Interspecific covariation in courtship displays, iridescent plumage, solar orientation, and their interactions in hummingbirds

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ABSTRACT

Many animals communicate using multiple signals. Historically, most attention was 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 vary within species, but little is known about how they vary among species, especially with regards to how the expression of particular signals may drive different signal interaction mechanisms. We studied the evolutionary relationships between iridescent plumage, courtship (shuttle) displays, solar environment, and male color appearance during a display (i.e. the signal interaction property) among six species of North American "bee" hummingbirds. We found that color appearances co-vary with behavioral and plumage properties, which themselves negatively co-vary, such that species with more exaggerated displays appeared flashier during courtship, while species with more exaggerated plumage appeared brighter/more colorful with minimal color-changes. By understanding how signal interaction properties co-vary with signals, we were able to discover the complex, multi-layered evolutionary relationships underlying these traits and uncover new potential drivers of signal evolution. Our results highlight how studying the interaction properties between animal signals provides a richer understanding of how those traits evolved and diversified.

INTRODUCTION

Animals communicate using a wide diversity of signaling traits (e.g. songs, colors, vibrations), and many animals use multiple signals (Bradbury and Vehrencamp, 2011). The question of why animals evolved multi-signal systems has generated many hypotheses, which mostly fall into two main groups: information-content hypotheses (e.g. multiple messages vs. redundant signals) and signal-efficacy hypotheses (Hebets and Papaj, 2005; e.g. how aspects of the environment influence signal transmission – sensory drive; Endler, 1992). 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 phenotypic properties (hereafter – *signal interaction properties*; Simpson and McGraw 2018*a*, 2018*b*). The interactive properties of multiple signals can enhance the efficacy of the individual signals or provide new information to receivers (Hebets and Papaj 2005). Thus, to better understand the evolution of multiple signals, it seems critical to investigate how these signals are interacting and how those interaction properties vary among species.

Although signals can interact both 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 mechanisms and evolution of signal interaction properties. Many colorful ornaments can be manipulated by behaviors during courtship to create a display-specific appearance of the colorful ornament (Hutton et al., 2015) and these color appearances are not solely driven by the color properties of the ornaments themselves (i.e. brighter/more colorful ornaments do not necessarily produce brighter/more colorful appearances; Simpson and McGraw, 2018*b*), but also by features of the courtship behavior and signaling environment (e.g. conditions for signal propagation, position of signal receivers). Interaction properties between color and behavior are especially conspicuous for iridescent 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 peafowl (Dakin and Montgomerie 2013), hummingbirds (Simpson and McGraw 2018*a*, 2018*b*), and butterflies (Rutowski et al. 2007; White et al. 2015) has elucidated how colorful ornaments (e.g. wing spots, feathers), display behaviors (e.g. flight patterns, tail rustles), and the sun orientation interact to 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 interaction properties can influence mating success – male peacocks that exhibited greater flashiness (produced by shaking their colorful tail eyespots) had more matings (Dakin and Montgomerie 2013). Although previous work has revealed the mechanisms (Simpson and McGraw 2018*b*) and functions (Dakin and Montgomerie 2013) of signal interaction properties, questions still remain about how signal interaction properties vary among species, evolve, and are driven by the expression of particular signaling traits.

The signaling environment can play a key role in the transmission and interactions of multiple signals (Endler 1992). For example, colorful ornaments can interact, through behaviors, postures, or gestures, with the visual environment to increase their detectability and signal efficacy, such as 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 certain bird and spider species will seek out specific light environments for courtship (Heindl and Winkler 2003; Gordon and Uetz 2011). To this effect, 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, 2018*a*, 2018*b*), and we propose that signal interaction properties will also vary among species due to the solar-positional environment.

The evolution of multiple signals can also be influenced by the relationships between the signaling traits themselves. By assessing the relationship between signaling traits among species, we can make inferences about how these signals co-vary among species and predict why/how selection might be acting upon them. For example, the positive correlation (i.e. positive signal covariance) between wood warbler song and coloration (Shutler and Weatherhead 1990) suggests selection can drive the elaboration of multiple signals at the same time, which leads to greater signal complexity and diversity. Alternatively, negative correlations between traits (i.e. negative signal covariance) can suggest selection driving the elaboration of one signal at the expense of another, due to tradeoffs in signal 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). But how do signal interaction properties covary with the signals that interact to produce them? It is possible that signal interaction properties evolve completely independently from signaling traits themselves, for example variation in flashy color appearances not co-varying among species with plumage brightness or behavioral complexity, though this seems unlikely based on recent signal interaction research. In hummingbirds, individuals with less colorful plumage can still appear bright and colorful due to behavioral modifications during display (Simpson and McGraw 2018*b*), and we suggest that similar relationships could occur among species. There could also be production costs or signal efficacy trade-offs between signal interaction properties and the signals themselves. For example, co-elaboration in the signals 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 any signal interaction properties (e.g. less bright/chromatic appearances).

In this study, we quantified inter-specific variation in colorful ornaments (figure 1 *A*), courtship displays (figure 1 *B*), and the solar signaling environment (figure 1 *C*) of six North American hummingbirds from the monophyletic "bee" tribe (Mellisugini; figure 2 *A*; McGuire et al., 2014) and evaluated the color appearance properties produced as male signals interact with each other and the environment during the display. Most "bee" hummingbirds vary in iridescent head coloration (figures 2 *B*, A1) and a stereotyped, rapid back-and-forth courtship flight (the shuttle display; figures 2 *B*, A1), which are presented simultaneously and close-up to a female during courtship (Videos A1, A2; Clark, 2011; Feo and Clark, 2010). In our previous work, we have shown, separately within two hummingbird species, that these visual traits interact with each other and the signaling environment during male courtship to produce unique male color appearances (namely, male flashiness and average color appearance throughout the display; figure 1 *D, E*; *sensu* Simpson and McGraw 2018*a*, 2018*b*).

Our goal here was to test, in a larger set of hummingbird species, if and how signal interaction properties vary *among* species and co-vary with the signals that interact to produce them and/or the signaling environment. We predicted that male shuttle displays, iridescent plumage, and display position relative to the sun will covary, either positively or negatively, and not exhibit independent (Ornelas et al. 2009) or de-coupled evolutionary relationships (Wiens 2000). We then predicted that inter-specific differences in male color appearance, as with intra-specific variation in color appearance (Simpson and McGraw 2018*a*, 2018*b*), will covary with display position relative to the sun, such that species with brighter, more colorful, and flashier color appearances will tend to face the sun as they shuttle. Additionally, or alternatively, we predicted that variation in male shuttle (i.e. width, speed) and/or plumage properties (i.e. feather reflectance, plumage patch size) will covary with male color appearance, as these traits vary more among species than they do within species (figure A1), which should cause them to play a bigger role in the interspecific covariance of signal interactions.

MATERIALS AND METHODS

Field sites & permissions

We 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 A1 for location coordinates). Species were pseudo-randomly selected for our study based on their geographic breeding location relative to our university (i.e. within a roughly 1200-kilometer radius, through all species but the Calliope were within a 500-kilometer radius). Species were not selected due to any *a priori* information about their shuttle display or plumage characteristics. Due to time and resource limitations, we were not able to fully sample this clade of 20 "bee" hummingbirds, which could influence the results of our study. All applicable national and institutional guidelines for the acquisition, care, and use of animals were followed (Please see text A1 for full permissions). All data used for statistical analyses have been deposited in the Dryad Digital Repository: doi:10.5061/dryad.t53g6p1 (Simpson and McGraw 2019)

Capture methods

We captured adult 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), we captured those adult males (broad-tailed: n = 11, black-chinned = 4, Costa's = 15, Calliope = 2, Allen's = 2; see below for Anna's) on their territories using 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 we were confident that the males we caught were those that we filmed (Simpson 2017). Unfortunately, we were unable to capture the six Anna's hummingbird males that we filmed, so we took measurements and 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, we plucked feathers (n = 7-10/bird) from their colorful throat patch (gorget), specifically from the area underneath their bill (Simpson and McGraw 2018*a*, 2018*b*), 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; Figure 1 *A*) of the iridescent plumage patch (Simpson and McGraw 2018*a*, 2018*b*).

Eliciting & filming male shuttle displays

To elicit male shuttle displays, we 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 (Clark and Feo 2010; Feo and Clark 2010; Clark 2011; Clark et al. 2011, 2013, Simpson and McGraw 2018*a*, 2018*b*). We did this during each species' breeding season (i.e. males were naturally displaying during this time) and under similar solar and visual environments to when males typically display (i.e. when the sun is visible, similar surrounding vegetation). Further, as males typically display in open areas (i.e. deserts, forest clearings etc.) with no overhead obstructions, we ensured there were no overhanging vegetation or structures that could possibly limit the selection of a male's display location. We 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 us to film both male shuttle movements/orientations and female position in the horizontal plane (Video A1; Simpson and McGraw, 2018*a*, 2018*b*). Because males display in the same plane as the female and do not move much vertically while shuttling (Video 2; Simpson and McGraw, *2018a*, 2018*b*), we did not measure vertical movement and focused on the horizontal component of the shuttle display (see text A2 for species-specific filming details).

Quantifying male shuttle displays

For each recorded display, we mapped the male's horizontal movement (i.e. display path) following the methods of Simpson & McGraw (2018*a*, 2018*b*), using the open-source video-analysis program Tracker (Brown 2017). Briefly, we 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 (Video A1; figure 3 *A*). We used these coordinates to calculate an average shuttle cycle (i.e. one back-and-forth movement, in cm) from the multiple back-and-forth movements conducted during a single display bout, or for Anna's hummingbirds (which do not shuttle, but perch stationary and sing to males in a fashion similar to the directed shuttles – with plumage presentation – of the other species; Video A2) an average singing position, for each display bout. From these average shuttle cycles, we calculated the shuttle width (the distance between the turnaround point and start point; figure 1 *B*) and average translational velocity (cm/s; both 0 for *C. anna*; figure 1 *B;* Clark and Russell, 2012).

We also quantified how each male oriented his iridescent plumage towards the female during shuttles by measuring the angle between the center of the male's gorget and the female's head (figure 3 *A*) at seven (Allen's, Calliope, and Costa's), nine (broad-tailed), or thirteen (black-chinned) representative points (selected based on shuttle shape and width; Simpson and McGraw, 2018*a*, 2018*b*). From these angles, we calculated an average male orientation angle relative to the female for each position in a shuttle display and then calculated an overall average and standard deviation in angle of orientation relative to the female for each shuttle (figure 1 *B*). To quantify male angles of orientation towards the female in Anna's hummingbirds, we 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), we collapsed them, using principal components analysis on individual-level data (n = 40), into a single principle 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, which we also define as more exaggerated shuttle displays (table A2).

We 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; figure 3 *B*). We 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 2018*a*, 2018*b*). Finally, we calculated the average of each shuttle and orientation property per individual (i.e. for individuals with multiple shuttle displays). While we calculated a single solar orientation angle per display, the variation in male solar orientation angles throughout a display are captured during our display re-creation methods (see below), to account for how this variation in male position relative to the sun during his display influences his color appearance.

Plumage reflectance & angle-dependence

We followed the spectrometric methods of Meadows *et al.* (2011) to quantify the reflectance properties of each male's feathers in a controlled laboratory setting. We 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 A1). We 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 previous methods, we did not alter the location of the receiver probe, as we were focused on male signals and interactions in this study, rather than the female (Simpson and McGraw 2018*b*). The feathers we measured here were the same feathers that we had plucked and photographed (see below) to quantify each male's color appearance during displays.

We averaged reflectance spectra for the feathers per male and used the average ultraviolet (UV) sensitive avian visual model in the R package *pavo* (Maia et al. 2013), since hummingbirds have UV-vis avian visual systems (Herrera et al. 2008), 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; Maia et al., 2013; Stoddard and Prum, 2008), and luminance. We then calculated the angle-dependence of each color variable by measuring the slope between all feather tilt angles.

Display re-creations & male color appearance

We quantified male color appearance during a display following the methods of Simpson & McGraw (2018*a*, 2018*b;* figure 3 *C,D*). Briefly, we moved the six gorget feathers that we 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 (figure 3 *D*; Simpson and McGraw, 2018*a*, 2018*b*). We calculated RGB hue, UV hue, and chroma (Stoddard and Prum 2008) for each position in each re-created display (figure 1) 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). For our measures of color appearance, we calculated flashiness during a display (i.e. absolute summed % change in color; figure 1 *D*) and average color appearance during a display (figure 1 *E*) from the tetrachromatic color variables (i.e. luminance, chroma, RGB hue and UV hue) across positions in a shuttle cycle (Simpson and McGraw 2018*a*, 2018*b*), and then averaged each color appearance variable per individual. Display re-creations for broad-tailed, black-chinned, and Calliope hummingbirds were conducted near Flagstaff, Arizona, and the re-creations for Costa’s, Anna’s, and Allen’s hummingbirds were conducted in Tempe, Arizona. We used a solar calculator to adjust the times/dates of these reconstructions so that the position of the sun closely matched the solar position when these males originally shuttled at their respective field sites (Hoffmann 2017), and all reconstructions were conducted when the sun was visible.

Comparative analyses

All statistical analyses were conducted in R (R Development Core Team 2017). For our analyses, we used a time-calibrated hummingbird phylogeny (figure 2 *A*; McGuire et al., 2014) and removed all other hummingbird species not included in our study using the R package *ape* (Paradis et al. 2004). We accounted for intra-specific variation in the properties of signals and signal interactions (i.e. multiple individuals per species) while using the R package *Rphylopars* (Goolsby et al. 2017) to calculate the inter-specific correlations between properties of male signals and signal interactions (e.g. color appearance, shuttle display behavior, plumage reflectance/patch size, and male display location relative to the sun; figure 4). We estimated the evolutionary variance-covariance matrices using a Brownian motion model, a Pagel's lambda model, 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. We created the variance-covariance matrix of the most supported model, converted each covariance measure into a Pearson's correlation coefficient, and only interpreted correlation coefficients greater than |0.65|.

RESULTS

Covariance among hummingbird signal properties

We first evaluated the evolutionary relationships among male signals and the signaling environment. Between our four evolutionary models, the Brownian motion model was most supported (table 1), so we interpreted the variance-covariance matrix from this model (figure 4). Among species, we found a significant negative correlation between exaggeration in shuttle behavior (shuttle behavior PC) and iridescent plumage patch size (r = -0.68; figure 4, 5 *A*), indicating that species with more exaggerated shuttles (e.g. wider, faster) had smaller plumage patches. We also found a positive correlation between plumage patch size and display orientation relative to the sun (r = 0.69; figure 4), meaning that species with males that have larger plumage patches tended to face the sun as they displayed to females. Further, we found that each measure of plumage reflectance (luminance, r = -0.69; chroma, r = -0.71; RGB hue, r = -0.65; and UV hue, r = -0.88; figure 4, 5 *B*) was negatively related to angle dependence in RGB hue (i.e. RGB hue slope), which means that species with males that have brighter, more chromatic, red-shifted and more UV-colored feathers had less iridescent feathers (i.e. less change in RGB hue due to changing angles of observation). Finally, we found that feather chroma (r = 0.92) and luminance (r = 0.94; figure 4, 5 *C*) were positively correlated to display position relative to the sun. In other words, species with males that had brighter, more colorful feathers tended to face the sun as they displayed.

Covariance between hummingbird signals and their interactions

We then tested how male signal interaction properties (flashiness and average color appearance) evolutionarily co-varied with their signals and the signaling environment among species. First, we found that both aspects of color appearance varied considerably among species (figure A2) and were negatively related to each other (figure 4). Specifically, we found a negative correlation between male flashiness in chroma (i.e. % change in chroma during a display) and average male appearance in luminance (r = -0.74), chroma (r = -0.72), and RGB hue (r = -0.70; figure 4, 5 *D*). Interestingly, we found a positive correlation between male flashiness in chroma and average male appearance in UV hue (r = 0.74; figure 4).

We found that male flashiness in chroma and UV hue were negatively correlated with iridescent plumage patch size (r = -0.75, -0.82; figure 4, 5 *E*). On the other hand, male flashiness in luminance (r = 0.80), RGB hue (r = 0.66), and UV hue (r = 0.82) were positively correlated with exaggeration in shuttle displays (figure 4, 5 *F*). In other words, species with males that have more exaggerated shuttles but smaller plumage patches appeared flashier while displaying. We also found that male flashiness in chroma was negatively correlated with feather reflectance in RGB hue (r = -0.74) and luminance (r = -0.76; figure 4), while male flashiness in RGB hue was positively correlated with feather chroma (r = 0.65; figure 4), demonstrating a mixed relationship between male flashiness and feather reflectance. Last, we found that male flashiness in chroma was negatively correlated with display position relative to the sun (r = -0.71; figure 4), meaning that species with males that have flashier color appearances tended to face away from the sun while displaying.

We also found male average appearance in luminance and chroma during displays to be positively correlated with feather reflectance in luminance (r = 0.95, 0.96) and chroma (r = 0.96, 0.96; figure 4, 5 *G*). Further, male average appearance in luminance and RGB hue during displays were positively correlated with feather reflectance in RGB hue (r = 0.74, 0.97; figure 4), while male average appearance in UV hue was negatively correlated with feather reflectance in RGB hue (r = -0.96; figure 4). So, species with males that have brighter, more chromatic, red-shifted, and less UV average color appearances during displays had brighter, more chromatic, and red-shifted feathers. We also found that male average appearance in luminance and chroma were negatively correlated with feather angle dependence in RGB hue (i.e. RGB hue slope; r = -0.69, -0.65; figure 4), indicating that species with males that appeared, on average, brighter and more chromatic during their displays had less iridescent feathers. Additionally, we found that male average appearance in chroma was positively correlated with male average orientation towards the female during his display (r = 0.79; figure 4), such that species with males that appeared more chromatic during displays tended to not face the female as directly while shuttling. Last, we found that male average appearance in chroma and luminance were positively correlated with display position relative to the sun (r = 0.86, 0.94, respectively; figure 4, 5 *H*), indicating that species with males that appeared brighter and more chromatic during their displays tended to face the sun.

DISCUSSION

We investigated evolutionary covariation between male hummingbird iridescent plumage, shuttling behavior, display orientation relative to the sun, and male color appearance during courtship displays, which is the property of interactions between the three aforementioned signaling traits. We found evidence for a negative evolutionary relationship between exaggeration in male iridescent plumage and shuttle displays. Additionally, our results suggest that male shuttle displays, plumage patch color and size, and how males oriented their displays relative to the sun all explain inter-specific variation in color appearance during courtship. Specifically, within our six focus species, we found that flashier color appearances positively covaried with exaggeration in shuttle displays and facing away from the sun, while brighter and more chromatic average appearances positively covaried with exaggeration in plumage properties and facing towards the sun. Altogether, these results support our hypothesis that visual signals within our focal hummingbirds exhibited correlated signal evolution and that signal interaction properties (i.e. male color appearance) do co-vary among species with the properties of the individual signaling traits themselves.

The negative relationship that we uncovered between exaggeration in male shuttles and iridescent plumage patch size in our focused dataset mirrors recent work on the acoustic signals in a larger dataset that includes our six focal hummingbirds, whereby a similar negative evolutionary relationship was uncovered between vocal and mechanical sounds produced during courtship (Clark et al. 2018). Clark et al. (2018) suggested that the negative relationship between male "bee" hummingbird wing trills and songs among species 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 negative 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). We propose, due to low levels of predation in these hummingbird species (especially during flight; Miller and Gass, 1985), that exaggeration in plumage serves similar efficacy functions (e.g. attention-grabbing, increasing signal detectability against the background) to exaggeration in shuttles, and perhaps environmental characteristics like display background (Cronin et al. 2014) or wind speeds (Pokorny et al. 2017) drive why certain species favor one signal over the other, with the caveat that our interpretation is based on results obtained with a small sample size and may not be robust to the inclusion of more species. 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), we suggest that these traits do not share a redundant function in terms of information content; future work is needed to test these predictions.

We found that inter-specific variation in both male shuttle behaviors and colorful plumage explained species differences in color appearance during courtship. Specifically, we found that species with more exaggerated shuttle displays but smaller plumage patches appeared flashier while they displayed, while species with more exaggerated plumage patches (i.e. brighter and more chromatic feather reflectance) appeared consistently bright and colorful throughout their displays. Because we also found that male shuttle display and plumage traits share a negative relationship among species, we hypothesize 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. Additionally, the two aspects of male color appearance were negatively correlated and had opposite relationships to male display position relative to the sun. Thus, it is possible that display position relative to the sun is the main mechanistic driver behind species either appearing flashy or appearing consistently bright/colorful.

Another possible explanation for why it might not feasible to appear maximally flashy and bright/colorful at the same time is trade-offs in the production costs of plumage and behavior, 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). Previous work has illustrated the nutritional costs of iridescent coloration (McGraw et al. 2002; Doucet and Montgomerie 2003*a*, 2003*b*; Hill et al. 2005; Kemp and Rutowski 2007) and that behavioral displays are costly in terms of the behavior itself or the maintenance of the required musculature, neuro-architecture, and/or skeletal structure (Byers et al. 2010; Clark 2012; Barske and Fusani 2014). Therefore, it is conceivable that the cumulative costs of these traits result in a trade-off between them. Additionally, while acoustic or olfactory signals are not always combined with color signals in the same way as behavioral displays are (e.g. *Monarcha* flycatchers; Uy and Safran, 2013), our results follow similar patterns to those for song and coloration in cardueline finches (Badyaev et al., 2002) and odor and coloration in *Sceloporus* lizards (Pruett et al. 2016), which are driven by signal trade-offs.

It is also possible that appearing both flashy and maximally bright/colorful during a display is unnecessary 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 displays to achieve high levels of conspicuousness (Galván 2008). However, because selection is likely acting on specific pairings of properties between one signal and color appearance (e.g. shuttles and flashiness), neither shuttle displays nor iridescent plumage can ultimately be lost, as both are needed to produce the signal interaction (Simpson and McGraw 2018*b*). Based on the combinations of relationships we uncovered between shuttle displays, plumage color and size, and color appearance, we suggest that these traits are evolving as two signal complexes among species, not one. With some species, we find flashy color appearances, exaggerated shuttle displays, and less exaggerated plumage patches (i.e. size and color), whereas in other species, we find consistent but bright/chromatic color appearances, less exaggerated shuttle displays, and more exaggerated plumage patches. We therefore hypothesize that this potential multi-signal complex selection is due to a combination of signal production trade-offs and redundancy in signal efficacy.

Interestingly, we found opposite relationships between male display position relative to the sun and color appearances among species compared to the relationships found within species (Simpson and McGraw 2018*b*, 2018*a*). Variation in the solar-positional environment is generally a strong driver of intra-specific (Klomp et al. 2017; Simpson and McGraw 2018*a*, 2018*b*), and inter-specific variation in color appearance and coloration in animals (Fleishman 1988; Persons et al. 1999; Heindl and Winkler 2003). The divergence between our previous intra-specific results and current inter-specific results could stems from the differences in display-sun position within the two species previously studied. Specifically, broad-tailed hummingbirds, which generally appear flashier compared to other species, exhibited high variation in male display position relative to the sun (Simpson and McGraw 2018*a*), while Costa's hummingbirds, which generally appeared consistently bright and colorful compared to other species, all tended to face the sun while they displayed (Simpson and McGraw 2018*b*). Alternatively, the opposite relationship we found here could be an artifact of low statistical power, and the inclusion of additional species in our analyses could reverse the pattern we found here.

In this study, we found one puzzling result, in that species with less iridescent feathers have brighter and more chromatic feathers, which is contrary to recent published work on iridescent coloration (Dakin & Montgomerie 2013; Gruson et al. 2018). This curious finding could be due to differences in feather structure among species (Prum 2006). Previously, the studies showing positive relationships between feather color and iridescence were performed on single species (Dakin & Montgomerie 2013; Gruson et al. 2018), where variation in feather structure is likely not as large (e.g. Shawkey *et al.* 2003; Doucet *et al.* 2006) as that between species (e.g. Maia *et al.* 2013; Eliason *et al*. 2015). Therefore, we suggest that feather-structure variation among species may be driving the odd, negative relationship we found between feather chroma/luminance and feather iridescence in our focal hummingbird species, and future work is needed to test this hypothesis.

Multiple signals can be perceived in several ways (Hebets and Papaj 2005), and based on our results, we can draw inferences about how these signals might be 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 phenotype only exists as the signals are co-expressed and interact. On the other hand, if all of these signals are evaluated together, then they may represent a multi-component, or composite, signal (Hebets and Papaj 2005; Clark 2011; Gumm and Mendelson 2011), similar to the diverse plumage ornaments in California Quail (*Callipepla californica*; Calkins and Burley, 2003). Due to our hypothesized divergent signal complexes in these hummingbird species, we predict that male color appearance is one component within a composite display involving color, plumage, and behaviors. Future work on receiver behavior is needed to determine if these signals and interaction properties 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. Previous studies have tested how multiple signals co-vary among species, but little is known about how the properties created by the interactions between signals also evolved. Here, we showcased the complex dynamics between signals and signal interaction properties, providing deeper insight into the evolution of multiple signals. Because we evaluated signal interaction properties, we were able to uncover more than just a simple linear relationship between two signals, creating new testable hypotheses with regards to the multi-layered relationships found here and whether signal interaction properties evolve similarly in other taxa. Additionally, our results highlight the importance of studying color in its behavioral and environmental contexts, because even among closely related species, individuals can dramatically differ in how manipulate and display their coloration. We hope that future studies on multiple signals and coloration will consider the interactions between signals and how these interaction properties can help explain the evolution and diversity of exaggerated animal traits and behaviors.

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Appendix A for: **Interspecific covariation in courtship displays, iridescent plumage, solar orientation, and their interactions in hummingbirds**

Text A1. Permissions and permit information

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 hummingbirds were granted by the United States Fish and Wildlife Services (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).

Text A2. Species-specific filming details.

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; our cage-stand set-up evolved as we progressed and worked under different environmental conditions). However, due to the extreme width of black-chinned hummingbird shuttles, we used two cameras positioned on either side of the caged female, pointing up, to ensure that we 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), we fitted a wire ring around the cage, at the same level of the female's perch, 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 we 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.

Table A1. Locations and GPS coordinates for each of our hummingbird field sites, and the average solar elevation for each species.

Species	Location	Coordinates	Avg. Solar Elevation
Broad-tailed	Elden Springs, AZ Lake Marshall, AZ	35.227336, -111.600045 35.130207, -111.533226	49°
Costa's	Boyd Deep Canyon Preserve, CA	33.648543, -116.376909	41°
Black-chinned	Appleton-Whittell Research Ranch, AZ Patagonia-Sonoita Creek Preserve, AZ	31.596682, -110.502764 31.529326, -110.769719	58°
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 A2. Principal components analysis on individual-level data for hummingbird shuttle characteristics and their interactions.

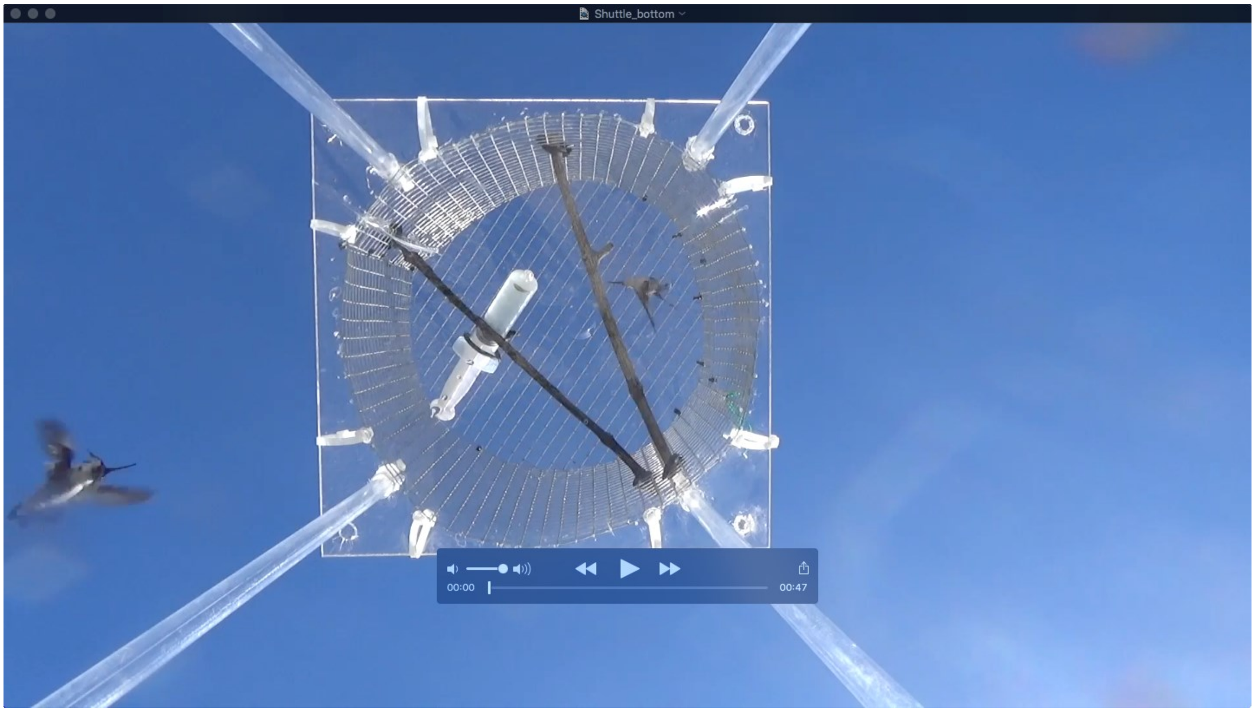
	Shuttle Behavior PC
Shuttle width	0.61
Shuttle velocity	0.61
Standard deviation in orientation angle	0.50
Variance explained	83%
Eigen value	2.50

NOTE – One principal component (PC) was generated and was 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.

Video A1. A view of each hummingbird species' shuttle display from below.

The shuttle is characterized by rapid back-and-forth movements while facing the female, and during these displays males erect their colorful throat patches. Displays were elicited by presenting a caged female at a visible location on a male's territory. Male Anna's hummingbirds do not shuttle, but perch and sing towards females, so the cage was fitted with a metal wire around it, for the male to perch and sing to the female. Calliope hummingbird shuttle is shown twice due to its brevity.

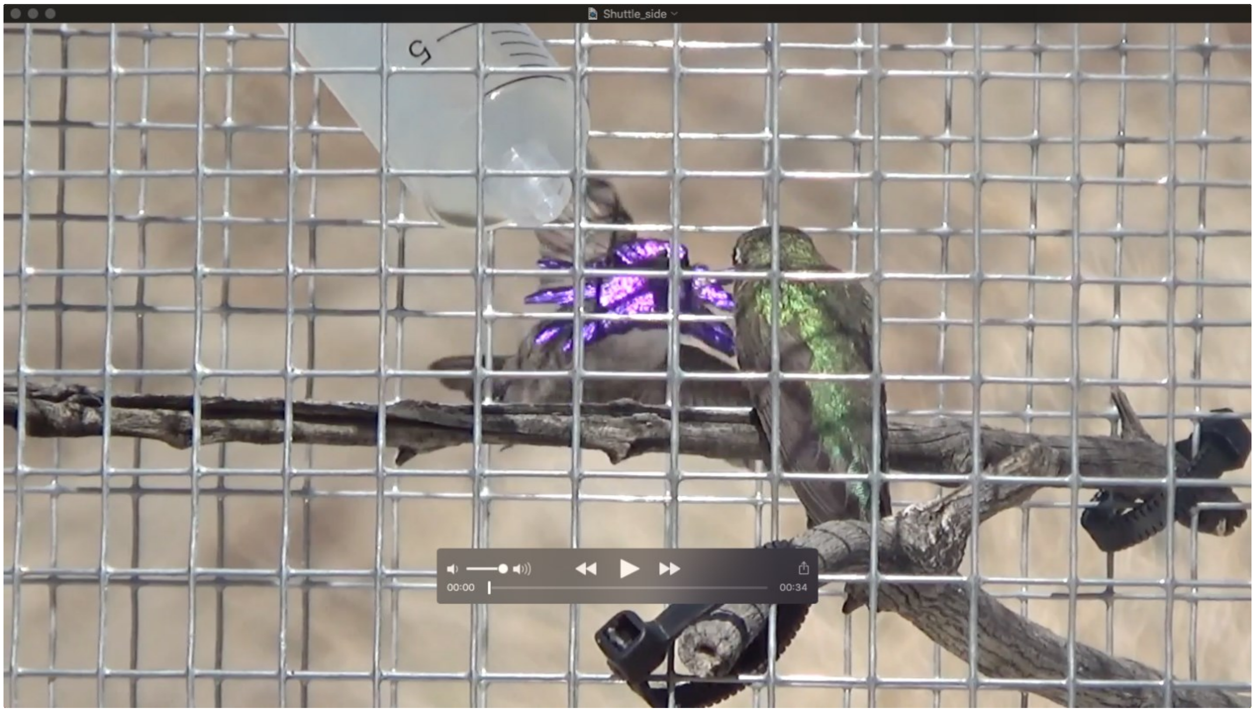
https://youtu.be/-Ja3gXP18r4



Video A2. A side view of each hummingbird species' shuttle display.

Male bee hummingbirds exhibit very little vertical movement during these displays. Male Anna's hummingbirds do not shuttle, but perch and sing towards females, so the cage was fitted with a metal wire around it, for the male to perch and sing to the female. We were unable to obtain a side view shuttle video for Calliope hummingbirds, and the black-chinned hummingbird shuttle is shown twice due to its brevity.

https://youtu.be/0nDzYv1Ow2Y



LITERATURE CITED:

Andersson, S., S. R. Pryke, J. Ornborg, M. J. Lawes, and M. Andersson. 2002. Multiple receivers, multiple ornaments, and a trade-off between agonistic and epigamic signaling in a widowbird. The American Naturalist 160:683–91.

Badyaev, A., G. Hill, and B. Weckworth. 2002. Species divergence in sexually selected traits :increase in song elaboration is related to decrease in plumage ornamentation in finches. Evolution 56:412–419.

Barske, J., and L. Fusani. 2014. Energetics of the acrobatic courtship in male golden-collared manakins (*Manacus vitellinus*). Proceedings of the Royal Society B: Biological Sciences 281:20132482.

Bradbury, J. W., and S. L. Vehrencamp. 2011. Principles of Animal Communication. Sinaeur Associates, Inc., Sunderland, MA.

Brown, D. 2017. Tracker: video analysis and modeling tool. Version 4.8.0. Open Source Physics.

Byers, J., E. Hebets, and J. Podos. 2010. Female mate choice based upon male motor performance. Animal Behaviour 79:771–778.

Calkins, J. D., and N. T. Burley. 2003. Mate choice for multiple ornaments in the California quail, *Callipepla californica*. Animal Behaviour 65:69–81.

Clark, C. J. 2011. Wing, tail, and vocal contributions to the complex acoustic signals of courting Calliope hummingbirds. Current Zoology 57:187–197.

Clark, C. J. 2012. The role of power versus energy in courtship: what is the ‘energetic cost’ of a courtship display? Animal Behaviour 84:269–277.

Clark, C. J., and T. J. Feo. 2010. Why do *Calypte* hummingbirds “sing” with both their tail and their syrinx? An apparent example of sexual sensory bias. The American Naturalist 175:27–37.

Clark, C. J., T. J. Feo, and I. Escalante. 2011. Courtship displays and natural history of scintillant (*Selasphorus scintilla*) and volcano (*S. flammula*) hummingbirds. The Wilson Journal of Ornithology 123:218–228.

Clark, C. J., T. J. Feo, and W. F. D. van Dongen. 2013. Sounds and courtship displays of the Peruvian sheartail, Chilean woodstar, oasis hummingbird, and a hybrid male Peruvian sheartail × Chilean woodstar. The Condor 115:558–575.

Clark, C. J., J. A. McGuire, E. Bonaccorso, J. S. Berv, and R. O. Prum. 2018. Complex coevolution of wing, tail, and vocal sounds of courting male bee hummingbirds. Evolution 72:630–646.

Clark, C. J., and S. M. Russell. 2012. Anna's hummingbird (*Calypte anna*). The Birds of North America. Ithaca: Cornell Lab of Ornithology.

Cronin, T. W., S. Johnsen, N. J. Marshall, and E. J. Warrant. 2014. Visual Ecology. Princeton University Press, Princeton, NJ.

Dakin, R., and R. Montgomerie. 2009. Peacocks orient their courtship displays towards the sun. Behavioral Ecology and Sociobiology 63:825–834.

———. 2013. Eye for an eyespot: how iridescent plumage ocelli influence peacock mating success. Behavioral Ecology 24:1048–1057.

Doucet, S. M., and M. G. Meadows. 2009. Iridescence: a functional perspective. Journal of The Royal Society Interface 6 Suppl 2:S115-32.

Doucet, S. M., and R. Montgomerie. 2003*a*. Structural plumage colour and parasites in satin bowerbirds *Ptilonorhynchus violaceus*: Implications for sexual selection. Journal of Avian Biology 34:237–242.

Doucet, S. M. S., and R. Montgomerie. 2003*b*. Multiple sexual ornaments in satin bowerbirds:

Ultraviolet plumage and bowers signal different aspects of male quality. Behavioral Ecology 14:503–509.

Doucet, S. M., M. D. Shawkey, G.E. Hill, R. Montgomerie. 2006. Iridescent plumage in satin bowerbirds: structure, mechanisms, and nanostructral predictors of individual variation in colour. Journal of Experimental Biology 209:380-390.

Eliason, C. M., R. Maia, and M. D. Shawkey. 2014. Modular color evolution facilitated by a complex nanostructure in birds. Evolution 169:357–367.

Endler, J. A. 1992. Signals, signal conditions, and the direction of evolution. American Naturalist 139:S125–S153.

Endler, J., J. Gaburro, and L. Kelley. 2014. Visual effects in great bowerbird sexual displays and their implications for signal design. Proceedings of the Royal Society B: Biological Sciences 281:20140235.

Feo, T. J., and C. J. Clark. 2010. The displays and sonations of the black-chinned hummingbird (Trochilidae: *Archilochus alexandri*). The Auk 127:787–796.

Fleishman, L. J. 1988. Sensory influences on physical design of a visual display. Animal Behaviour 36:1420–1424.

Galván, I. 2008. The importance of white on black: unmelanized plumage proportion predicts display complexity in birds. Behavioral Ecology and Sociobiology 63:303–311.

Goolsby, E. W., J. Bruggeman, and C. Ané. 2017. Rphylopars: fast multivariate phylogenetic comparative methods for missing data and within-species variation. Methods in Ecology and Evolution 8:22–27.

Gordon, S. D., and G. W. Uetz. 2011. Multimodal communication of wolf spiders on different substrates: evidence for behavioural plasticity. Animal Behaviour 81:367–375.

Gruson, H. C. Anraud, W. Daney de Marcillac, S. Berthier, M. Elias, D. Gomez. 2018. Quantitative characterization of iridescent colours in biological studies: a novel method using optical theory. Journal of the Royal Society Interface Focus 9:20180049.

Gumm, J. M., and T. C. Mendelson. 2011. The evolution of multi-component visual signals in darters (genus *Etheostoma*). Current Zoology 57:125–139.

Hebets, E. A., and D. R. Papaj. 2005. Complex signal function: developing a framework of testable hypotheses. Behavioral Ecology and Sociobiology 57:197–214.

Hebets, E., and G. Uetz. 2000. Leg ornamentation and the efficacy of courtship display in four species of wolf spider (Araneae: Lycosidae). Behavioral Ecology and Sociobiology 47:280–286.

Heindl, M., and H. Winkler. 2003. Vertical lek placement of forest-dwelling manakin species (Aves, Pipridae) is associated with vertical gradients of ambient light. Biological Journal of the Linnean Society 80:647–658.

Herrera, G., J. C. Zagal, M. Diaz, M. J. Fernández, A. Vielma, M. Cure, J. Martinez, et al. 2008. Spectral sensitivities of photoreceptors and their role in colour discrimination in the green-backed firecrown hummingbird (*Sephanoides sephaniodes*). Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology 194:785–94.

Hill, G. E., S. M. Doucet, and R. Buchholz. 2005. The effect of coccidial infection on iridescent plumage coloration in wild turkeys. Animal Behaviour 69:387–394.

Hoffmann, T. 2017. SunCalc.

Hutton, P., R. A. Ligon, K. J. McGraw, B. M. Seymoure, and R. K. Simpson. 2015. Dynamic color communication. Current Opinion in Behavioral Sciences 6:41–49.

Kemp, D. J., and R. L. Rutowski. 2007. Condition dependence, quantitative genetics, and the potential signal content of iridescent ultraviolet butterfly coloration. Evolution 61:168–83.

Klomp, D. A., D. Stuart-Fox, I. Das, and T. J. Ord. 2017. Gliding lizards use the position of the sun to enhance social display. Biology Letters 13:9–12.

Maia, R., C. M. Eliason, P. P. Bitton, S. M. Doucet, and M. D. Shawkey. 2013. pavo: An R package for the analysis, visualization and organization of spectral data. Methods in Ecology and Evolution 4:906–913.

Maia, R, D.R. Rubenstein, M.D. Shawkey. 2013. Key ornamental innovations facility diversification in an avian radiation. Proceedings of the National Academy of Sciences 10:10687-10692.

Martins, E. P., A. G. Ossip-Klein, J. J. Zúñiga-Vega, C. Vital García, S. M. Campos, and D. K. Hews. 2015. Evolving from static to dynamic signals: evolutionary compensation between two communicative signals. Animal Behaviour 102:223–229.

McGraw, K. J. 2006. Mechanics of carotenoid-based coloration. Pages 177–242 *in* G. E. Hill and K. J. McGraw, eds. Bird Coloration Volume 1: Mechanisms and Measurements. Harvard University Press, Cambridge.

McGraw, K. J., E. A. Mackillop, J. Dale, and M. E. Hauber. 2002. Different colors reveal different information: how nutritional stress affects the expression of melanin- and structurally based ornamental plumage. Journal of Experimental Biology 205:3747–55.

McGuire, J. A., C. C. Witt, J. V. V. Remsen, A. Corl, D. L. Rabosky, D. L. Altshuler, and R. Dudley. 2014. Molecular phylogenetics and the diversification of hummingbirds. Current Biology 24:910–916.

Meadows, M. 2012. *The costs and consequences of iridescent coloration in anna's hummingbirds (Calypte anna)*. Arizona State University.

Meadows, M. G., N. I. Morehouse, R. L. Rutowski, J. M. Douglas, and K. J. McGraw. 2011.

Quantifying iridescent coloration in animals: a method for improving repeatability. Behavioral Ecology and Sociobiology 65:1317–1327.

Miller, R. S., and C. L. Gass. 1985. Survivorship in hummingbirds: is predation important? The Auk 102:175--178.

Olea, P. P., F. Casas, S. Redpath, and J. Viñuela. 2010. Bottoms up: great bustards use the sun to maximise signal efficacy. Behavioral Ecology and Sociobiology 64:927–937.

Ornelas, J. F., C. González, and A. E. de los Monteros. 2009. Uncorrelated evolution between vocal and plumage coloration traits in the trogons: a comparative study. Journal of Evolutionary Biology 22:471–484.

Paradis, E., J. Claude, and K. Strimmer. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20:289–290.

Partan, S., and P. Marler. 1999. Communication goes multimodal. Science 283:272–273.

Persons, M. H., L. J. Fleishman, M. A. Frye, and M. E. Stimphil. 1999. Sensory response patterns and the evolution of visual signal design in anoline lizards. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology 184:585–607.

Pokorny, T., I. Vogler, R. Losch, P. Schlütting, P. Juarez, N. Bissantz, S. R. Ramírez, et al. 2017. Blown by the wind: The ecology of male courtship display behavior in orchid bees. Ecology 38:42–49.

Pruett, J. A., J. J. Zúñiga-Vega, S. M. Campos, H. A. Soini, M. V. Novotny, C. Vital-García, E. P. Martins, et al. 2016. Evolutionary interactions between visual and chemical signals: chemosignals compensate for the loss of a visual signal in male *Sceloporus* lizards. Journal of Chemical Ecology 42:1164–1174.

Prum, R. O. 2006. Anatomy, physics, and evolution of structural colors. Pages 295–353 *in* K. J.

McGraw and G. E. Hill, eds. Bird Coloration, Volume 1: Mechanisms and Measurements. Harvard University Press, Cambridge.

R Development Core Team. 2017. R: a language and environment for statistsical computing. Version 3.4.1. R foundation for statistical computing, Vienna.

Russell, S. M., and R. O. Russell. 2001. The North American Banders' Manual for Banding Hummingbirds. North American Banding Council, Point Reyes Station, California.

Rutowski, R. L., J. M. Macedonia, J. W. Merry, N. I. Morehouse, K. Yturralde, L. Taylor-Taft, D. Gaalema, et al. 2007. Iridescent ultraviolet signal in the orange sulphur butterfly (*Colias eurytheme*): spatial, temporal and spectral properties. Biological Journal of the Linnean Society 90:349–364.

Shutler, D., and P. Weatherhead. 1990. Targets of sexual selection: song and plumage of wood warblers. Evolution 44:1967–1977.

Simpson, R. K. 2017. Courtship and territorial behaviors of three hummingbird species in Arizona. Arizona Birds 2017:1–7.

Simpson, R. K., and K. J. McGraw. 2018*a*. Two ways to display: male hummingbirds show different color-display tactics based on sun orientation. Behavioral Ecology 29:637–648.

———. 2018*b*. It's not just what you have, but how you use it: solar-positional and behavioural effects on hummingbird colour appearance during courtship. Ecology Letters 21:1413–1422.

———. 2019. Data from Interspecific covariation in courtship displays, iridescent plumage, solar orientation, and their interactions in hummingbirds. American Naturalist, Dryad Digital Repository, doi:10.5061/dryad.t53g6p1.

Stoddard, M. C., and R. O. Prum. 2008. Evolution of avian plumage color in a tetrahedral color space: a phylogenetic analysis of new world buntings. The American naturalist 171:755–76.

Taylor, L. A., D. L. Clark, and K. J. McGraw. 2011. Condition dependence of male display coloration in a jumping spider (*Habronattus pyrrithrix*). Behavioral Ecology and Sociobiology 65:1133–1146.

Troscianko, J., and M. Stevens. 2015. Image calibration and analysis toolbox - a free software suite for objectively measuring reflectance, colour and pattern. Methods in Ecology and Evolution 6:1320–1331.

Uy, J. A. C., and R. J. Safran. 2013. Variation in the temporal and spatial use of signals and its implications for multimodal communication. Behavioral Ecology and Sociobiology 67:1499–1511.

White, T. E., J. Zeil, and D. J. Kemp. 2015. Signal design and courtship presentation coincide for highly biased delivery of an iridescent butterfly mating signal. Evolution 69:14–25.

Wiens, J. J. 2000. Decoupled evolution of display morphology and display behaviour in phrynosomatid lizards. Biological Journal of the Linnean Society 70:597–612.

TABLES

Table 1. Akaike (AIC) and Bayesian information criterion (BIC) results for the estimation of trait variance-covariance matrices under four different evolutionary models.

Evolutionary Model	AIC score	BIC score
Brownian Motion	**210.3**	**2177.8**
Univariate Ornstein-Uhlenbeck	213.0	2185.2
Multivariate Ornstein-Uhlenbeck	212.2	2184.4
Pagel’s Lambda	212.3	2184.5

NOTE – We found the Brownian motion model to be the best supported model using both AIC and BIC scores.

FIGURE LEGENDS

Figure 1. Hummingbird sexually dimorphic iridescent plumage (A), shuttle displays (B), and display-to-sun orientation (C) interact to produce color appearance during displays, which we measured as % change in color (flashiness) and average color appearance (D-E). Male iridescent plumage, depicted by the broad-tailed hummingbird (A), was quantified by plumage patch size, feather reflectance (from gorget feathers), and angle-dependence in reflectance. Male shuttle displays (B) were characterized by shuttle width, velocity, and how males oriented themselves relative to the female; an example shuttle display path/orientation is depicted by the male Calliope hummingbird. Display orientation relative to the sun (C) is depicted by examples of a male black-chinned hummingbird directly facing the sun and directly facing away from the sun. For each position in a re-created male display (see Materials and Methods for details), we measured the luminance, chroma, RGB hue, and UV hue of that male's feathers, which is depicted in the bottom four graphs (red lines/points corresponds to brighter, more colorful, and flashier appearance, while black lines/points correspond to a duller, less colorful, and less flashy appearance). We quantified male color appearance as flashiness (i.e. percent change in color appearance; D) and average color appearance (E) during a display from six gorget feathers. Male flashiness (D) is depicted by the sets of Allen's hummingbirds (bottom left), with the top set of images depicting a male with a flashy color display and the bottom images depicting a consistenly colored display. Average male color appearance (E) is also represented by two sets of Allen's hummingbirds (at bottom right), with the top images depicting a brighter and more chromaic average color appearance and the bottom images depicting a darker and less chromaic average appearance.

Figure 2. Our focal hummingbird species vary markedly in their shuttle displays and iridescent plumage.

The phylogenetic relationships between each species (*A*), and example shuttle displays of one individual per species that are arranged by shuttle width, with photographs of an individual's head per species (*B*); Note: the distances 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 position from a perched singing male to a female.

Figure 3. A four-panel depiction of the display re-creation methods.

A mock example of three male Costa's hummingbird x-y positions in the horizontal plane during a shuttle display, with a cartoon of the iridescent crown and gorget (purple shape) and bill (black line) indicating male angle of orientation to the female (blue arrows; dashed line aligns with the male's bill while the solid line aligns with the female's head, and the angle in between the two lines is the angle of orientation; *A*). We measured male display location relative to the sun by specifically measuring the angle between the location of the male at the start of his shuttle, relative to the solar azimuth, with the female being the central point of the angle (*B*). Next is a cartoon of the translation from the quantified male x-y positions and angles of orientation to the lazy-Susan apparatus (*C*). The plucked feathers (represented by three purple teardrop shapes; note that six feathers were used in the actual display reconstructions), in addition to white and black standards (circles), were moved as if they were the male displaying and are oriented (by rotating the lazy-Susan) to match the angle of orientation that the male exhibited at those positions (blue arrows). The camera (black shape) at bottom represents the female's point of view and is attached to the lazy-Susan apparatus by a black line. Lastly, a picture of the lazy-Susan apparatus connected to our modified, full-spectrum DLSR camera (*D*).

Figure 4. The estimated evolutionary variance-covariance matrix of hummingbird signals and signal interactions under a Brownian motion model.

Each covariance measure was converted to a Pearson's correlation coefficient. Each cell represents an inter-specific correlation coefficient between two traits. All cells are color coded based on the strength of their correlation (blue for strong negative, green for strong positive, and white for no correlation). Only correlations greater than 0.65 or less than -0.65 were interpreted (see main text for details).

Figure 5. Male hummingbird visual signals and signal interaction properties exhibit complex evolutionary relationships, leading to two distinct evolutionary trajectories.

More specifically, species with males that have more exaggerated shuttle displays had smaller plumage patches (A). Species with males that have more chromatic feathers had less iridescent feathers (i.e. lower angle dependence; B). Species with males that have brighter feathers tended to face the sun as they displayed (C). Species with males that appeared flashier during displays appeared less chromatic as well (D). Species with males that have flashier appearances had smaller plumage patches (E), but more exaggerated shuttle displays (F). Finally, species with males that appeared on average more chromatic had more colorful feathers (G), and species with males that appeared brighter tended to face the sun as they displayed (H). Evolutionary correlation co-efficients for each relationship are presented at the top of each plot, and each point is color-coded by species.

Figure A1. Interspecific variation in the properties of male shuttle behavior, plumage patch characteristics, and display orientation relative to the sun.

Specifically, we show variation among species in plumage feather reflectance (*A-D*), plumage angle dependence (*E-H*), shuttle display properties (*I-L*), male display orientation relative to the sun (*M*), and plumage patch size (*N*). Bars depict species averages and error bars represent standard error. Species labels for each bar are color-coded (see panel *I*) and are located at the bottoms of lower panels (*K-N*).

Figure A2. We found great interspecific variation in the color appearances of males during their displays.

We found variation among species in % change in male luminance (*A*), chroma (*B*), RGB hue (*C*), and UV hue (*D*) during a display, and variation among species in average male luminance (*E*), chroma (*F*), RGB hue (*G*), and UV hue (*H*) appearance during a display. Species labels for each bar are color-coded based on figure A1 and are located on the bottoms of *E-H*. Error bars represent standard error.