, hummingbird flashiness during a display positively covaries with courtship display properties (e.g. display width, velocity), whereas how bright and colorful a species appears during a display positively covaries with plumage properties (i.e. feather brightness; Chapter 1). These results lead to questions about how optimized a hummingbird's plumage is for its particular behavioral display (and vice versa) and how optimized one or both signals are to produce specific signal interactions. There is evidence that selection does favor specific trait pairings (e.g. evolution of hummingbird bill shape with flower shape; Snow and Snow 1980; Stiles 1981; Smith et al. 1996), including for specific forms of communication (e.g. the evolution of birdsong to match a species' habitat;

Tobias et al. 2010). If I can disrupt the pairing between two different signals in a species (i.e. have species A perform a behavioral display with the plumage of a species B), I can test how tightly or diffusely these signal pairings have co-evolved, based on if/how the interaction properties change relative to how they appear naturally in each species.

The evolutionary linkage between two co-expressed signaling traits can be disrupted, both naturally and experimentally. One example of a natural disruption between two signals is found within *Sceloporus* lizards, where most species within the genus possess a colorful underbelly and exhibit push-up/head-bob displays, which they use to communicate to rival males (Martins et al. 2015). However, some species have lost their underbelly coloration, and this has led to changes in their behaviors, such that males of the species without underbelly coloration exhibit more behavioral displays (Martins et al. 2015). While *Sceloporus* lizards provide an example of a natural disruption of the links between multiple signals, experimental disruptions of signal linkages, especially signals with known and quantifiable signal interaction properties, may also provide key insights into the coevolved links and interactions between multiple signals.

My aim in this study was to experimentally disrupt the evolved linkage between courtship displays and angle-dependent structural coloration in a monophyletic clade of North American “bee” hummingbirds (Tribe Mellisugini, McGuire et al., 2014), by creating signal mis-matches (i.e. plumage/behavior mismatches: running the feathers of one species through the courtship display of different species; Figure 6; see more below) and quantifying if/how these mis-matches alter the signal interaction properties (i.e. color appearance) previously studied in this system (Chapter 1). Prior work on *Schizocosa* wolf

spiders tested the effects of signal alterations, or mis-matches, on female choice, and found that the addition of leg ornaments on males did not affect female choice for species that do not naturally have visual components to their courtship displays (Hebets and Uetz 2000). On the other hand, adding or removing leg ornaments increased or decreased, respectively, female receptivity in species that did have visual components to their displays (Hebets and Uetz 2000). However, in this study I aim to test the effect of mis-matches on signal interaction properties, and I am not completely removing or adding signaling trait properties, as in the spider work, but expanding or reducing male signals based on natural, interspecific variation within the clade. These mis-matches allow me to quantify the robustness of each evolved signal combination (plumage-behavior-appearance), providing a tool to measure the strength of the evolutionary pairing between those signals.

Hummingbirds from this clade vary in their striking angle-dependent head/throat plumage coloration and a stereotyped courtship display: the shuttle display (Chapter 1). Shuttles are characterized by a male flying rapidly back and forth in front of a female while erecting his colorful plumage (Feo and Clark 2010; Clark et al. 2012; Clark et al. 2013; Simpson and McGraw 2018a,c). In my previous work, I tested the correlational relationships between properties of male shuttles, plumage, display environment, and their interactions to begin to understand how these signals co-evolved (Chapter 1). However, to better understand the evolution of multiple signals and their interactions, I aimed to experimentally disrupt the evolved links (i.e. species-specific signal forms) between these signals and test how this altered the signal interaction properties (i.e. male color appearance during displays). Previously, I conducted shuttle display re-constructions by moving

plucked feathers from a given species through that species' courtship display, to quantify male color appearance while shuttling (Simpson and McGraw 2018a,c). However, here I took colorful feathers from each of five "bee" hummingbird species and also moved them through the shuttle display paths of the other four species, to quantify male color appearance during these mis-matched color-displays. With these plumage/behavior mis-matches, I can test if/how the mis-matched color appearances are different from the species-specific color appearances during a display, such that the magnitude of the deviations between the two color appearances represents the degree of specificity of the evolved signal pairing.

In general, I predicted that plumage/behavior mis-matches would cause large departures in each species' color appearance – both in terms of color flashiness (changes in color throughout the display) and average brightness/coloration during the display. Specifically, I predicted that the greater the difference between properties of the plumage patch (e.g. patch size, feather reflectance), courtship-display (e.g. shuttle width, speed), and/or display orientation to sun for the natural and mis-matched signal pairings, the greater the deviation in color appearance. Based on my current understanding of the co-evolution between signal properties and interactions in these hummingbirds (Chapter 1), I also sought to determine the specific properties of the signals themselves that might be driving inter-specific deviations in color appearance. For example, because plumage patch size and feather reflectance positively covary with how bright and colorful a male appears during a display (Chapter 1), I predicted that species with larger plumage patches and/or more colorful feathers would have greater deviations in average color appearance during

mis-matches. I also predicted that species with more exaggerated display movements (e.g. wider shuttles) should exhibit greater deviations in color appearance during mis-matches, because of the positive covariance between shuttle behaviors and flashiness (Chapter 1). Finally, I predicted that species that normally appeared flashier and/or more colorful during their displays would exhibit greater deviations in their color appearance during mis-matches.

METHODS

Field shuttle and plumage data collection

For this study, I used previously recorded shuttle displays (from 2014-2017) and plucked feathers for each hummingbird species (Simpson and McGraw 2018a,c; Chapter 1): broad-tailed (*Selasphorus platycercus*) and black-chinned (*Archilochus alexandri*) hummingbirds, which were studied in Arizona, and Costa's (*Calypte costae*), Calliope (*Selasphorus calliope*), and Allen's (*Selasphorus sasin*) hummingbirds, which were studied in California (see Chapter 1 for location and permit details). Anna's hummingbirds were excluded from this study due to the fact that they do not move during their courtship display, making it difficult to mis-match their displays with other species. Captured female hummingbirds from each species were used to elicit male shuttle displays from their own species in the field (Simpson and McGraw 2018a,c). I presented a female in a wire-mesh cylindrical cage with a clear plexiglass bottom (see Simpson and McGraw 2018c for full description of cage set up) to males on their territories, and then I placed a high-definition video camera (Sony HDR-CX330) underneath the caged female, pointing up, which

enabled me to film male shuttle orientations and movements in the horizontal plane (Simpson and McGraw 2018c). After I filmed male shuttles, I captured the males that displayed on their territories and plucked feathers from their colorful throat patch (gorget), specifically from the area underneath their bill (Simpson and McGraw 2018c). I also quantified male plumage patch size by photographing each side of the male's head (before I plucked feathers, area in mm^2 ; Simpson and McGraw, 2018a).

Quantifying male shuttle displays

I used the quantified shuttle-display data from my previous work (Chapter 1) to recreate male displays in the field. Briefly, I measured the x-y coordinates of each male throughout his display path, tracking the position of his gorget relative to the female. I then used these coordinates to calculate an average shuttle cycle, from which I calculated shuttle width (cm, distance between the turn-around point and starting point) and average translational velocity (cm/s). I also quantified how males oriented themselves towards the female during shuttles by measuring the angle between the center of a male's gorget and the female's head at representative points (see Simpson and McGraw 2018c for additional details). I then calculated an average angle of orientation during a display, and the variance (standard deviation) in male angle of orientation relative to the female during a display. Finally, based on the direction of compass north, the female's location, and the time/date of each display, I calculated the solar azimuth and elevation for each display (Hoffmann 2017). I then quantified each male's display location relative to the solar azimuth and female (see Simpson and McGraw 2018c for additional details).

Plumage reflectance and angle-dependence measurements

To determine the color properties of feathers outside of the context of the display (in other words, their inherent reflectance when measured under controlled conditions), I used UV-Vis spectrometry to quantify reflectance and angle-dependence of each male's plucked gorget feathers (Simpson and McGraw 2018c). These reflectance measurements were taken from individual feathers following the methods of Meadows et al. (2011). From these reflectance measurements, I used an ultraviolet (UV) sensitive avian visual model (Vorobyev and Osorio 1998; Herrera et al. 2008) to calculate standard tetrachromatic color variables (Stoddard and Prum 2008) in R using the *pavo* package (Maia et al. 2013). Specifically, I calculated hue theta (hereafter, "red-green-blue" or "RGB hue"), hue phi (hereafter, "UV hue"), chroma (i.e. r.achieved; Stoddard and Prum 2008; Maia et al. 2013), and luminance. I also tilted the feathers from 0° to 20° to the side and measured the feather reflectance in 5° increments. From these tilted reflectance measures, I measured the slope between all angles for each tetrachromatic color variable, as my measures of feather angle dependence (Simpson and McGraw 2018c).

Display re-creations, plumage-display mis-matches, and quantifying male color appearance

In my previous work, I determined male color appearance during his display by taking a male's plucked feathers into the field, positioning them relative to the sun as that male had positioned himself while shuttling, moving the feathers through that male's re-created shuttle display, and photographing the feathers to acquire my color-appearance

metrics (Simpson and McGraw 2018c). For this study, I re-created plumage-display mismatches in the field by moving feathers from each of the hummingbird species through the average shuttle display paths for the other species (Figure 6). For example, I moved the six gorget feathers that I plucked from a Costa's hummingbird through the quantified average shuttle cycles of an Allen's, black-chinned, broad-tailed, and Calliope hummingbird (Figure 6). Because I was focused on how plumage/behavior mismatches would affect male color appearance, I re-created each mis-match in similar environmental (lighting) conditions of the non-mis-matched species (in the example above – all Costa's display mismatches occurred under Costa's environmental conditions). Further, I kept the mis-matched displays positioned relative to the sun based on the original display (Figure 6). By re-creating the mis-matches under the same environmental conditions of the original species, I could eliminate any added effects of species/environment mismatches to better determine the evolutionary link between the plumage and behavioral traits themselves and their interactions. I randomly selected (using a randomly generated sequence from random.org, using an atmospheric noise model without replacements) feathers/behaviors from five individuals per species for these mis-matches. I was able to pluck feathers from only two male Allen's and four male black-chinned hummingbirds, but I had multiple displays from each individual, so I was able to use a unique feather/behavior combination for my mis-matches (i.e. a different Allen's/black-chinned display path per mis-match replicate). Unfortunately, I only filmed three displays from two Calliope male hummingbirds, so I randomly (as above) selected which two of the three shuttles would be used twice in order to achieve five mismatches with this species. Overall, this resulted in

20 plumage/behavior mis-match sets and a total of 100 re-created mis-matches (n = 5 species, 4 mismatches/species, 5 replicates/mismatch).

During each of these re-created mis-matches, I followed the exact same methods as I did previously to determine color appearance of male feathers during his shuttle display (i.e. the within-species matches) by moving plucked feathers through re-created shuttles and then photographing the feathers from the female's point of view at each representative position, using a full-spectrum DSLR camera (Canon 7D with a quartz sensor) equipped with an EI Nikkor 80 mm enlarging lens and two Bradaar light filters (Stevens et al. 2007; Troscianko and Stevens 2015) attached to a lazy-Susan apparatus (described in Simpson and McGraw 2018c). For each position, I calculated RGB hue, UV hue, and chroma using the relative cone stimulation values from each multispectral photo through *pavo* (Maia et al. 2013), and calculated luminance from the double-cone stimulation values from each photo using the *Multispectral Imaging package* (Troscianko and Stevens 2015) in ImageJ (Schneider et al. 2012). Finally, as my measures of trait interactions (i.e. degree of match/mismatch between plumage and behavior), I used the tetrachromatic color variables taken from the multispectral photos to calculate the average color appearance (i.e. how males appeared on average during their display) and % change in color appearance during a display for each mis-match (Simpson and McGraw 2018a).

Statistical methods

i) Testing the effects of plumage/behavior mis-matches on color appearance

All statistical analyses were conducted in R (R Development Core Team 2012). To initially test if the plumage-display mis-matches caused divergences in male color appearance from the species-appropriate matches, I compared the average species-specific color appearance to the mis-matched color appearances for each species. First, using principal components analysis (PCA), I collapsed the % color change variables (luminance, chroma, RGB hue, and UV hue; $n = 20/\text{variable}$) into a single principal component (PC): “% change in color PC,” with higher values indicating males that had higher percent changes in luminance, chroma, RGB hue, and UV hue color appearance during a display (Table 7). I also collapsed the variables for how males appeared on average during a display into a single PC: “average color PC,” with higher values indicating males that appeared brighter, more chromatic, more red-shifted, and exhibited lower UV reflectance during a display (Table 7).

I used analysis of variance (ANOVA) to test whether the average color appearance from each mis-match resulted in a departure from the natural (i.e. species-specific) color appearance within each species (see example below; and see Chapter 1 for samples sizes per species). I then used Tukey-Kramer post-hoc tests to evaluate the significant differences in male color appearance due to mis-matches with different species. For example, I tested whether the average color appearance of an Allen’s hummingbird was different than the color appearance from mis-matches of: 1) Allen’s feathers with black-chinned shuttle, 2) Allen’s feathers with broad-tailed shuttle, 3) Allen’s feathers with

Calliope shuttle, and 4) Allen's feathers with Costa's shuttle (Figure 7a). For the purposes of this study, I ignored all post-hoc results between different mis-match groups (i.e., Allen's:Costa's vs. Allen's:black-chinned) and only focused on whether each mismatch differed from the one species-appropriate match. Through these analyses, I found that plumage-behavior mis-matches had little to no effect on how males appeared on average during a display (see Results for more details) and thus I focused on understanding what about these mis-matches might be driving deviations in % color change during a display.

ii) Testing if/how differences in signaling traits between focal and mis-matched species predict deviations in color appearance

Next, I aimed to understand what about these mis-matches might be driving the deviations in % change in color during a display, such as differences in shuttle display behavior, plumage, or male display position relative to the sun between the focal species and mis-matched species. I first averaged the % change in color appearance variables from the five individual re-creations per mis-match pair per species. For example, I calculated single average % change in luminance during a display for the 1) Allen's-black-chinned mis-match, 2) Allen's-broad-tailed mis-match, 3) Allen's-Calliope mis-match, and 4) Allen's-Costa's mis-match. I then took each of these average % change in color mis-match variables (luminance, chroma, RGB hue, and UV hue) and found the absolute difference between them and the average natural % change in color to create a "deviation in % color change" per each color variable. I also calculated the absolute difference in shuttle

behavior, plumage, and solar environment properties for each mis-match (e.g. |Allen's shuttle width - black-chinned shuttle width|).

I collapsed (separate PCA from above) the deviation in male color change variables into a single PC: "deviation in % color change PC," with higher values indicating larger differences between the focal species' and mis-matched species' variables for % change in luminance, chroma, RGB hue, and UV hue during a display (Table 8). I also collapsed differences in the three shuttle-display properties between the focal and mis-match species (width, velocity, and variation in angles of orientation) into a single PC: "differences in shuttle behavior PC," with higher values indicating larger differences between the focal species' and mis-matched species' shuttle behaviors (Table 8). Additionally, I collapsed differences in feather reflectance between the focal and mis-matched species into two PCs: 1) "differences in feather hue PC," with higher values indicating larger differences between the focal species' and mis-matched species' feather RGB/UV hue (Table 8); and 2) "differences in feather luminance/chroma PC," with higher values indicating larger differences between the focal species' and mis-matched species' feather luminance/chroma (Table 8). Finally, I collapsed differences in feather angle-dependence between the focal and mis-matched species into two PCs: 1) "differences in hue angle-dependence PC," with higher values indicating larger differences between the focal species' and mis-matched species' angle-dependence in RGB/UV hue (Table 8); and 2) "differences in luminance/chroma angle-dependence," with higher values indicating larger differences between the focal species' and mis-matched species' angle-dependence in luminance/chroma (Table 8).

I used an information-theoretic, model-averaging approach to test whether differences between the focal species' and mis-matched species' traits explained variation in the deviations from their natural % change in color during a display. I built a global mixed liner model, with deviations in % color change PC as my response variable, species as a random effect (as these mis-match data are not necessarily species-specific data points but data on differences between species), and differences in plumage patch size, feather hue PC, feather luminance/chroma PC, hue angle-dependence PC, luminance/chroma angle-dependence PC, shuttle behavior PC, average male orientation angle towards the female, male display position relative to the sun, and solar elevation between the focal and mis-matched species as my fixed effects. Then I used Akaike weights from the global and all subsequent models to calculate the summed weight for each fixed effect while also calculating the average beta value for each fixed effect. While relative importance (RI) values are informative, they can be misleading (Galipaud et al. 2014), so I created a final mixed linear model, with deviations in % color change PC as my dependent variable, using fixed effects that had a summed weight greater than 0.5 (Simpson and McGraw 2018c). I only interpreted fixed effects that were significant in this final model.

iii) Testing if/how natural signal variation predicts deviations in color appearance

Lastly, I tested the prediction that interspecific variation in signaling traits (i.e. shuttle behavior, plumage size, natural color appearance) predicts variation in deviations in % change in color due to mis-matches. For example, I predicted that species with wider shuttles will exhibit greater deviations between their natural and mis-matched % change in

color. Thus, I calculated a species-average for each courtship trait and for their deviations in % change in color due to mis-matches. However, I only tested whether plumage patch properties, shuttle properties, and natural color appearances predicted interspecific variation in deviations in % change in color, as these variables were previously found to strongly, evolutionarily covary (Chapter 1).

I was unable to neatly collapse species-average deviations in % color change variables into PCs, so I retained them as individual variables in analyses. I did, however, collapse species-average natural % change in color appearance and how males naturally appeared on average during a display into a single PC (in a separate PCA than above) each: 1) “% change in color PC” and 2) “average color PC,” both of which had the same relationships as in *Statistical methods: section 1*. (Table 9). I collapsed shuttle velocity, shuttle width, and variation in male orientation towards the female into a single PC: “shuttle behavior PC,” with higher values indicating species with faster and wider shuttles and more variation in how they oriented towards the female during a display (Table 9). I collapsed species-average feather reflectance variables into a single PC: “feather reflectance PC” with higher values indicating species with brighter, more chromatic, more red-shifted, and more UV reflecting feathers (Table 9). Finally, I collapsed species-average feather angle-dependence variables into two PCs: 1) “Angle-dependence PC1,” with higher values indicating species with more angle-dependence in chroma, RGB hue, and UV hue; and 2) “Angle-dependence PC2,” with higher values indicating species with more angle-dependence in chroma and luminance (Table 9).

I then created univariate models for each independent variable explaining each of the four species-average deviations in % change in color variables separately (n = 20 models). I calculated and evaluated the AIC values and weights for each model, per color variable (i.e. five for luminance, five for chroma, etc.), to test which (if any) variables best explained variation in each deviation in % color change variable.

RESULTS

Effects of plumage-shuttle mis-matches on color appearance

I found significant differences in the % change in color PC between species-specific and mis-matched plumage-shuttle pairings for all five species: Allen's (ANOVA: $F_{4,21} = 7.8$, $p < 0.001$), black-chinned (ANOVA: $F_{4,24} = 17.9$, $p < 0.001$), broad-tailed (ANOVA: $F_{4,29} = 6.1$, $p = 0.001$), Calliope (ANOVA: $F_{4,18} = 11.6$, $p < 0.001$), and Costa's (ANOVA: $F_{4,48} = 14.4$, $p < 0.001$). Specifically, I found that the natural flashiness (i.e. % change in color) of displaying male black-chinned hummingbirds was significantly higher than that for all the mis-match groups (Table 10, Figure 7b). For Allen's hummingbirds, I only found that flashiness during a display was significantly higher during a black-chinned shuttle, compared to the natural flashiness (Table 10, Figure 7a). With broad-tailed hummingbirds, I found significantly greater natural flashiness compared to the flashiness of a broad-tailed feathers during an Allen's shuttle, but no significant differences for other mis-matches. Lastly, for Costa's and Calliope hummingbirds, the natural flashiness was significantly lower than the flashiness of Costa's/Calliope feathers during a black-chinned shuttle (Table 10, Figure 7d, e). Thus, I found that species that naturally tended to have flashier color

appearances during displays (black-chinned and broad-tailed) had their flashiness reduced by plumage/behavior mis-matches, whereas the mismatches increased flashiness in species that naturally tended to have more consistent color appearances (Allen's, Costa's, and Calliope). I found no significant differences between species-specific and mis-matched groups in average color PC for any species (Table 10).

Signal differences predicting color appearance deviations

I found that the best predictor of deviations in % color change PC was differences in shuttle behavior PC between focal and mis-matched species (RI = 0.99; Figure 8), and differences in shuttle behavior PC was significant in the final model ($mR^2 = 0.85$; Table 11). Thus, I supported out prediction that greater differences between species-specific and mis-matched shuttles (i.e. width and velocity and variation in male orientation angle towards the female during a display) led to greater deviations between natural and mis-matched flashiness (Table 11; Figure 7f). However, I did not find support for my other predictions about how differences in plumage or environmental properties between focal and mis-matched species predict deviations in % color change PC with an RI greater than 0.5 (Figure 8).

Species-average signals predicting variation in color appearance deviations

I found that the best univariate model explaining variation in species-average deviation in % change in luminance was plumage patch size (weight = 0.66; Table 12), such that species with larger plumage patches had greater deviations between their natural

and mis-matched flashiness in luminance (Figure 9a). I found that the best model explaining deviation in % change in chroma was flashiness during a display (% change in color PC; weight = 0.86; Table 12). Specifically, species that naturally appeared flashier during their displays had greater deviations between their natural and mis-matched flashiness in chroma (Figure 9b). Additionally, I found that the best model explaining variation in deviation in % change in RGB hue was shuttle behavior PC (weight = 0.54; Table 12), as species with more exaggerated shuttles had greater deviations between their natural and mis-matched flashiness in RGB hue (Figure 9c). Finally, I found that the best model explaining variation in deviation in % change in UV hue was feather reflectance PC (weight = 0.91; Table 12). Species with more exaggerated feather reflectance had greater deviations between their natural and mis-matched flashiness in UV hue (Figure 9d). Overall, I supported my predictions that inter-specific variation in courtship signals predict the extent to which plumage-behavior mis-matches affect species-specific color appearance during a display.

DISCUSSION

I employed a novel experimental signal-mismatch procedure in attempt to understand the co-evolved links between two signaling traits (ornamental plumage and courtship behavior) and their interactions in several species from the “bee” hummingbird tribe. I have previously shown that hummingbird plumage, shuttle behaviors, and display orientation relative to the sun can interact in complex ways to produce different male color appearances during courtship (Simpson and McGraw 2018a,c), and that these interactions

co-evolved with their originating traits (Chapter 1). By tracking color changes of feathers from the different species as they were moved through the paths of other species' shuttle displays, I found that one of the two color appearance variables (color flashiness) differed significantly between the natural species condition and the heterospecific mis-matches. Additionally, I found that the differences between the natural and mis-matched flashiness were positively related to deviations between focal and mis-matched shuttle parameters (e.g. shuttle width). Altogether, these results illustrate the close species-specific pairings between shuttle displays and color appearance, but not between plumage and color appearance, because as differences between species-specific and mis-matched shuttles increased, there was an increased disruption in the natural flashiness of a males and this was not the case with differences in plumage patch sizes.

I found that differences in shuttle behavior between focal and heterospecific mis-matched species predicted deviations in male flashiness, suggesting that the orientation- and-position-specific movements that produce flashy color displays are finely tuned and specially paired within each “bee” hummingbird species. The importance of behavioral movements, orientations, and postures in creating a flashy color-display has also been demonstrated in two species of butterflies and in peacocks, albeit intraspecifically in each case (*Pavo cristatus*; Rutowski et al. 2007; Dakin and Montgomerie 2013; White et al. 2015). Further, some colorful ornaments are only observable due to specific behavioral manipulations, such as uncovering a colorful patch (e.g. lifting a wing and showing an underwing color patch; Hansen and Rohwer 1986; Zanollo et al. 2013), positioning the color patch towards the receiver (e.g. male *Habronattus* jumping spiders ensuring they

directly face a female throughout their courtship display; Echeverri et al. 2017), or re-positioning various body parts to create a color-display not possible in a natural body position (e.g. superb bird-of-paradise, *Lophorina superba*; Laman and Scholes 2012). Thus, for many animals, behaviors are important for optimizing color presentations during displays and/or coloration is important for accentuating behavioral performances (Byers et al. 2010), and my results here shed further light on this behavior/color appearance relationship by demonstrating how alterations in a species' natural display behavior reduce the efficacy of their natural color-display.

Interestingly, I did not find that plumage/behavior mis-matches affected how bright/colorful males appeared on average during a display. In my previous work (Simpson and McGraw 2018a,c), I found that within-species plumage properties did not strongly predict variance in how colorful/bright males appear on average, suggesting that intra-specific variation in plumage properties may be relatively less important or play a secondary role to receivers during courtship displays. For example, colorful ornaments have been suggested to amplify or enhance behavioral displays in *Anolis* lizards and bowerbirds (Fleishman 1988; Endler et al. 2014) instead of being the primary trait of interest. Additional work in *Schizocosa* wolf spiders has demonstrated lower female receptiveness to male ornamental traits that are presented alone as opposed to when paired with behavioral displays or compared to behaviors presented alone (Hebets and Uetz 2000; Uetz et al. 2009). However, among species, I found that how hummingbird species appear during a display did co-vary with species-specific plumage properties (i.e. feather reflectance; Chapter 1), indicating that inter-specific variation in how bright/colorful a

species appears during a display is due to the color properties of male feathers and not behavior. Further inter-specific variation in average color appearance during a display did not co-vary with shuttle display properties (Chapter 1). Thus, it is possible that average color appearance is more robust to changes in behavior among species due to the specific pairings between plumage patch properties and average color appearance, while within a species, average color appearance is mainly influenced by how displays are oriented relative to the sun and overall less sensitive to plumage variation (perhaps because there is little variation in plumage properties within most species). Altogether these results showcase the complex, multi-level relationships among co-expressed, interacting behavioral and color signals.

When comparing deviations between natural and mis-matched flashiness to the individual male signaling traits (e.g. shuttle behaviors, plumage size), I found that species with flashier and more exaggerated shuttle displays had greater deviations from their natural flashy color appearance due to mis-matches, which further supports the aforementioned specific co-evolutionary pairing between behavior and flashy color appearance. Additionally, the negative relationship between species-average feather reflectances and deviations from their natural flashy color appearance suggests that the natural flashiness of species with more exaggerated plumage (which often have more consistent color appearances throughout a shuttle; Chapter 1) are less sensitive to plumage/behavior mis-matches (also mentioned above). Because exaggeration in feather reflectance and bright/colorful consistent appearances co-evolved, whereas feather reflectance and flashiness did not (Chapter 1), it is possible that brighter and more colorful

feathers are better designed to produce a consistent color-display, similar to more conventional non-angle-dependent plumage patches, such as carotenoid-based plumage (McGraw 2006), melanin-colored ornaments (D’Alba et al. 2014), or bright white color patches (Ferns and Hinsley 2004), especially when presented under relatively static light environments (i.e. male display orientation relative to the sun does not vary much within a species).

The positive relationship between plumage patch size and deviations from species natural flashy color appearance is interesting considering the negative co-evolutionary relationship between these two traits, which I previously uncovered in this clade (Chapter 1). It seems that, despite this antagonistic co-evolution, the flashy color appearance of species with larger plumage patches (which is also typically a consistent color-display) is more sensitive to plumage-behavior mis-matches, suggesting that these plumage patches might be more prone to imprecise presentation if not oriented properly (i.e. as occurs in a non-species-specific shuttle) and that plumage patch sizes specifically co-evolved with shuttle displays. Previous work on widowbirds (*Euplectes ardens*) and barn swallows (*Hirundo rustica*) have suggested that exaggerated plumage ornaments like long tails are unwieldy and difficult to fly with (Barbosa 1999; Pryke et al. 2001; Andersson et al. 2002), so because hummingbirds erect their plumage while shuttling, they may have compensated for their large plumage patches by evolving shuttles with less exaggerated movements to avoid this issue (Møller 2008) and/or more optimally show off their larger plumage patches (Simpson and McGraw 2018a). However recent work on peacocks, hummingbirds, and rhinoceros beetles (*Trypoxylus dichotomus*) found that longer, and theoretically more

unwieldy, tails/horns do not negatively impact various aspects of flight (Clark and Dudley 2009; McCullough et al. 2012; Askew 2014), potentially dismissing this idea. Instead, the evolved pairing between display flashiness and plumage patch size among species may have arisen because a bigger area of feathers may be more difficult to coordinate and orient to produce uniform/specific flash patterns. Future work manipulating gorget size is needed to better test these hypotheses.

Animals exhibit a great diversity of signaling traits, and understanding the evolutionary patterns of this diversity has long interested biologists. In this study, I have provided a new and unique set of methods to more deeply probe the potential causes of specific signal pairings among species. My results in this “bee” hummingbird clade show specific pairings between flashiness and behavior and also a tight coupling between consistent color-displays and plumage reflectance; together these results further illustrate the complex evolutionary dynamics of multiple signals and their interactions. I hope that this study further demonstrates the importance of incorporating and understanding the role of signal interactions in the evolution of multiple signals, instead of solely focusing on the properties of the signaling traits in isolation.

CHAPTER 3

CONCLUDING REMARKS

The overarching aim of my dissertation was to understand the mechanisms and evolution of inter-signal interactions in a hummingbird clade with complex coloration and courtship behaviors. To date, although several studies have been conducted testing the information-content and signal-efficacy hypotheses for multiple signaling (Hebets and Papaj 2005), few have considered the signal-interaction hypothesis. Those who have studied signal interactions to date have focused on how receivers react when one or more signals that are thought to interact in their natural state are removed/altered (Fleishman 1992; Persons et al. 1999; Hebets and Uetz 2000), but neglected to describe the complex dynamics of spatiotemporal interactions among simultaneously displayed and changing signals. Which specific properties of the organism, behaviors, environment, or their interactions vary most among males or species during signaling events? Does one feature of the organism or environment control which others are featured or modified during courtship? Are display properties (e.g. environment, organism, behaviors, and their interactions) consistent among hummingbird species? Do signal interaction properties co-evolve with their interacting signals, or do they evolve independently? These are the specific questions I set out to answer through my dissertation.

In my first (Appendix C) and second chapters (Appendix D), I focused on understanding intra-specific variation in male color appearance (i.e. signal interaction properties) in two “bee” hummingbird species. I found that the positioning of males relative to the environment (i.e. sun) was the strongest predictor of variation in color appearance

for both species, such that males who tended to face the sun as they displayed appeared flashier, brighter, and more colorful. However, the two species differed in how displaying males positioned themselves relative to the sun. Costa's hummingbirds consistently faced the sun while displaying, suggesting that there is an optimal environmental orientation for males to present their plumage to females. Many animals display facing the sun, to best show off their ornaments to signal receivers (Rutowski et al. 2007; Dakin and Montgomerie 2009; Olea et al. 2010; Bortolotti et al. 2011; White et al. 2015), supporting the idea that male Costa's hummingbirds are doing the same. In contrast, male broad-tailed hummingbirds greatly varied in their orientation relative to the sun during displays, which suggests relaxed directional selection on lighting conditions for display in this species. Instead male broad-tailed hummingbird may either be making the best of a bad job with regards to their ornaments/behaviors (Kempnaers et al. 1995; Brockmann 2001; e.g. hiding a bad molt year; Webster et al. 2008) or dynamically adjusting their courtship displays based on other environmental variation (Bro-Jørgensen 2010; e.g. background, wind), including female position or territory quality. Alternatively, males might be exhibiting alternative courtship strategies (Chapman et al. 2009; Han et al. 2016) based on aspects of their signaling traits, age/experience, and/or condition. Another possibility is that females are directing where males display to evaluate specific aspects of their signaling traits (Hutton et al. 2015). For example, if a female wanted to better evaluate male shuttle displays, having the male display while facing the sun, causing him to appear flashier, might make evaluating the behavior easier (i.e. flashiness as a signal amplifier; Hebets and Papaj 2005; Byers et al. 2010). Although I did not measure territory quality or the fitness

outcomes of such male courtship variation, these will be important metrics to quantify in future work in order to understand why male Costa's and broad-tailed hummingbirds significantly differed in their sun-orienting shuttle tactics.

In my third chapter (Chapter 1 in text), I focused on understanding inter-specific variation and the macroevolutionary trajectories of signal interaction properties. I found that male color appearance varied significantly across hummingbird species and covaried with plumage and display traits. By incorporating signal interaction properties into a comparative analysis of multiple signals, I was able to fill key gaps in our understanding of trait evolution, because without the signal interactions, I would have only found a negative evolutionary relationship between plumage and shuttle displays. Based on how I found hummingbird signal interaction properties to covary with male signal properties, I can hypothesize why this antagonistic relationship between plumage and shuttle exaggeration exists. For example, one hypothesis is that there is a resource/cost trade-off, either with regards to signal production (Badyaev et al. 2002) or maintenance (i.e. maintaining clean plumage and/or large flight muscles; Møller 2008). While the energetic costs of structural coloration are still debated (Meadows 2012), these bright and conspicuous color patches could be easy to detect by predators (Journey et al. 2013) and/or unwieldy to display if they are too large (Andersson et al. 2002). On the other hand, hummingbird shuttle displays should require the coordination of several physiological systems, such as powerful muscles that enable their high-speed movement (Dakin et al. 2018) or controlling food intake to limit weight gain and having to expend extra energy

during displays (Calder et al. 1990), making them both costly in terms of energy or system maintenance.

Another hypothesis is that it is not mechanically possible to appear optimally bright and colorful, while also appearing flashy, which is in part supported by the fact that I found plumage exaggeration to co-vary with how bright and colorful males appear while displaying, whereas shuttle exaggeration co-varied with flashiness in these hummingbirds (Chapter 1 in text). Additionally, through the results in my fourth chapter (Chapter 2 in text), it seems that flashiness and shuttle behaviors exhibit specific evolved pairings among species. A third hypothesis for the negative relationship between plumage and shuttle exaggeration is that these signals, and their associated color appearances, might serve similar signal efficacy functions, in that it is not necessary to both appear very bright/colorful and appear flashy to attract females (Galván 2008; Endler et al. 2014). Overall, investigations into the evolutionary dynamics between multiple signals and their signal interactions can lead to a much deeper understanding of the evolutionary relationships between the signals themselves and perhaps why these signals are so diverse.

In previous work on color/behavior signal pairings, some have hypothesized that colorful ornaments evolved secondarily to behavioral displays, as a means of highlighting the specific, often complex courtship movements in space and time (Byers et al. 2010; Endler et al. 2014). Previous work in *Schizocosa* wolf spiders, *Anolis* lizards, and bowerbirds supports this idea, in that the colorful/exaggerated ornaments possessed by these species evolved to enhance the behavioral displays of these animals and are not the primary trait of interest (Fleishman 1988; Persons et al. 1999; Hebets and Uetz 2000;

Endler et al. 2014) or that color is primarily used for species recognition, since it is still highly variable across species (Santana et al. 2012; Macedonia et al. 2014; Taylor et al. 2014; Secondi et al. 2015). In fact, in *Schizocosa* wolf spiders, female receptivity was still high during displays without ornamental traits (i.e. leg tufts), but much less so when tufts were present alone (Hebets and Uetz 2000; Uetz et al. 2009). The results from my fourth chapter (Chapter 2 in text) suggest that color may have evolved secondarily to behavioral displays within my focal clade of hummingbirds, since differences in shuttle behavior properties between focal and mis-matched species, not plumage properties, best predicted deviations from natural male color appearance among species, demonstrating the specificity of the evolved behavior/color appearance pairings and that plumage properties (e.g. feather coloration, patch size) may be more arbitrary. I would also predict, based on my findings here, personal observations in the field, and previous work, that females should attend more to behavioral displays and color appearances, as opposed to natural plumage properties. One reason is that behavioral displays and the resulting color appearances may provide current and more complete information on the condition and physiological state of an individual (Byers et al. 2010; Barske et al. 2011), whereas plumage properties provides information on the past condition of males (i.e. during molt; McGraw et al. 2002). Further, I predict that behavioral displays, like those in hummingbirds, are likely to be index signals, because males can only display as fast or long as their muscles allow them. Since behaviors seem like more robust targets for females assessment and choice of mates, especially in these hummingbirds, it would then make sense that coloration – in this case color appearance – evolved to amplify the shuttles or make them more detectable/discriminable

(Hebets and Papaj 2005). However, it is also possible that hummingbird courtship displays are a multicomponent signal, where each component is necessary to elicit a receiver response (Hebets and Papaj 2005), but overall my results do demonstrate the central importance of behavior in this signaling system and that plumage properties may play a more secondary role.

Through my dissertation, I have demonstrated that signal interactions are complex and can vary as a function of environmental and organismal traits, both within and among species. By incorporating signal interactions into research on multiple signals, we can gain a much deeper understanding of the evolutionary relationships between signals, the mechanisms of signal use and production, and a better understanding of why animals signals are so diverse (as signal interaction properties are another trait that can be selected upon by different habitats, predation pressures, signal modalities, etc.). Additionally, signal interactions, whether they are part of a multi-component display (i.e. a composite trait) or are an emergent property, can provide additional or new information to receivers, either by allowing aspects of the other traits to be better detected or discriminated or by providing new links to additional non-signaling trait(s) (Hebets and Papaj 2005). Further, through studying signal interactions, we can better understand how signals can be flexibly used and/or adjusted due to particular environmental circumstances (Bro-Jørgensen 2010; Hutton et al. 2015). Overall, I hope that my dissertation validates the need to understand and incorporate signal interactions into current signal theory.

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APPENDIX A

CHAPTER 1 TABLES AND FIGURES

Table 1. Locations and GPS coordinates for each of my hummingbird field sites, and the average solar elevation for each species.

Species	Location	Coordinates	Avg. Solar Elevation
Broad-tailed	Elden Springs, AZ	35.227336, -111.600045	49°
	Lake Marshall, AZ	35.130207, -111.533226	
Costa's	Boyd Deep Canyon Preserve, CA	33.648543, -116.376909	41°
Black-chinned	Appleton-Whittell Research Ranch, AZ	31.596682, -110.502764	58°
	Patagonia-Sonoita Creek Preserve, AZ	31.529326, -110.769719	
Anna's	Arizona State University, AZ	33.418812, -111.933368	46°
Allen's	University of California-Riverside, CA	33.971204, -117.324853	33°
Calliope	Sagehen Creek Field Station, CA	39.432464, -120.240191	72°

Table 2. Results from principal components analysis on individual-level data for % change in color appearance variables (luminance, chroma, RGB hue, UV hue), average color appearance variables (luminance, chroma, RGB hue, UV hue), feather reflectance variables (luminance, chroma, UV hue), feather angle-dependence variables (angle dependence in luminance, chroma, and UV hue), and shuttle behavior variables (shuttle width, shuttle velocity, and variation in male angle of orientation relative to the female). Five principal components (PC) were generated, one for each of the above categories of variables, and these were used for the evolutionary variance-covariance estimations. Values in all but the last two rows of the table indicate the loadings of each variable to their respective PC.

	% Change in Color PC	Avg. Color PC	Feather Reflectance PC	Feather Angle Dep. PC	Shuttle Behavior PC
% change in luminance	0.41				
% change in chroma	0.52				
% change in RGB hue	0.51				
% change in UV hue	0.55				
Avg. luminance		0.46			
Avg. chroma		0.41			
Avg. RGB hue		-0.53			
Avg. UV hue		-0.58			
Feather luminance			0.54		
Feather chroma			0.60		
Feather UV hue			0.58		
Feather lum. slope				0.49	
Feather chr. slope				0.66	
Feather UV slope				0.57	
Shuttle width					0.60
Shuttle velocity					0.60
Orientation angle sd					0.52
Variance Explained	70.0%	60.0%	79.6%	71.0%	86%
Eigen Value	2.80	2.42	2.39	2.13	2.58

Table 3. Akaike (AIC) and Bayesian information criterion (BIC) results for the estimation of trait variance-covariance matrices under three different evolutionary models: 1) Brownian motion, 2) univariate Ornstein-Uhlenbeck, and 3) multivariate Ornstein-Uhlenbeck. No model stood out above the others, with the Brownian motion model only marginally better than the other two.

Evolutionary Model	AIC score	BIC score
Brownian Motion	1718.5	2262.0
Univariate Ornstein-Uhlenbeck	1719.0	2263.6
Multivariate Ornstein-Uhlenbeck	1718.9	2262.4

Table 4. Results from principal components analysis on species-level data for % change in color appearance variables (luminance, chroma, RGB hue, UV hue), average color appearance variables (luminance, chroma, RGB hue, UV hue), feather reflectance variables (luminance, chroma, UV hue), feather angle-dependence variables (angle dependence in luminance, chroma, RGB hue, and UV hue), and shuttle behavior variables (shuttle width, shuttle velocity, and variation in male angle of orientation relative to the female). Six principal components (PC) were generated, one for each of the above categories of variables (except angle-dependence, which produced 2 PCs), and these were used for the ancestral state reconstructions and PGLS. Values in all but the last two rows of the table indicate the loadings of each variable to their respective PC.

	% Change in Color PC	Avg. Color PC	Feather Reflectance PC	Lum/Chr Angle Dep. PC	Hue Angle Dep. PC	Shuttle Behavior PC
% change in luminance	0.54					
% change in chroma	0.48					
% change in RGB hue	0.41					
% change in UV hue	0.55					
Avg. luminance		0.47				
Avg. chroma		0.50				
Avg. RGB hue		-0.54				
Avg. UV hue		-0.49				
Feather luminance			0.46			
Feather chroma			0.46			
Feather RGB hue			0.53			
Feather UV hue			0.55			
Feather lum. slope				0.71		
Feather chr. slope				0.71		
Feather RGB slope					0.71	
Feather UV slope					0.71	
Shuttle width						0.63
Shuttle velocity						0.58
Orientation angle sd.						0.52
Variance Explained	78.5%	77.4%	73.0%	81.7%	92.9%	81.8%
Eigen Value	3.14	3.09	2.92	1.63	1.86	2.46

Table 5. Results from PGLS model analysis testing the effects of male hummingbird traits (behavior, plumage, and environmental) on each other and on color appearance during displays. All possible independent models of hummingbird behavior/plumage/ environment predicting color appearance were tested, and only relationships between variables with a correlation coefficient of |0.7| and higher from the evolutionary variance-covariance matrix (see Figure 6 in main text) were tested. Relationship between shuttle PC and plumage patch size italicized, because it was explored further (see Table 6). Significant effects in bold.

Response Variable	Fixed Effect	Estimate	Std. Err.	t-value	p-value
% Change in Color PC R² = 0.89	Intercept	0.00	0.27	0.00	1.00
	Shuttle PC	1.07	0.19	5.75	<0.01
% Change in Color PC R ² = 0.01	Intercept	0.76	1.46	0.52	0.63
	Average Orient. Angle	0.11	0.72	0.16	0.88
% Change in Color PC R ² = 0.08	Intercept	1.81	2.40	0.75	0.49
	Male Dist. To Female	-0.02	0.04	-0.57	0.60
% Change in Color PC R ² = 0.38	Intercept	2.54	1.61	1.58	0.19
	Plumage Patch	-0.01	0.01	-1.56	0.19
% Change in Color PC R ² = 0.38	Intercept	0.00	0.64	0.00	1.00
	Feather Reflectance PC	0.64	0.41	1.57	0.19
% Change in Color PC R ² = 0.63	Intercept	0.61	1.37	0.45	0.68
	Lum./Chr. Angle Dep.	-0.36	0.44	-0.80	0.47
% Change in Color PC R ² = 0.35	Intercept	0.00	0.65	0.00	1.00
	Hue Angle Dep.	0.82	0.56	1.47	0.21
% Change in Color PC R ² = 0.01	Intercept	0.64	1.85	0.35	0.75
	Male-solar Position	0.00	0.01	0.09	0.93
Average Color PC R ² = 0.26	Intercept	0.00	0.69	0.00	1.00
	Shuttle PC	-0.57	0.48	-1.17	0.31
Average Color PC R ² = 0.20	Intercept	-0.21	0.90	-0.23	0.82
	Average Orient. Angle	0.69	0.70	0.99	0.38
Average Color PC R² = 0.75	Intercept	-18.54	5.21	-3.56	0.02
	Male Dist. to Female	4.45	1.28	3.49	0.03
Average Color PC R ² = 0.06	Intercept	-0.88	1.90	-0.46	0.67
	Plumage Patch	0.00	0.01	0.51	0.64
Average Color PC	Intercept	0.00	0.39	0.00	1.00

R² = 0.77	Feather Reflectance PC	0.90	0.25	3.66	0.02
Average Color PC	Intercept	0.00	0.76	0.00	1.00
R² = 0.10	Lum./Chr. Angle Dep.	0.42	0.61	0.68	0.53
Average Color PC	Intercept	0.00	0.73	0.00	1.00
R² = 0.18	Hue Angle Dep.	-0.58	0.62	-0.93	0.40
Average Color PC	Intercept	3.56	1.50	2.37	0.08
R² = 0.70	Male-solar Position	-0.02	0.01	3.06	0.04
<i>Shuttle PC</i>	<i>Intercept</i>	<i>1.81</i>	<i>0.77</i>	<i>2.35</i>	<i>0.08</i>
R² = 0.64	<i>Plumage Patch</i>	<i>0.00</i>	<i>0.00</i>	<i>-2.66</i>	<i>0.06</i>
Male Dist. to Female	Intercept	20.92	10.93	1.91	0.13
R² = 0.67	Male-solar Position	0.29	0.06	4.94	0.01
Plumage Patch	Intercept	4.69	0.33	13.82	<0.01
R² = 0.67	Male-solar Position	0.01	0.00	2.84	0.05
Feather Reflectance PC	Intercept	0.00	0.54	0.00	1.00
R² = 0.52	Hue Angle Dep.	-0.96	0.46	-2.07	0.11
Feather Reflectance PC	Intercept	-3.52	1.33	-2.64	0.06
R² = 0.75	Male-solar Position	0.02	0.01	3.43	0.03

Table 6. Results from PGLS model analysis testing the effects of male hummingbird shuttle properties individually (not combined in a PC) on each other and on color appearance during displays. Significant effects in bold.

Response Variable	Fixed Effect	Estimate	Std. Err.	t-value	p-value
Shuttle width R² = 0.65	Intercept	277.09	35.3	7.84	<0.01
	Plumage Patch	-36.93	13.65	-2.71	0.05
Shuttle velocity R² = 0.65	Intercept	313.85	43.45	7.24	<0.01
	Plumage Patch	-31.90	11.70	-2.73	0.05
Var. in orient angle R² = 0.65	Intercept	5.44	0.28	19.53	<0.01
	Plumage Patch	-0.82	0.30	-2.76	0.05

Figure 1. Representative average shuttle displays of each hummingbird species (color-coded based on legend within figure), with an accompanying photo of the male's angle-dependent structurally-colored plumage. Shuttle displays are arranged by size, and the distance from the female (located at the origin (0,0)) are not accurate in this figure. All distances are in centimeters, and error bars are not shown, to improve clarity of visual presentation. Male Anna's hummingbirds do not move as they shuttle, but instead perch and sing towards females (Clark and Russell 2012), so they are represented by a single point showing the average distance/position from a perched singing male to a female.

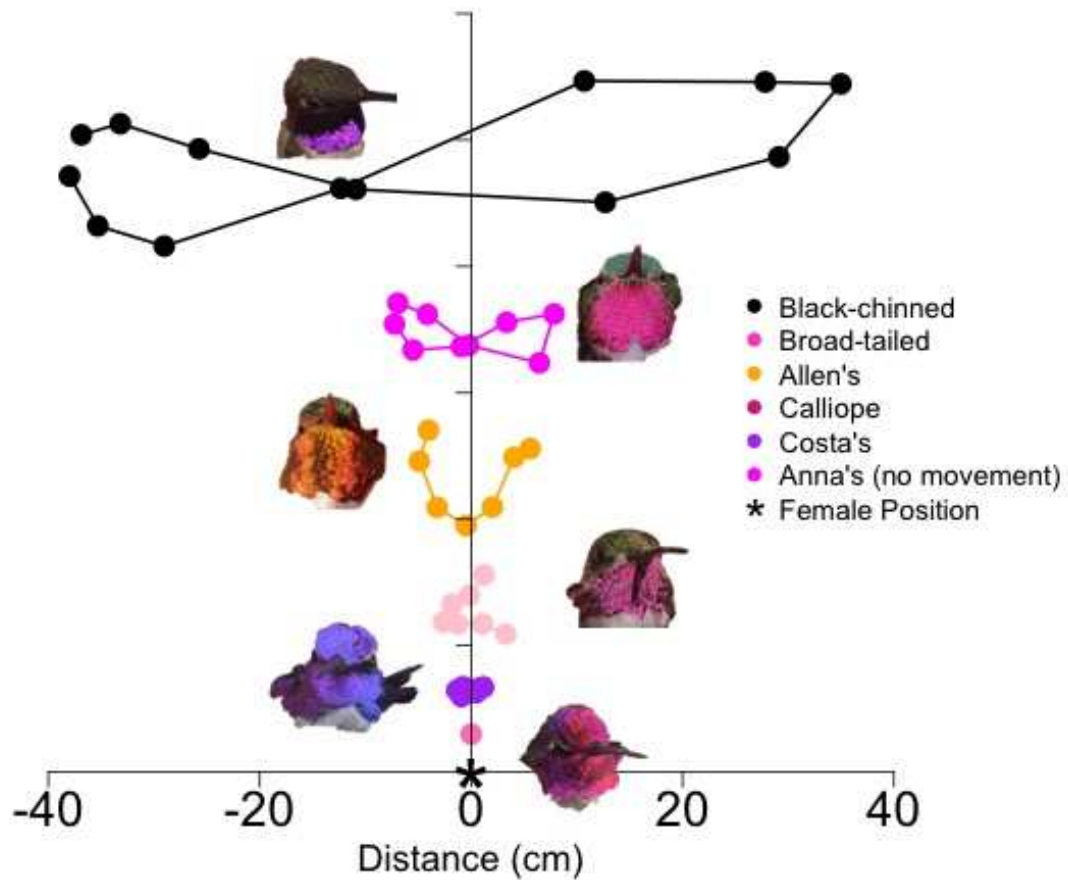


Figure 3. Variation in male color appearance among species. Error bars represent standard error and species labels for each bar (which are also color-coded based on figure 1) are located on the bottoms of E-H. A-D) Variation among species in percent change in male luminance (A), chroma (B), RGB hue (C), and UV hue (D) during a display. E-H) Variation among species in average male luminance (E), chroma (F), RGB hue (G), and UV hue (H) appearance during a display.

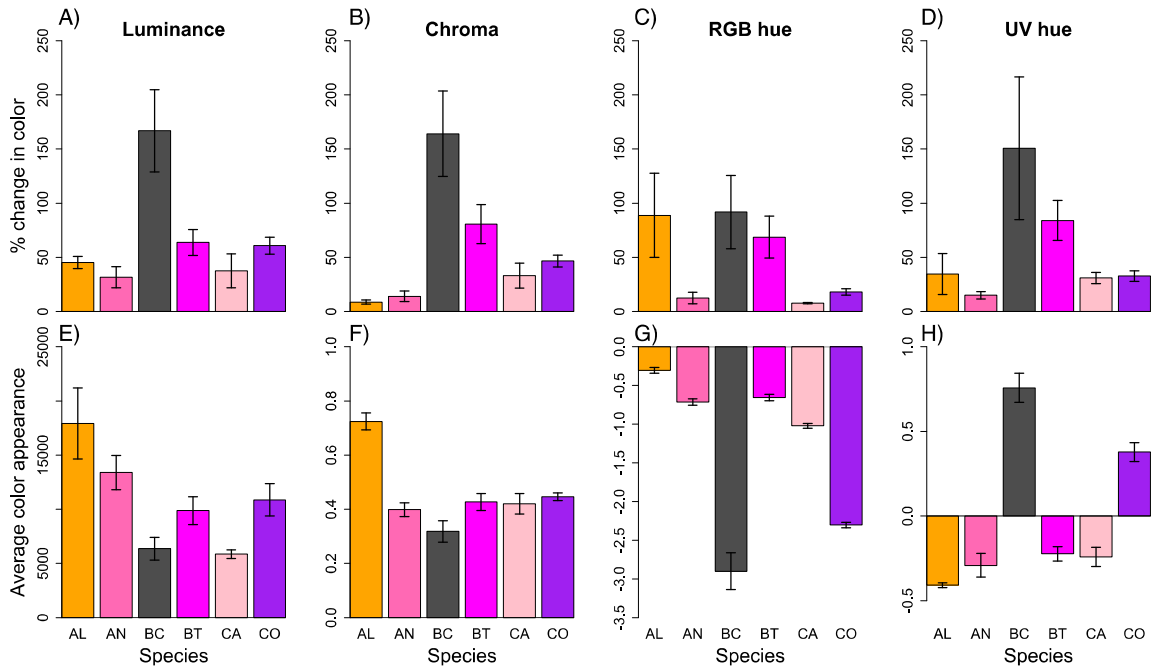


Figure 4. The estimated evolutionary variance-covariance matrix under a Brownian motion model. Each covariance measure was converted to a Pearson's correlation coefficient. The diagonal black line separates the coefficients for the within-species correlations (upper values) and among-species correlations (lower values). All cells are color coded based on the strength of their correlation (red for strong negative, green for strong positive, and white for no correlation). The L-shaped outlined area represents the specific correlations between color appearance during a display (Avg. color PC and % Change PC) and male plumage, shuttle, and environmental traits. All other values are the correlations between male plumage, shuttle, and environmental traits themselves. Only correlations greater than 0.7 or less than -0.7 were interpreted (for details, see text), and values marked with two asterisks (**) are correlations that I interpreted and were also significant in my PGLS models, while values marked with one asterisk (*) are correlations that I interpreted but were not significant in my PGLS models (for details, see main text).

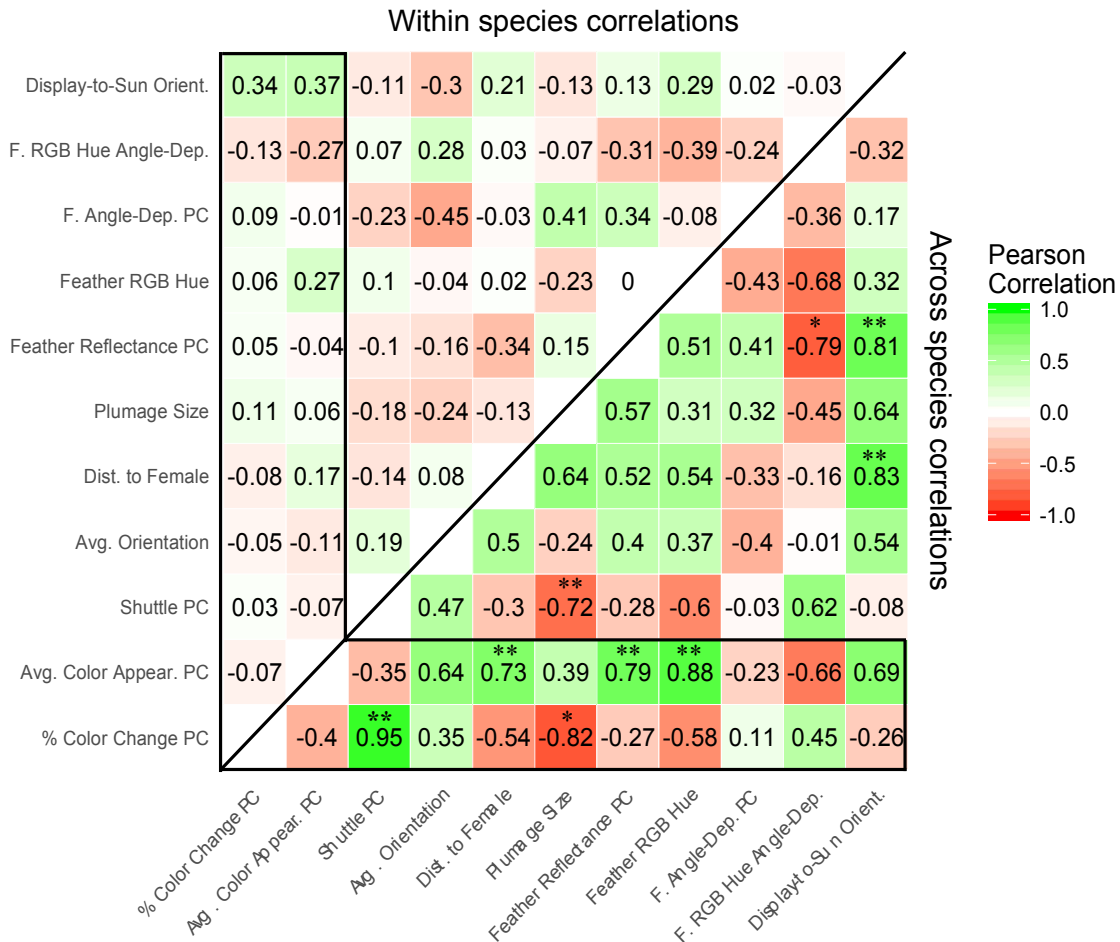
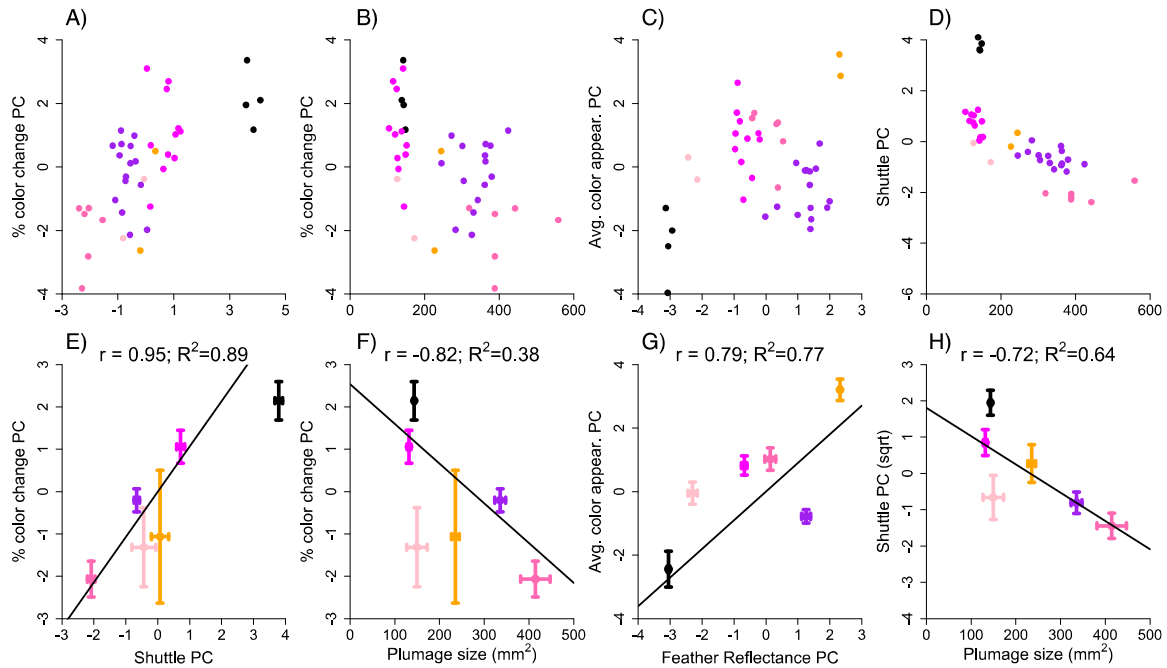


Figure 5. Main interpreted evolutionary correlations between hummingbird plumage, shuttles, and color appearance from variance-covariance matrix (Figure 4). A-D depict individual level data, and are color-coded by species based on Figure 1. E-H depict species means with error bars representing standard errors, and the trendlines based on correlation (r value) and PGLS (R^2 value) results. A,E) Species that have more exaggerated shuttle displays appear flashier during displays. B,F) Species that have smaller plumage patches appear flashier during displays. C,G) Species with more exaggerated feather reflectances appeared on average brighter and more colorful during displays. D,H) Species with more exaggerated displays had smaller plumage patches.



APPENDIX B

CHAPTER 2 TABLES AND FIGURES

Table 7. Results from principal components analysis on % change in color appearance variables (luminance, chroma, RGB hue, UV hue) and average color appearance variables (luminance, chroma, RGB hue, UV hue). Two principal components (PC) were generated, one for each color appearance variable, and these were used to test if plumage-behavior mis-matches affect a species' natural color appearances during a display. Values in all but the last two rows of the table indicate the loadings of each variable to their respective PC.

	% Change in Color PC	Avg. Color PC
% change in luminance	0.47	
% change in chroma	0.50	
% change in RGB hue	0.52	
% change in UV hue	0.51	
Avg. luminance		0.43
Avg. chroma		0.47
Avg. RGB hue		0.53
Avg. UV hue		-0.56
Variance Explained	70.0%	67.1%
Eigen Value	2.78	2.68

Table 8. Results from principal components analysis on data for deviations from natural % change in color appearances (luminance, chroma, RGB hue, UV hue), and differences in 1) feather reflectance variables (luminance, chroma, UV hue), 2) feather angle-dependence variables (angle dependence in luminance, chroma, and UV hue), and 3) shuttle behavior variables (shuttle width, shuttle velocity, and variation in male angle of orientation relative to the female) between focal and mis-matched species. Six principal components (PC) were generated, one for deviations in % change in color and one for differences in shuttle behavior each, and two for differences in feather reflectance and feather angle-dependence each. These PCs were used to test how differences between focal and mis-matched plumage, shuttle, and disop traits predict deviations in % color change due to mis-matches. Values in all but the last two rows of the table indicate the loadings of each variable to their respective PC.

	Deviations/Differences in:					
	% Color Change PC	Shuttle Behavior PC	Feather Hue PC	Feather Lum/Chroma PC	Feather Hue angle-dep. PC	Feather Lum/Chroma angle-dep. PC
% change in luminance	0.45					
% change in chroma	0.56					
% change in RGB hue	0.43					
% change in UV hue	0.54					
Shuttle width		0.59				
Shuttle velocity		0.58				
Orientation angle sd		0.56				
Feather luminance			0.31	0.64		
Feather chroma			0.36	0.60		
Feather RGB hue			-0.64	0.29		
Feather UV hue			-0.60	0.38		
Feather lum. slope					0.16	0.69
Feather chr. slope					0.10	0.70
Feather RGB hue slope					0.70	0.11
Feather UV hue slope					0.69	0.14
Variance Explained	65.0%	60.0%	50.1%	40.0%	39.5%	34.0%
Eigen Value	2.60	2.42	2.04	1.61	1.58	1.36

Table 9. Results from principal components analysis on species-level data for % change in color appearance variables (luminance, chroma, RGB hue, UV hue), average color appearance variables, feather reflectance variables, feather angle-dependence variables (angle dependence in luminance, chroma, and UV hue), and shuttle behavior variables (shuttle width, shuttle velocity, and variation in male angle of orientation relative to the female). Six principal components (PC) were generated, one for each of the above categories of variables, except feather angle-dependence, which has two PCs, and PCs were used to test what species-level traits predicted variation in deviations between natural and mis-matched male % change in color. Values in all but the last two rows of the table indicate the loadings of each variable to their respective PC.

	% Color Change PC	Average color appearance PC	Shuttle Behavior PC	Feather Reflectance PC	Feather Angle- dependence PC1	Feather Angle- dependence PC2
% change in luminance	0.55					
% change in chroma	0.47					
% change in RGB hue	0.57					
% change in UV hue	0.38					
Average luminance		0.45				
Average chroma		0.53				
Average RGB hue		0.52				
Average UV hue		0.51				
Shuttle width			0.59			
Shuttle velocity			0.59			
Orientation angle sd.			0.55			
Feather luminance				0.46		
Feather chroma				0.46		
Feather RGB hue				0.53		
Feather UV hue				0.55		
Feather lum. slope					0.05	0.74
Feather chr. slope					0.43	0.59
Feather RGB hue slope					0.64	-0.24
Feather UV hue slope					0.64	-0.21
Variance Explained	74.0%	78.5%	92.6%	73.2%	48.8%	45.2%
Eigen Value	2.96	3.14	2.78	2.93	1.95	1.81

Table 10. Results from Tukey-Kramer post-hoc tests, testing whether species-specific % change in color appearance was significantly different during a plumage-behavior mis-match. The names of the species of the feathers used for the mis-match (and the natural % change in color) are in the first column, followed by the names of the species of the display used in the mis-match, and the p-value in the final column. Significant effects are in bold.

Focal species (feathers that were used)	Mis-matched species' display	p-value
Allen's	Black-chinned	<0.01
Allen's	Broad-tailed	0.09
Allen's	Calliope	0.92
Allen's	Costa's	0.99
Black-chinned	Allen's	<0.01
Black-chinned	Broad-tailed	0.01
Black-chinned	Calliope	<0.01
Black-chinned	Costa's	<0.01
Broad-tailed	Allen's	0.05
Broad-tailed	Black-chinned	0.24
Broad-tailed	Calliope	0.43
Broad-tailed	Costa's	0.17
Calliope	Allen's	0.80
Calliope	Black-chinned	<0.01
Calliope	Broad-tailed	0.15
Calliope	Costa's	0.97
Costa's	Allen's	0.60
Costa's	Black-chinned	<0.01
Costa's	Broad-tailed	0.16
Costa's	Costa's	0.91

Table 11. Results from final linear mixed model, containing fixed effects with a relative importance (RI) of 0.5 or greater, in which I tested the influence of differences in male shuttling behaviors between focal and mis-matched species on deviations natural % change in male color appearance during courtship displays. Marginal R^2 value are listed below the response variable, which measures 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. Significant effects are in bold.

Response Variable	Fixed Effects (RI)	Estimate	Std. Err.	t-value	P-value
Dev. In % Change in Color PC $R^2_m = 0.85$	Intercept	<0.01	0.14	<0.01	1.00
	Shuttle Behavior PC (0.99)	0.89	0.08	10.55	<0.01

Table 12. Results from AIC model comparisons testing which species-average signaling traits (color appearance, plumage, and shuttle behaviors) best predicted species-average deviations from natural % change in color variables (luminance, chroma, RGB hue, and UV hue, separately). The top model for each of the four comparisons is bolded.

Response variable	Fixed effect	AICc	ΔAIC	Akaike weight
Deviation in % Lum. Change	Plumage Patch Size	37.3	0.00	0.66
	Feather Reflectance PC	41.2	3.90	0.10
	% Change in Color PC	41.4	4.06	0.09
	Average Color PC	41.6	4.30	0.08
	Shuttle PC	41.6	4.33	0.08
	Deviation in % Chroma Change	% Change in Color PC	25.3	0.00
	Shuttle PC	29.7	4.49	0.09
	Average Color PC	31.9	6.60	0.03
	Feather Reflectance PC	33.2	7.98	0.02
	Plumage Patch Size	35.5	10.22	0.01
Deviation in % RGB Hue Change	Shuttle PC	38.2	0.00	0.54
	% Change in Color PC	39.8	1.63	0.24
	Plumage Patch Size	41.8	3.66	0.09
	Average Color PC	42.2	4.07	0.07
	Feather Reflectance PC	43.2	4.11	0.07
	Deviation in % UV Hue Change	Feather Reflectance PC	24.6	0.00
	Average Color PC	29.7	5.10	0.07
	Shuttle PC	34.0	9.37	0.01
	% Change in Color PC	34.8	10.18	0.01
	Plumage Patch Size	35.4	10.80	<0.01

Figure 6. A visual representation of an original, species-specific shuttle re-creation, in this instance a Costa's hummingbird (A), and three plumage-behavior mis-matches (B-D). In each figure, the camera on the tripod represents the modified DSLR camera I used to photograph male feathers from the female's point of view (also indicated by the female symbol). The wood block on grey squares represents my lazy-Susan apparatus, where I could move the feathers around the camera to simulate male display movements, and also orient the feathers to simulate male angles of orientation relative to the female. The grid represents the horizontal plane in which my measurements and re-creations are done (see Chapter 1 for further details). In each panel, the lines represent the shuttle display path and the points indicate the representative points where I photographed the feathers (see Chapters 1 and 2 for examples/species). For each re-creation, I start the display based on the starting location of this male Costa's display, keeping the starting position of each display relative to the sun constant. A) Represents a re-creation of a Costa's hummingbird shuttle, with that Costa's male's feathers. B) Represents a re-creation of a black-chinned hummingbird shuttle with the same Costa's male's feathers. C) Represents a re-creation of a broad-tailed hummingbird shuttle with the Costa's feathers. D) Represents a re-creation of an Allen's hummingbird shuttle with the Costa's feathers. Note: display paths not accurately scaled by size, and the Calliope hummingbird path was not included for an even number of panels.

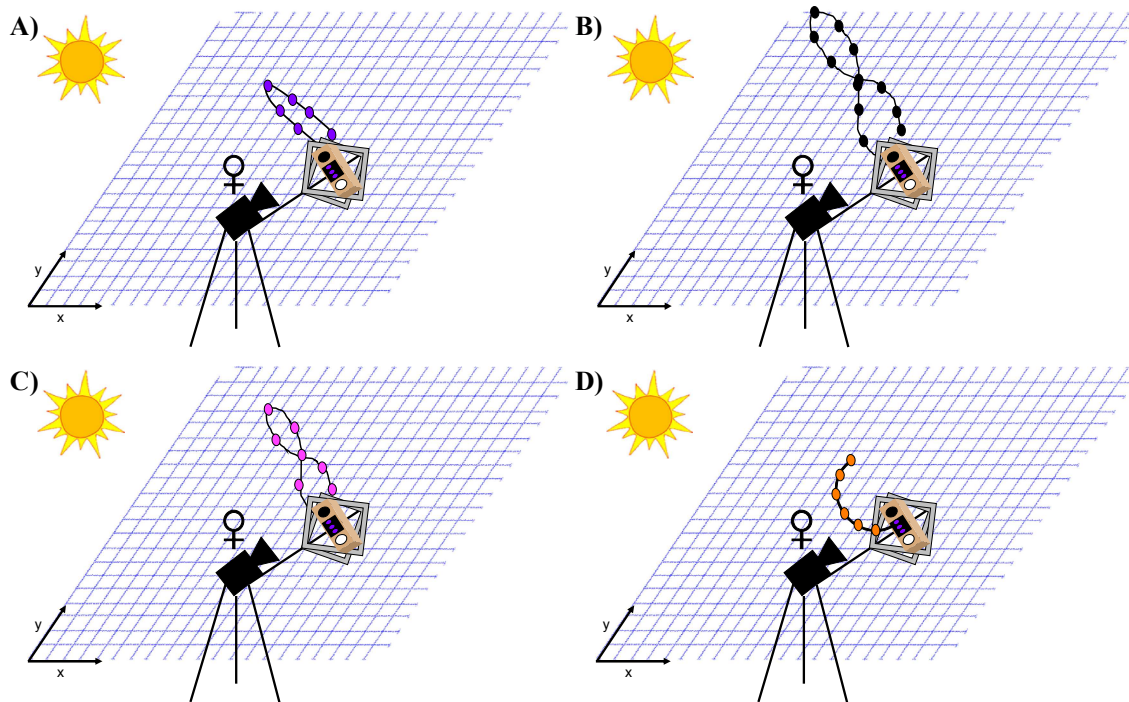


Figure 7. A-E) Plumage-shuttle mis-matches significantly affected natural male color appearance during a display – specifically % change in male coloration, or flashiness. In each barplot, the natural plumage-shuttle combinations are the first column, while the other columns represent the mis-matches. The species whose feathers were used is indicated in the title, and the species whose display was used for each re-creation is along the x-axis (AL = Allen’s, BC = black-chinned, BT = broad-tailed, CA = Calliope, and CO = Costa’s). Asterisk above a bar indicate a significant difference between the natural and mis-matched color appearances. Error bars represent standard errors. F) The positive relationship between differences in male shuttle behavior PC and deviation from natural % change in color appearance PC.

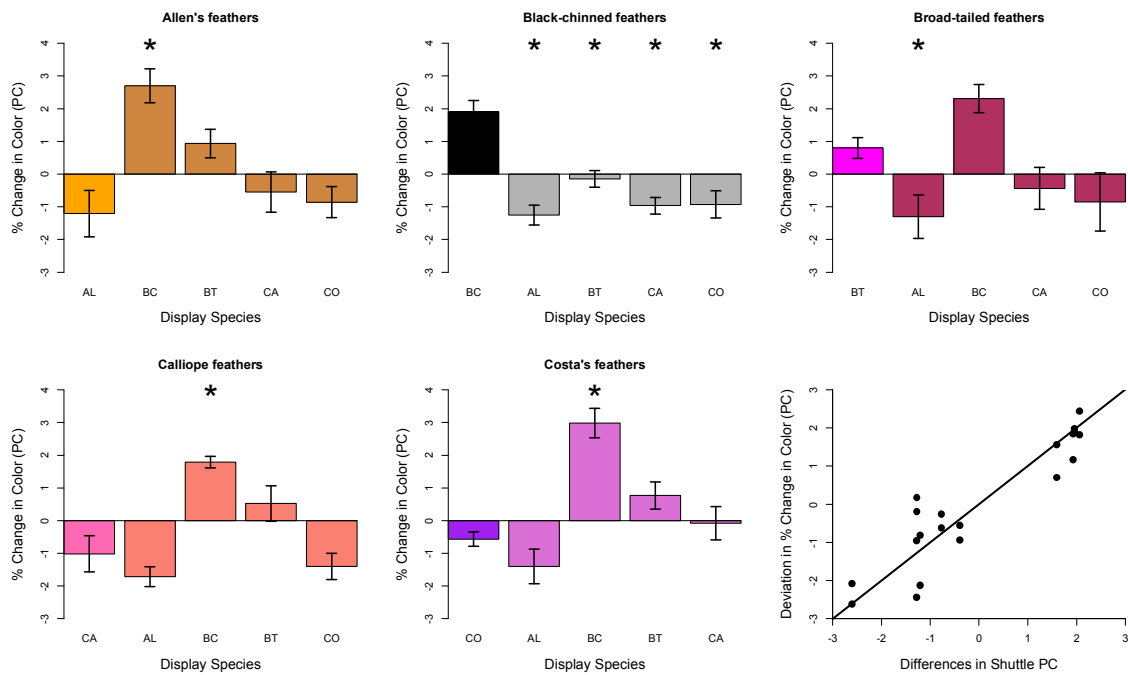


Figure 8. Results from information-theoretic model-averaging analyses, illustrating the relative importance (RI) of each fixed effect (left-hand side) on deviations from natural % change in color appearance PC. Fixed effects with an RI greater than 0.7 are indicated with green bars, while fixed effects below 0.7 are in black. The average beta for each effect is on the right-hand side of the plot, with betas lower than 0.01 indicated by a dash.

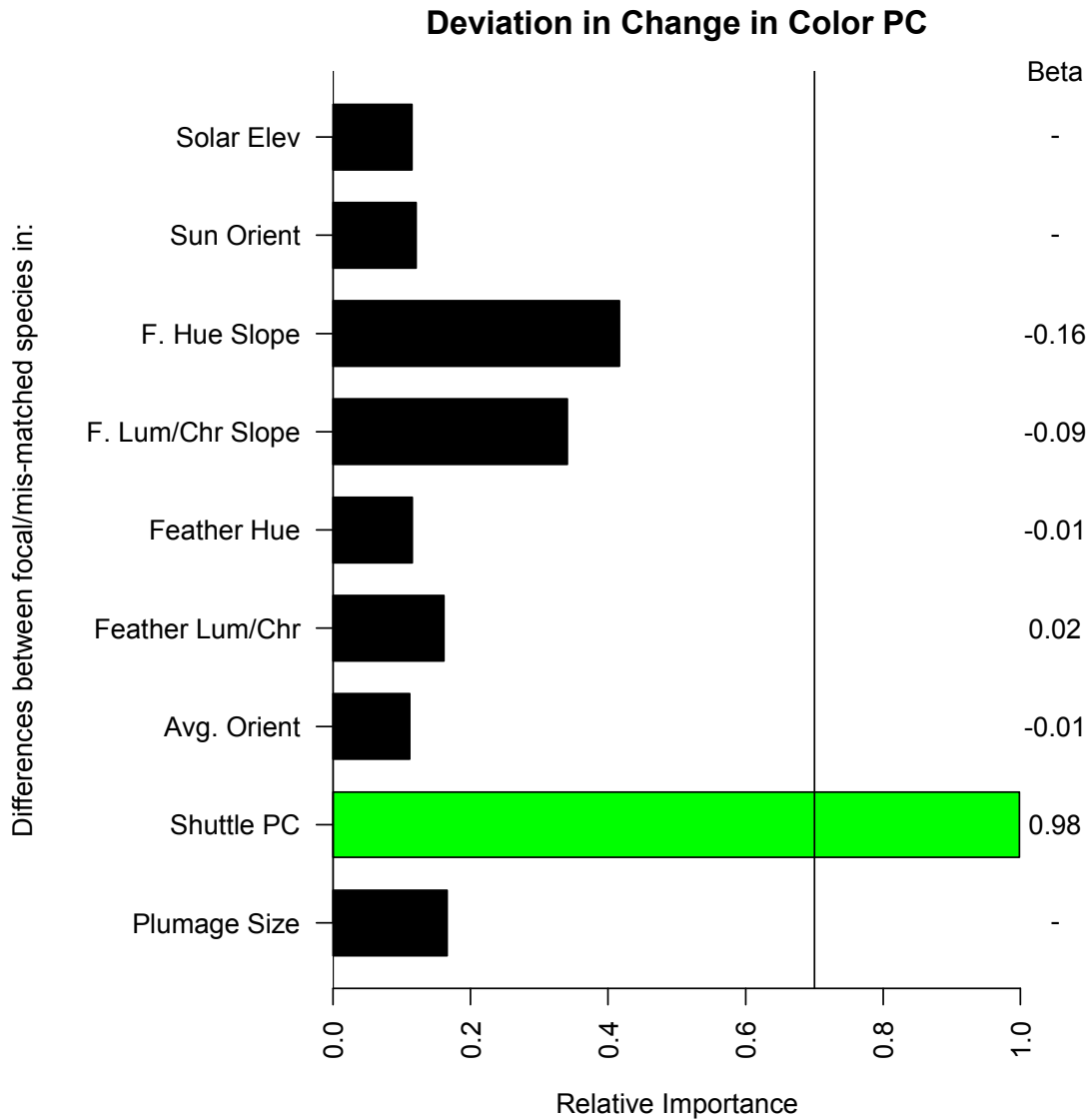
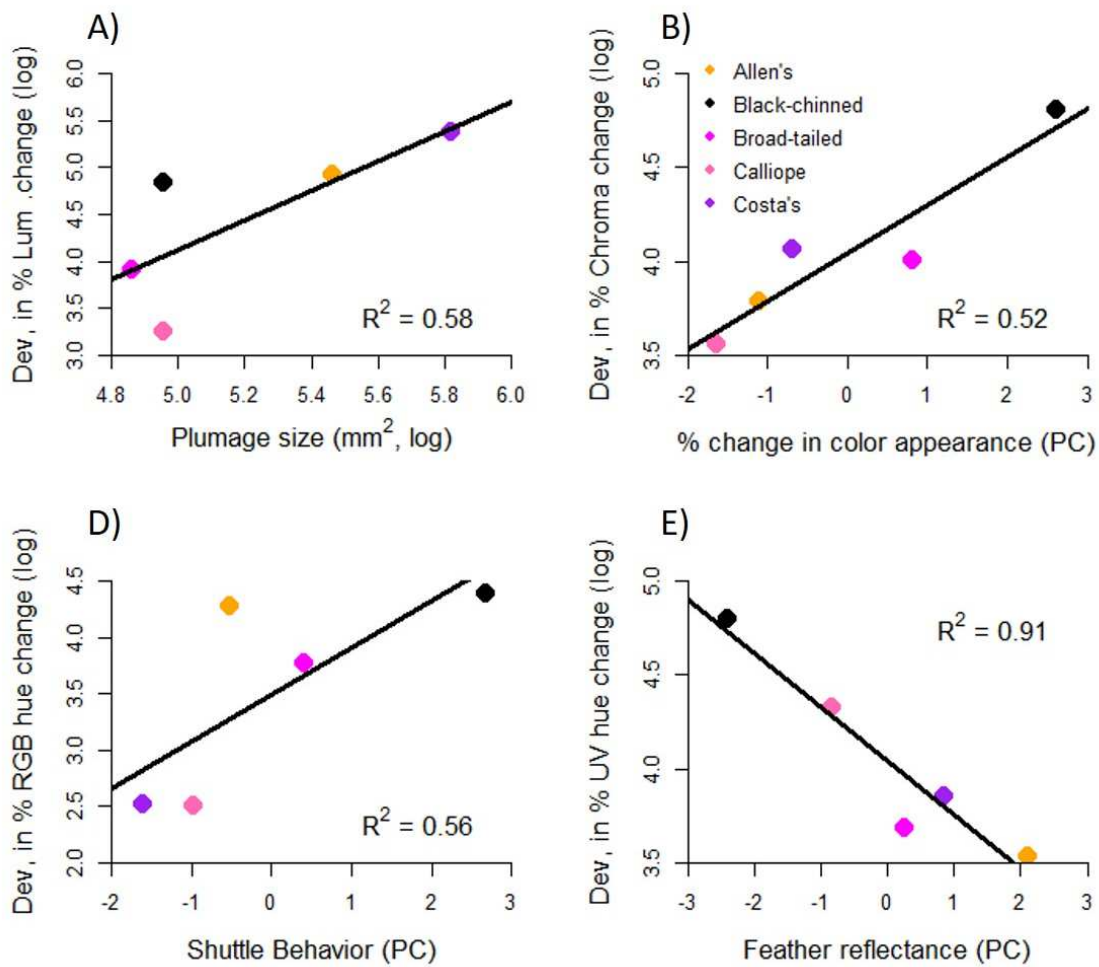


Figure 9. The relationship between species-average shuttle and plumage signals and species-average deviations from natural % change in color appearance. A) A significant positive relationship between plumage patch size and deviations from natural % change in luminance. B) A significant positive relationship between naturally occurring flashiness (% change in color appearance PC) and deviations from natural % change in chroma. C) A significant positive relationship between shuttle behavior PC and deviations from natural % change in RGB hue. D) A significant negative relationship between feather reflectance PC and deviations in from natural change in UV hue. Each point is color coded by species, with the color-species legend in panel B. R^2 for each relationship are also present in each panel.



APPENDIX C

TWO WAYS TO DISPLAY: MALE HUMMINGBIRDS SHOW DIFFERENT COLOR-
DISPLAY TACTICS BASED ON SUN ORIENTATION



Original Article

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

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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 toward 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.

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 (Anderson 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 (Emler 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 (Emler 1992; White and Kemp 2015), and has been used to explain ornament diversity across environments in several clades (e.g. manakins: Pipridae, Emler and Thery 1996; Heindl and Winkler 2003a; surperch: Embiotocidae, Cummings 2007; and African cichlids: Cichlidae, Siehausen et al. 2009). 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 Rohrer 1986) or alter the environment in which they display (Uy and Emler 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 2015; Hutton et al. 2015; White 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 (Emler 1992; Emler 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 (Emler and Thery 1996; Jalanson 2000; Heindl and Winkler 2003a, b; Chapman et al. 2009) and/or the background against which the color is presented (Uy and Emler 2004; Uetz et al. 2010). Animals may orient

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their displays toward the sun (Rutowski et al. 2007; Dakin and Montgomerie 2009, 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, b). 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, b). 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; Hutton et al. 2015; White 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. 2015). 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* and *S. roseni*) 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 toward the sun during courtship (Olea et al. 2010) or *Anolis* lizards perform a pushup alert display to increase the 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 mitchells*) 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 schreiberi* lizards, Pérez i de Lanuza and Font 2014). Thus, iridescent coloration may rapidly change as animals move or change how they orient themselves toward 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. 2015), 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 nonavian 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 toward the sun and/or female to produce a more flashy (i.e., on/off) display (White et al. 2015). 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, Pfalzheim, Germany) and subsequently used to elicit male shuttle displays (see “Eliciting and filming courtship displays”). 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. Males were consistently found at their same territories before and after filming/capture, 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; 4000×3000 pixels). In ImageJ (Schneider et al. 2012), we measured the pixels occupied by each half of the male's gorget and summed the 2 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 \times 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 (Simpson RK, personal observation; 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 h) from 3 to 13 July 2014 and 4 to 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 toward 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).

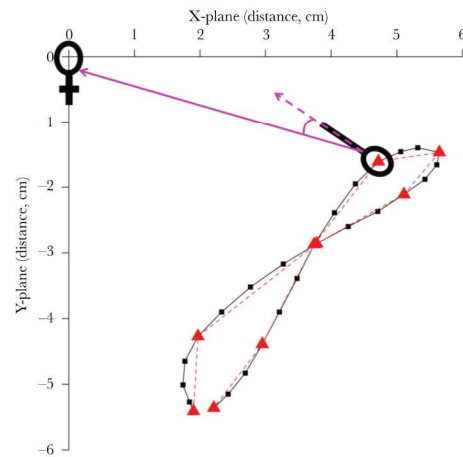


Figure 1 Representative mean path for a male broad-tailed hummingbird's shuttle display. This display path was taken by averaging all shuttle cycles (one back-and-forth movement) from a single display bout (black squares and line) by one male. From this average display path, we selected 9 representative 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 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 (circle) and bill (line) is in black. Error bars are not shown for a clearer presentation.

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 4 males and found that the shuttle cycle width of the average shuttle cycle was not significantly different from 5 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 toward the female during the display; Figure 1). To measure male orientation toward the female throughout the shuttle, we selected 9 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 9 points. We then calculated an average male-to-female orientation angle for each of the 9 positions. We also calculated the standard deviation (SD) of these 9 averaged angles as a measure of how variably a male orients himself toward 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 toward 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 2017) to test whether or not males were orienting toward 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; $0/360^\circ$: 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 recreations 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 9 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 9 points of a male's average shuttle cycle, we also recreated 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 next paragraph). 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 4 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 stimulation values of gorget feathers through the eyes of a bird using a newly developed digital photography technique that creates and analyzes multispectral color photographs (Stevens et al. 2007; Troscianko and Stevens 2015). We mounted 6 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>; 5184×3456 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 Supplementary 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., 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 3 measures of color variation (SD, 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 Supplementary Text S2 for details). PCA resulted in 2 dynamic plumage-color principal components: “% change in coloration PC,” with higher values indicating that 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 to 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° (SD: $\pm 4.0^\circ$) different for the solar azimuth and 1.9° ($\pm 1.1^\circ$) different for the solar elevation compared to the position of the sun during the original display. Thus our recreations 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 5 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 2 levels, which could cause issues with our models, removing year as a random effect did not change our results qualitatively, so 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 toward the female as fixed effects predicting our 5 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 mixed linear models, due to the multiple comparisons. The results after this P -value adjustment were overall similar, 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 2017). 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 2017). 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, state, 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 US Fish and Wildlife Services (MB088806-3), Arizona Game and Fish Department (SP772725), and Coconino National Forest (PEA0943).

RESULTS

Male orientation toward the sun during displays

We found that, on average, shuttling male broad-tailed hummingbirds did not significantly orient themselves toward or away from the sun, but instead displayed in a uniform spatial pattern with no specific mean angle toward 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 toward the sun during shuttles was significantly positively related to % change in gorget luminance and % change in gorget color PC (Figure 3a, b; Figure 4a–d; Table 2; Supplementary Table S6), such that the

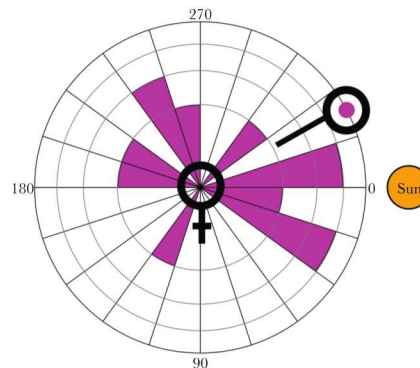


Figure 2 Distribution of shuttle displays by male broad-tailed hummingbirds relative to the sun, which was statistically indistinguishable from uniform. Inner circles represent the number of males in a given sun-orientation bin ($n = 1, 2, 3$, respectively; bins = 18°). The magenta point on the outer circle represents average sun orientation for males. This average was not statistically different from 180° (facing toward the sun) and $0^\circ/360^\circ$ (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, were always roughly facing inward toward the female (indicated by the cartoon of the male head around the average sun orientation point).

Table 1
Circular average solar orientation (\pm circular SD) 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 (<i>n</i>)	Average solar orientation \pm SD	Solar orientation vector length	Rayleigh test of uniformity <i>P</i> -value ^a	Rayleigh test with alternative mean direction (180°) <i>P</i> -value ^a	Rayleigh test with alternative mean direction (0°) <i>P</i> -value ^a
All displays (14)	322.1° \pm 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.

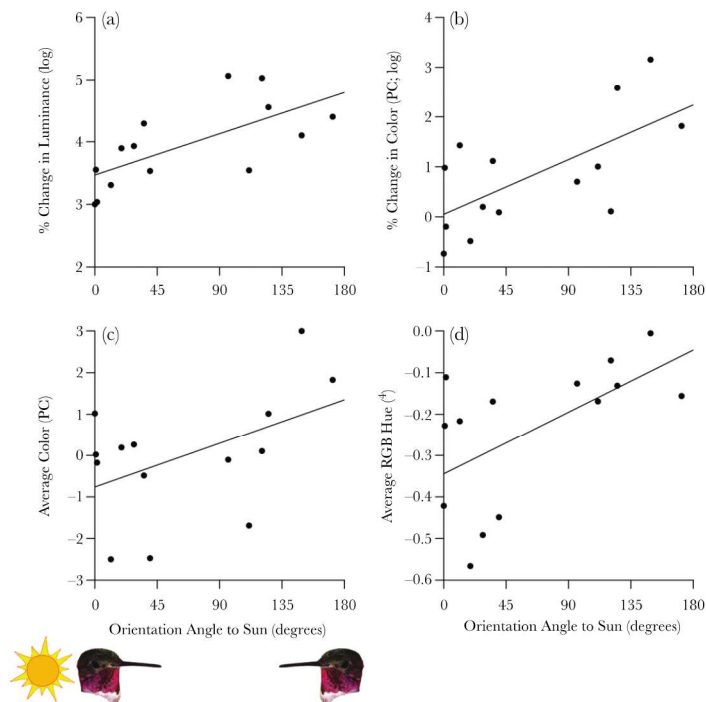


Figure 3

Male sun orientation was significantly positively related to (a) % change in gorget luminance during a display, (b) % change in gorget color PC (chroma and RGB hue) during a display, (c) average gorget color PC (luminance, chroma, and 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.

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 toward the sun during shuttling was significantly positively related to average color PC and average perceived RGB hue of iridescent plumage (Figure 3c, d; Figure 4a–d; Table 2; Supplementary Table 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; Supplementary Table 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

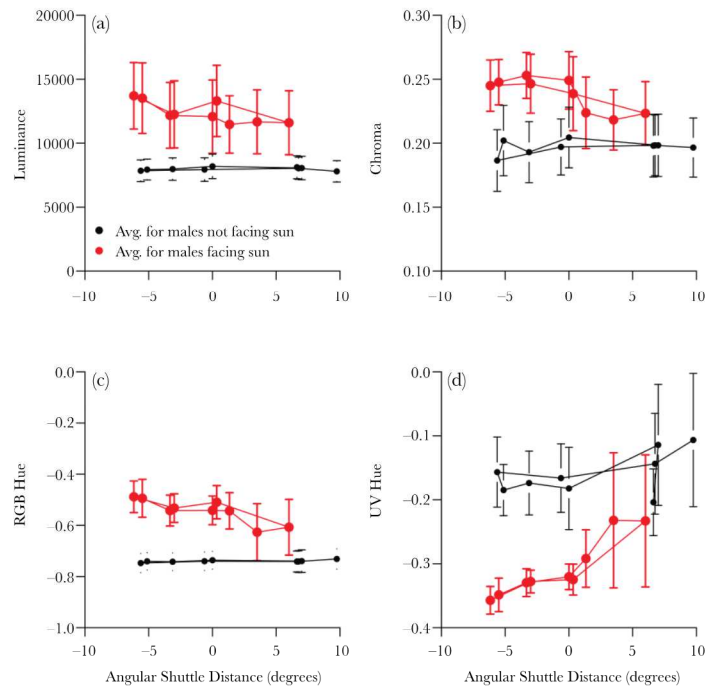


Figure 4

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 2 display tactics along the sun-orientation continuum. Males who tended to face away from the sun had orientations from 0° to 90° and 270° to 360°, while males who tended to face the sun had orientations from 90° to 270°. Further breaking down sun orientation into multiple bins resulted in too little data per group, which is why we chose 2 groups. Flashiness is illustrated by the nonflatness of the lines. Error bars represent standard errors, and in some cases (a and 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.

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

gorget coloration and solar elevation and orientation (Table 2; Supplementary Table 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 toward the female (marginal $R^2 = 0.34$; Figure 5; Table 3; Supplementary Table 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 toward 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 toward the female (marginal $R^2 = 0.17$; Figure 5; Table 3; Supplementary Table 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 toward 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 toward the female (marginal $R^2 = 0.50$; Figure 5; Table 3; Supplementary Table 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 toward the female. We found no other relationships between % change in color and male traits (Figure 5; Table 3; Supplementary Table 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; Supplementary Table 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; Supplementary Table S7), meaning that males with wider

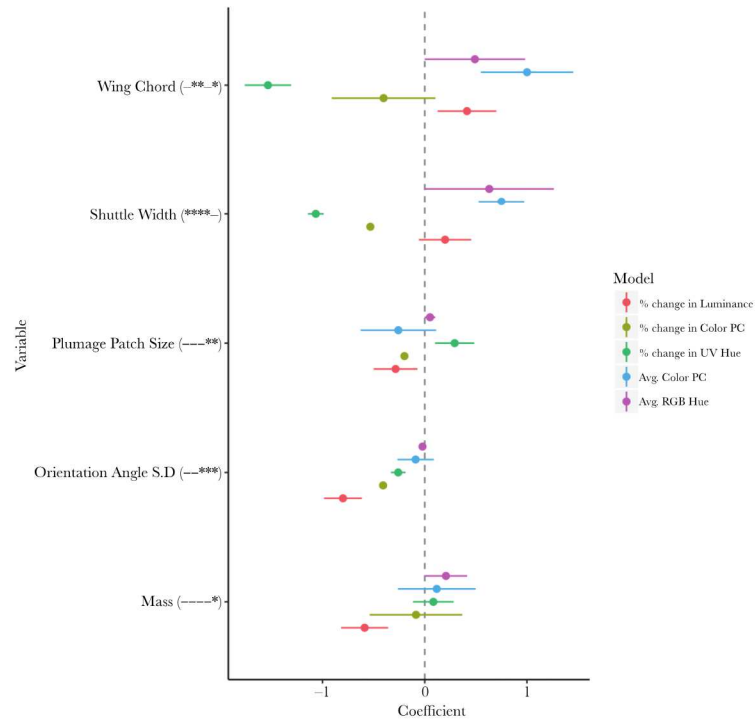


Figure 5

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 SD 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 nonsignificant effects on the response variable, respectively.

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	Standard error	t-value	P-value
% Change in Luminance $R^2_m = 0.84$	Gorget Size*	-0.01	0.005	-2.61	0.03
	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 $R^2_m = 0.17$	Gorget Size	-0.02	0.001	-31.13	<0.01
	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 $R^2_m = 0.50$	Gorget Size	0.02	0.007	3.00	0.07
	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 $R^2_m = 0.30$	Gorget Size	-0.02	0.019	-1.37	0.22
	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 $R^2_m = 0.29$	Gorget Size	<0.01	0.002	0.27	0.80
	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.

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; Supplementary Table 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 toward 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 2 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 2 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, b) 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. 2015; Klomp et al. 2017), although, unlike these other species, male broad-tailed hummingbirds do not all specifically orient toward 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 nondirectional 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 toward the female, these

departures would have less of an effect on perceived color, due to the nondirectional 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. 2015), 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 toward 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 toward the female during their display, because the orientation toward the female was relatively fixed, while the angle toward the sun was variable. These variable angles of orientation toward 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 2 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 with regards to the displays of other males and female preferences, however testing this would require an assessment how these different color displays stimulate females and how the color display 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 (personal communication, C.J.C.), which suggests that males can govern where they display to females and are attempting to get 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, 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 near 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 (Fco 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 Fco 2008; 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, multimodal 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 toward and away from). Further, we hypothesized that male traits—plumage patch size, behavioral performance—would be better emphasized through one of the 2 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.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

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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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APPENDIX D

IT'S NOT JUST WHAT YOU HAVE, BUT HOW YOU USE IT: SOLAR-
POSITIONAL AND BEHAVIORAL EFFECTS ON HUMMINGBIRD COLOR
APPEARANCE

LETTER

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

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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.

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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

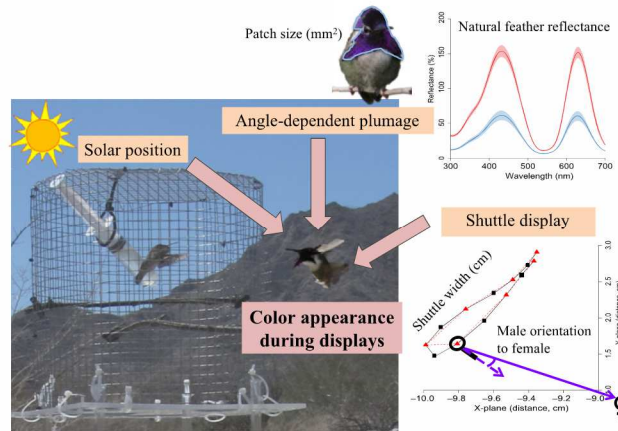


Figure 1 A schematic of the tripartite set of variables that may influence male colour appearance during displays. In the centre of the diagram is a male in mid-shuttle displaying to a female in a cage (centre-left), representing male colour appearance during displays. Illustrated at top-right are the 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 re-creations 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 ($n = 7\text{--}10$ 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 mm^2) 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

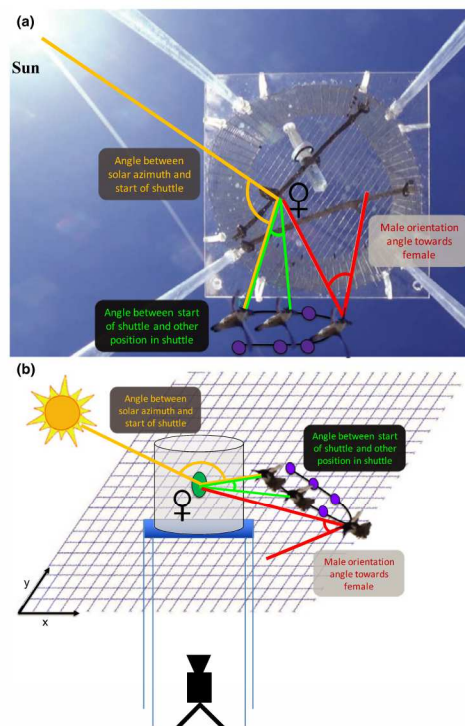


Figure 2 Two-dimensional (2D; a) and three-dimensional (3D; b) visual representation of how we re-created male shuttle displays and the angles we measured. In both panels, the orange text and angles depict how we measured the angle between the solar azimuth and the starting position of the male shuttle 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.

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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 & Håstad 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

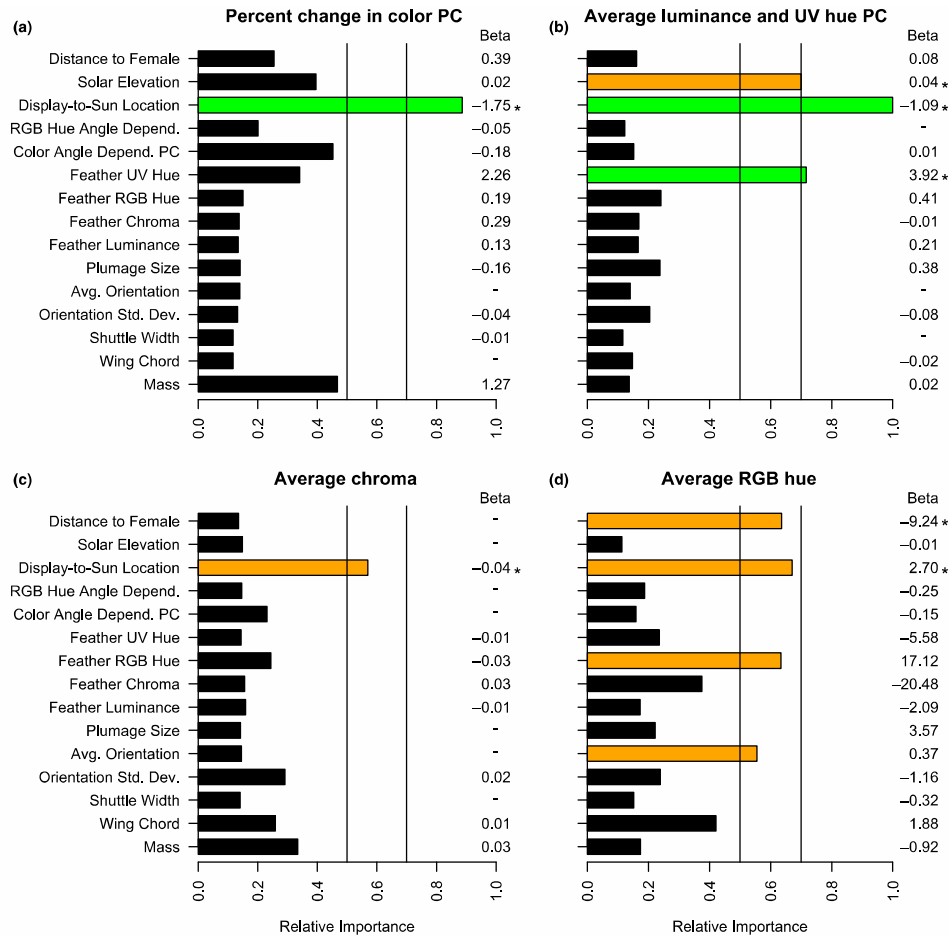


Figure 3 Results from information-theoretic model-averaging analyses, illustrating the relative importance (RI) of each fixed effect (left-hand side) on male colour appearance: (a) Percent change in colour PC during a display; (b) Average luminance and UV Hue PC during a display; (c) Average chroma during 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 $RI > 0.5$.

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

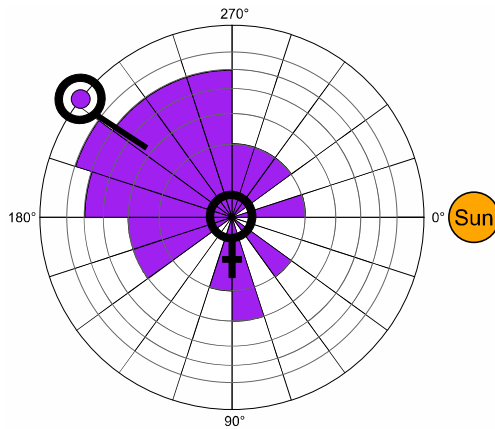


Figure 4 A circular distribution diagram of shuttle displays by male Costa's hummingbirds relative to the sun, which significantly face the sun in a non-uniform pattern. Filled in purple cells in the inner circles represent the number of males in a given male position bin ($n = 1-6$, respectively; bins = 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°/360° (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.

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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>.

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SUPPORTING INFORMATION

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

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APPENDIX E

COAUTHOR PERMISSIONS FOR INCLUSION OF PUBLISHED WORKS



17 July 2018

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To Whom It May Concern:

I am the dissertation committee chair for Mr. Richard Simpson and co-author on his recent dissertation-chapter publications, 1) "Two ways to display: male hummingbirds show different color-display tactics based on sun orientation," in Behavioral Ecology (doi:10.1093/beheco/ary016) and 2) "It's not just what you have, but how you use it: solar-positional and behavioral effects on hummingbird colour appearance during courtship," in Ecology Letters (doi:10.1111/ele.13125). I hereby give my permission for these journal publications to be used as a chapter in his dissertation.

Sincerely,

A handwritten signature in black ink that reads "Kevin J. McGraw".

Dr. Kevin J. McGraw
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