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Appendix 1

What animals perceive as colour is an integrated sensation of multiple physical stimuli received by the eye. Colour can be deconstructed into two components; the chromatic signal with at least three constituents (hue, chroma, and ultra-violet reflectance) and the achromatic signal, which is usually described as brightness (Montgomerie 2006). The human eye cannot detect shorter wavelengths in the ultra-violet (UV) range whereas birds and a number of other organisms are sensitive to UV and violet light.

Birds are tetrachromats and they perceive colour through the use of four cone photoreceptor classes with differing sensitivities to incoming light (Hart 2001). Most bird species are capable of perceiving a broader range of incoming light that encompasses portions of the UV spectrum than are humans. They do this through the use of two short wavelength sensitive (SWS1 and SWS2) photoreceptors (mammals have SWS2 only) where one has peak sensitivity 355–445 nm and the second, shared with primates, has peak sensitivity 400–470 nm (Hunt et al. 2009). To obtain measures of hue, chroma, and brightness free from human perception and relevant to model avian visual systems, we used the program TETRACOLORSPACE (Stoddard and Prum 2008). For the reflectance curve from each individual, TETRACOLORSPACE calculates the photon catch for all colour receptors present in the avian visual system, and plots the position of that colour in a tetrahedral colour space using the following formula,

$$Q_I = \int_{300}^{700} R(\lambda)C_r(\lambda)d\lambda$$

where Q_I is the idealized stimulus for each of four avian cone types sensitive to ultraviolet wavelength, short wavelength, medium wavelength, and long wavelength light integrated across all wavelengths (λ) between 300nm and 700nm. $R(\lambda)$ is the reflectance spectrum of the plumage patch, $C(\lambda)$ is the spectral sensitivity function for each of the four avian cone types, and $d(\lambda)$ is a constant. We did not use an irradiance spectrum. We calculated photon catches using average spectral sensitivity curves for a theoretical model ultraviolet sensitive bird. The stimulation values from all cones are used to calculate the three-dimensional coordinates (X, Y, Z) of each colour point in tetrahedral space following Stoddard (2008).

The Cartesian coordinates for a colour in the tetrahedron are converted to spherical coordinates, θ , r and ϕ that represent components of the colour signal. The horizontal angular displacement from the positive x-axis around the origin is θ . This value lies purely within the x-y plane and is equivalent to the hues visible to humans. This value can be thought of as lying on a superimposed colour wheel at the base of the tetrahedron. Any point in the vertical direction within the tetrahedron is attributed to the UV cone. The variable r represents chroma, the purity of a hue and a measure of how much white exists in that hue. The angular measurement ϕ describes the UV contribution to hue, but is not equivalent to percent UV because it describes a direction, and thus must include the value of r ; or the distance of a colour from the origin to capture how much UV reflectance is present. A constant angle ϕ could represent different amounts of UV reflectance as r varies. Because ϕ by itself does not represent percent UV and could be misleading (Stoddard pers. comm.), we decided not to include it in the analysis and instead obtained Z from the ultraviolet cone stimulus values. The Z-axis in the tetrahedron then represents percent UV. These colour variables are processed independently from brightness, which can affect how a colour is perceived. We thus used TETRACOLORSPACE to calculate normalized brilliance (total reflectance / $N \times 100$) of each colour patch as our measure of plumage brightness (Stoddard and Prum 2008). See below for the exact variables we used from TETRACOLORSPACE.

We collected three body feathers from the rump of adult bluebirds captured from Bermuda and the coastal (sedentary) and continental (migratory) regions of North America during June and July of 2007 and 2008. We chose to use feathers from the blue rump patch to be consistent with previous studies on bluebirds (Shawkey et al. 2005, Siefferman and Hill 2005). Also, we found that rump feather coloration is highly correlated with the coloration of feathers collected from other body patches (i.e. head and back, data available on request). We quantified plumage colour with an Ocean Optics USB4000 spectrometer and Ocean Optics PX-2 pulsed xenon light source. To approximate their natural arrangement on the bird, we stacked the feathers collected from each individual on a black velvet background with zero reflectance (Siefferman and Hill 2003, Shawkey et al. 2005).

All measurements of reflectance were calculated relative to a diffuse white standard (Ocean Optics WS-1) using SpectraSuite (2006). Each measurement of a plumage patch is an average of 10 scans computed within the spectrasuite software during data collection. We inserted the measurement probe into a matte black plastic sleeve that prevented ambient light from entering the read fibers, thus creating a measurement distance of 5 mm. We made five repeated measurements of each sample by removing the probe and replacing it within the blue plumage. We calculated measurement error (ME) (Bailey and Byrnes 1990) and found it to be consistent with the range of values reported in other studies of structural coloration (Budden and Dickinson 2009). Male and female hue was most repeatable (9.9% and 7.0% ME respectively) and UV had moderate repeatability (37.4 and 32.7 respectively). Both male and female chroma (65.9% and 45.8%) and brightness (51.85% and 56.5%) exhibited higher ME. We then averaged the five repeated measurements to generate a single reflectance curve for each individual. The range of wavelengths captured by this process included 300 to 700 nm and thus includes all parts of the avian visible spectrum.

We understand that some researchers prefer to examine plumage differences as a single unit, a 'likely' representation of how a bird perceives colour (Stoddard and Prum 2008), but there

is considerable merit in looking at each component individually (Montgomerie 2006). One of the principal insights of separating colour into its constituent parts is that the mechanisms behind colour production and evolution vary considerably across these colour components (Badyaev and Hill 2003, Owens 2006, Stoddard and Prum 2008). To satisfy both perspectives, we test for differences among whole colours, and when such differences are found, we proceed to test for differences among individual components of colour. These colour components are as follows, and are described in detail above: hue, chroma, percent ultra-violet (% UV), and brightness.

All statistical analyses were performed using the program R ver. 2.14.1. We first used the Cartesian coordinates of each colour point to compare overall differences in whole colour between island and mainland individuals, and to calculate measures of dichromatism between the sexes. We tested for separation between sexes and regions (mainland migratory, mainland sedentary, Bermuda) using a PERMANOVA (Anderson 2001) with 1000 permutations and the Euclidean distance measure in the package VEGAN (Oksanen et al. 2011). The Cartesian coordinates X, Y, and Z were the dependent multivariate response variables used to calculate linear Euclidean separation of colour and region (three levels) and sex (two levels) were the independent variables. We used a PERMANOVA because each Cartesian response variable was non-normal and correlated with one another and we first wanted to test for separation among groups of colour points. This procedure is ideal for data that do not follow normal distributions and exhibit collinearity (Anderson 2001). This test also allowed us to make inferences about sexual dichromatism and set the stage for tests of individual colour descriptors (e.g. hue, chroma, UV and brightness). Our initial model included region and sex, as well as the interaction between region and sex. Due to the nature of the PERMANOVA, we were unable to determine the exact behavior of the interaction terms. Thus, we followed this global model with a PERMANOVA for each sex using region as the independent variable to test for sex-specific differences across regions.

Given a statistically significant PERMANOVA for the overall differences in plumage colour, we conducted univariate tests of differences among regions in the colour components hue

(θ), chroma (r), UV, and brightness. We used general linear models (GLM) with Tukey's posthoc tests because the data were unbalanced with unequal numbers of observations across regions. Males and females were significantly different in overall coloration, therefore we ran the GLMs for each sex independently and included region as the explanatory variable. All individual colour variables with the exception of male chroma followed a normal distribution. We were unable to satisfactorily transform male chroma and therefore used the non-parametric test described above to look for differences across regions. The results were essentially the same as a univariate GLM so we reported the test statistics from a GLM to match output for other variables.

To quantify differences in sexual dichromatism within a region, we built a matrix of all possible Euclidean distances between male and female colour points in our dataset using the three Cartesian coordinates. To visualize region-specific mean dichromatism, we bootstrapped the inter-sexual distances from this matrix for each region, repeating this sampling routine 10,000 times. We then calculated the mean dichromatism value from each of the 10,000 iterations. We calculated 95% confidence intervals on the bootstrapped means to examine overlap in levels of sexual dichromatism among regions.

Museum dataset results

The PERMANOVA we conducted on the museum data was similar to the live feather dataset (population: $F_{1,232} = 6.54$, $p = 0.013$; sex: $F_{1,232} = 430.23$, $p = 0.001$; population \times sex: $F_{1,232} = 0.19$, $p = 0.742$). The tests we performed on each sex individually did not result in significant differences in whole colour (males, population $F_{1,140} = 1.88$, $p = 0.137$; females, population $F_{1,92} = 2.52$, $p = 0.095$). Keep in mind that this model does not include a measure of brightness, as this is calculated independently from the perceptual model and is not represented by the Cartesian coordinates. The lack of a dimension for brightness, as well as the age of the museum specimens likely makes it more difficult to detect differences in whole colour. However, we do detect the same differences in hue and brightness as we observed in the live feather dataset when we test

individual colour components. We interpret this to mean that these patterns existed at least 100 years ago when the majority of Bermudan specimens were collected, but that the pattern was weaker as a result of structural feather changes that resulted from the storage of specimens, or weaker phenotypic differences at the turn of the century. As an additional note, we did not detect differences in museum feather coloration between migratory and non-migratory groupings. We checked this by splitting the data into different categories: north-east, north-central, south-east and south-central groups that correspond to known migratory flyways and sedentary populations (Gowaty and Plissner 1998). In other words, birds from the nominate subspecies appear to have relatively uniform blue rump coloration across their range.

Table A1. Univariate GLM results for each live colour variable as measured in males and females. We used region (Bermuda, migratory mainland, and sedentary mainland) as the independent variable in all tests. Significant tests describe which components of blue coloration contribute to overall differences among populations

Trait	Male			Female		
	DF	F	p	DF	F	p
Hue	2, 54	15.73	0.000	2, 55	31.32	< 0.001
Chroma	2, 54	0.97	0.385	2, 55	0.35	0.704
UV	2, 54	2.39	0.102	2, 55	9.14	< 0.001
Brightness	2, 54	16.40	0.000	2, 55	7.05	0.002

Figure A1. Eastern bluebird *Sialia sialis* subspecies for comparison. Individuals from the island of Bermuda (*S. s. bermudensis*) are arranged across the top (four males and two females). Arranged along the bottom are male/female pairs from the mainland starting with birds from Guatemala on the left (*S. s. guatemalae*), New York, Massachusetts, and Texas to the right (*S. s. sialis*).



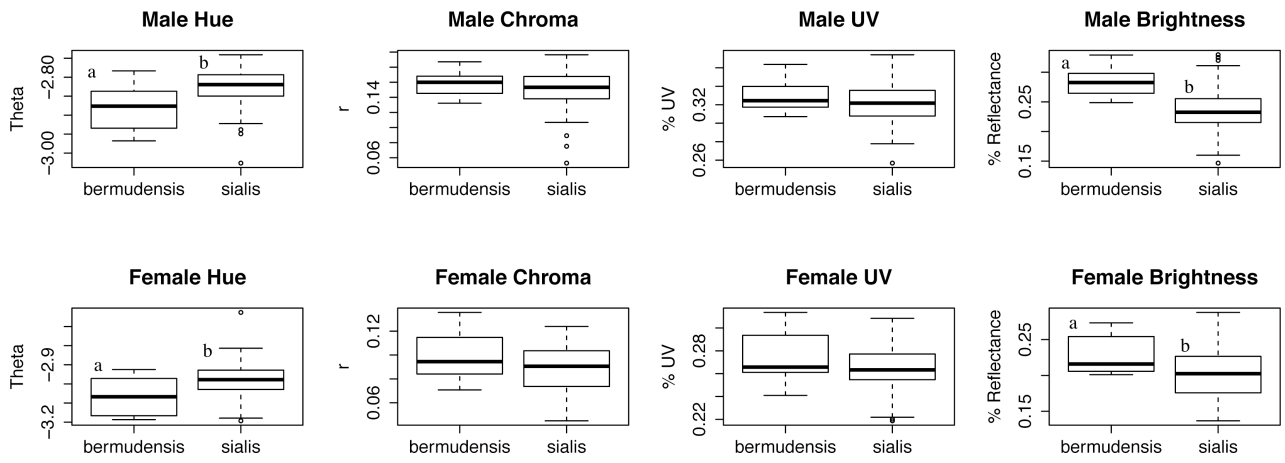


Figure A2. Boxplots of individual colour components in the museum dataset for island bluebirds (*Sialia sialis bermudensis*) and the nominate mainland subspecies (*Sialia sialis sialis*)

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