VARIATION IN PLUMAGE COLORATION OF EASTERN BLUEBIRDS (*SIALIS SIALIS*) IN RELATION TO WEATHER AND GEOGRAPHY

by

Mary-Catherine Warnock

Honors Thesis

Appalachian State University

Submitted to the Department of Biology
and The Honors College
in partial fulfillment of the requirements for the degree of

Bachelor of Science in Ecology, Evolution, and Environmental Biology

May, 2017

Lynn Siefferman, Ph.D. Biology, Thesis Director

Robin Hale, M.A. Geography, Second Reader

Lynn Siefferman, Ph.D. Biology, Departmental Honors Director

Ted Zerucha, Ph.D., Interim Director, The Honors College

Abstract

Sexual selection is understood to help communicate a message from a signaler to a receiver about the signaler's overall quality, even if it compromises survivability. Plumage coloration of birds is a type of ornamentation thought to convey individual quality and influence reproductive success for many different species. Many studies have shown that plumage coloration is condition dependent and influenced by the environment; yet, few studies have investigated how variation in weather during molt impacts ornamental traits. Eastern bluebirds (*Sialis sialis*) are an insectivorous passerine that display sexually dichromatic ultraviolet (UV)-blue structural based and chestnut melanin-based plumage. The UV-blue coloration is likely to be sexually selected as it is sensitive to nutritional stress, reliably indicates individual quality, and influences reproductive success via male-male interactions. The melanin coloration of the breasts of eastern bluebirds is less sensitive to environmental conditions during molt and is likely an indicator of age but not necessarily mate quality. In my thesis, I examine how annual variation in precipitation and temperature during molt (August to October) affects UV-blue and chestnut plumage in eastern bluebirds. I do this by 1) following an Alabama population and investigating whether plumage coloration tracks weather during molt and 2) using museum specimens dating back to 1895 and investigating how geography, weather, and year of specimen collection influence plumage coloration. My results demonstrate that, in the non-migratory Alabama population, birds displayed more ornamented UV-blue plumage in years following late summers with lower temperatures and greater precipitation. In the museum study, birds displayed brighter UV-blue plumage (more ornamented) and chestnut-plumage (less ornamented) when collected in more easterly locations and in locations with higher average temperature. Overall, the Alabama population results suggest that structural based plumage is more affected by climate variation

than melanin-based coloration. The museum specimen results suggest that both plumage types appear to be affected by geographical location and climate, yet structural coloration appears to be more affected by year of specimen collection.

Chapter 1: Annual Variation in Precipitation and Temperature During Molt Influence Structural Plumage Coloration in Eastern Bluebirds (*Sialis sialis*)

Abstract

Annual variation in precipitation and temperature has been shown to affect food availability for insectivores and thus may influence condition-dependent sexually selected plumage of insectivorous birds. Yet, few studies have investigated how natural variation in weather during molt influences ornamental traits. Eastern bluebirds (*Sialis sialis*) are an insectivorous passerine that display sexually dichromatic ultraviolet (UV)-blue structural-based and chestnut melanin-based plumage. The UV-blue coloration is likely sexually selected as it has been shown to be sensitive to nutritional stress, indicate individual quality, and influence reproductive success via male-male competitive interactions. Here, I examine whether precipitation and temperature during the molt period (August to October) influenced male and female coloration measured during the following breeding season at an Alabama field site over a 12-year period. Birds displayed more-ornamented UV-blue plumage in years following late summers with lower temperatures and greater precipitation. Further, these patterns were stronger in males but, in general, consistent between the sexes and age classes. The data suggest that structural-based plumage appears to be more affected by climate variation than melanin-based coloration and precipitation has stronger effects on structural coloration than does temperature. Overall, this study corroborates research suggesting that structural coloration may be more sensitive to environmental conditions during molt than melanin coloration and thus be a more reliable sexually selected signal.

Introduction

Why any animals would be conspicuous in an environment riddled by predators was a source of great debate between Charles Darwin and Alfred Wallace because elaborate conspicuous traits should reduce survivorship (Cronin 1993). Darwin argued that these ornamental traits evolve via sexual selection; they are often sexually dimorphic and influence access to mates, either via mate choice or male-male competitive interactions (Darwin 1871; reviewed in Anderson 1994). Ornaments, including avian plumage coloration, are often reliable signals of mate quality, because they are costly to produce and maintain (reviewed in Hill and McGraw 2006). Within populations, body condition has been shown to be positively correlated with plumage coloration, thus the environment the individual experiences during molt may influence ornamentation (reviewed in Hill 2006).

Avian plumage coloration is produced via two different mechanisms: pigments allocated to feathers and feather microstructure. The two common types of plumage pigments are: 1) melanins (including eu-melanins and phaeo-melanins) which create black, tan and chestnut coloration and 2) carotenoids, which produce colors ranging from yellow to red (McGraw 2006a; b). Carotenoids are generally regarded as the most clearly condition-dependent form of plumage coloration; the pigments are derived from the diet and thus are directly linked to ingesting food (Hill 2002). Melanin coloration, however, are produced *de novo* and are less affected by environmental conditions than carotenoid coloration, although it is possible that access to scarce amino acids minerals might limit expression (McGraw 2006a; b). Structural coloration is different from pigment-based coloration, as green, blue, purple, and iridescent coloration are achieved by reflecting short wavelengths and scattering long wavelengths with the reflective keratin in the feather (Prum 2006). Unlike carotenoid pigments, structural plumage coloration is not directly dependent on ingesting nutrients. However, field correlative studies suggest that variation in elaborate structural coloration is positively associated with greater body condition (*Volatinia jacarina*; Doucet 2002) and with higher quality and older males (*Guiraca caerulea;* Keyser and Hill 1999; 2000). Moreover, aviary studies using restricted food access have shown that structural coloration is influenced by nutritional stress (McGraw et al. 2002; Siefferman and Hill 2005a) while melanin ornaments are not (McGraw et al. 2002).

For species that undergo a complete molt post-breeding season, food availability can be critically important for the acquisition of high-quality plumage (Hill and Montgomerie 1994), and many songbird species molt only one time annually, directly after the summer breeding season when the energetic cost of molt is at the lowest (Payne 1972). Using a multiple year (11 year) correlative approach, Reudink et al. (2015) showed the carotenoid-based orange plumage of American redstarts (*Setophaga ruticilla*) is influenced by rainfall during the preceding molt. This positive co-variation between precipitation and coloration was attributed to higher abundance of insect prey (Reudink et al. 2015).

Here, I investigate how weather patterns during molt influence plumage coloration of Eastern bluebirds (*Sialis sialis*). Eastern bluebirds are socially monogamous passerines that occur year-round in the Southeast, southern Midwest, and along the Atlantic coast of the United States. Eastern bluebirds are partial migrants; the migratory populations breed in the northern latitudes and over-winter in the southern latitudes, while the populations that breed in the southern latitudes do not migrate. Eastern bluebirds are secondary cavity nesters that readily breed in nest-boxes. Female eastern bluebirds build the nest, lay 3-6 eggs and incubate eggs and brood nestlings; however, both male and females feed the young (Pinkowski 1977; Gowaty and Plissner 1998). Bluebirds' diet consists of primarily of terrestrial arthropods and they feed on the ground (Gowaty and Plissner 1998); the arthropods commonly consumed by bluebirds include: Orthoptera, Araneae, and Lepidoptera (Pinkowski 1978). Abundance of terrestrial arthropods has been shown to be correlated with higher precipitation and stable temperatures (e.g. Williams 1961). Adult eastern bluebirds molt once annually and replace all plumage each autumn following the breeding season (Gowaty and Plissner 1998). Juveniles undergo an incomplete molt in late summer and autumn, in which the primaries, secondaries, primary coverts, and alula are retained while all other feathers are molted (Pinkowski 1976).

Eastern bluebirds have ultraviolet (UV)-blue (structural-based) plumage on their entire dorsum and chestnut (melanin-based) plumage on their chests (Shawkey et al. 2003; McGraw et al. 2004). Males and females are sexually dimorphic: male have brighter and more chromatic UV-blue and darker chestnut coloration compared to females (Gowaty and Plissner 1998; Shawkey et al. 2005). The chestnut plumage coloration is a combination of phaeo- and eu-melanin (McGraw et al. 2004). Structural coloration in eastern bluebirds might be affected by inadequate nutrients ingested as feathers are built from molecules derived from food (Hill 2006). Plumage coloration is most likely driven by sexual selection for both male and female eastern bluebirds. Colorful UV-blue plumage in both males and females is positively associated with pairing success, an increased rate of provisioning to nestlings and mates, and higher reproductive success (Siefferman and Hill 2003; 2005a; b). Moreover, males with more-ornamented UV-blue plumage are mated to females that invest relatively more in offspring care (Ligon and Hill 2010 a; b). As eastern bluebirds are secondary cavity nesters, establishing a territory (i.e. nest-box) is critical for reproductive success. Males with more-ornamented UV-blue plumage are better able to compete for access to high-quality territories (Siefferman and Hill 2005b). UV-blue plumage coloration is likely condition dependent in both sexes of eastern bluebirds (Siefferman and Hill

2005a; c). Male plumage, however, appears to be more sensitive to environmental fluctuation than does that of females (Siefferman and Hill 2007; Doyle and Siefferman 2014). Both UV-blue and chestnut plumage of males also varies with age, with older males possessing lighter and less chromatic chestnut color (i.e. more female-like) and brighter and more chromatic UV-blue color (i.e. more male-like; Siefferman et al. 2005). Additionally, chestnut breast coloration may allow males to distinguish adult from juvenile conspecifics (Ligon and Hill 2009). Although research shows that males with both darker chestnut (melanin-based coloration) and brighter UV-blue (structurally-based coloration) breed earlier and feed offspring more often (Siefferman and Hill 2003), this study used an analysis that combined the two plumage types into one variable. Thus, the ability to tease about the signaling roles of the two feather types is limited. Further, far less research has focused on role of chestnut (melanin-based) than UV-blue (structurally-based) coloration in eastern bluebirds (L. Siefferman, personal communication). Yet, to date, there is little compelling evidence that chestnut coloration is as important as UV coloration in signaling mate quality or body condition (L. Siefferman, personal communication).

In this study, my objective was to document plumage coloration variation in an eastern bluebird population over a 12-year period to investigate associations between weather during molt and plumage ornamentation during the following year's breeding season. The population occurs in Auburn, Al and is non-migratory. Individuals commence breeding between March and April and can have up to three successful broods each summer; birds begin to molt between August and October of that same year (L. Siefferman, personal communication). I hypothesized that birds would be more ornamented after years with high precipitation and moderate temperatures during molt. The assumption is that arthropod abundance would be greater in years with rain and stable temperatures (reviewed in Williams 1961). I also predict that, because the

UV-blue coloration is more closely tied to sexual selection than is chestnut coloration in bluebirds, UV-blue coloration will be more closely associated with weather during molt than will melanin coloration. Further, I predict that males would be more sensitive to environmental fluctuations than females.

Methods

Researchers from Auburn University studied a nest-box breeding population of eastern bluebirds in Lee County, Alabama (32.5889° N, 85.3963° W) from 1999 to 2015 (excluding 2009, 2010, 2013, and 2014). Nest-boxes were monitored weekly for nest building, and when complete nests were found, nests were monitored daily from clutch initiation until fledging and all pairs were followed throughout the breeding season. Adults were captured during the breeding season (March-April) either via mistnet or trapped at the nest. Researchers marked each bird with a unique combination of three colored plastic bands and one U.S. Fish and Wildlife Service aluminum band.

In most years, researchers estimated the age of all newly banded birds as either Second Year (SY) (having undergone only one post-nestling molt) or After Second Year (ASY) on the shape of the 10th primary feather (Pitts 1985). Like all primary feathers, the 10th primary feather is not molted with the rest of the juvenal plumage and instead is retained from the natal plumage (Pitts 1985; Pinkowski 1976). Birds can be aged as SY or ASY by the amount of wear on the 10th primary (Pitts 1985). Males were labeled as SY if the 10th primary feather was frayed and not sharply pointed, and the color was grayish brown, or are labeled ASY if the 10th primary feather was sharply pointed and the pigment area is distinctly blue (Pitts 1985). Females were labeled as SY if the 10th primary feather was frayed and not sharply pointed, the feather is nonsymmetrical, and was pigmented brown, or as ASY if the tip of the 10th primary feather was distinctly pointed, shows some blue color, and was symmetrically shaped (Pitts 1985). Eastern bluebirds undergo an annual, complete molt post breeding season in their second year of life and every year following.

Color Analysis

At time of capture, 9 breast and 9 rump feathers were collected from each bird. Feather samples were carefully plucked from the same location on all birds. Feathers were stored in separate envelopes in a climate-controlled environment until spectrophometric analyses were conducted. Feathers were placed on low reflectivity black paper, mimicking the way feathers naturally lay on the bird. Spectral data was recorded with an Ocean Optics S2000 spectrometer (range 250-880nm: Dunedin, Florida, USA) using a micron fibre-optic probe at a 90-degree angle to the feather surface (see detailed methods in Siefferman and Hill 2003). For each individual, five measurements were recorded from each plumage region and then averaged.

Reflectance data were summarized by calculating two standard descriptors of reflectance spectra: chroma and brightness. For the UV-blue rump feathers, UV-chroma, a measure of spectral purity, was calculated as the ratio of the UV reflectance (300-400 nm) to the total reflectance (300-700 nm). For the chestnut breast feathers, red-chroma was calculated as the ratio of the total reflectance in the orange-red range (500-700 nm). Higher chroma scores for each body region is considered more-ornamented (Siefferman and Hill 2003). Brightness, or total amount of light reflected by the feather, is the summed reflectance from 300 to 700 nm. For UV-blue plumage, a *higher* brightness value is considered more-ornamented while, for chestnut plumage, a *lower* brightness score is considered more-ornamented (Siefferman and Hill 2003).

Weather Data

I collected precipitation and temperature data for the Auburn, AL breeding site from the National Oceanic and Atmospheric Administration (NOAA). I obtained weather data from August-October 1998-2014 (excluding 2008, 2009, 2012, and 2013 due to lack of feather data) to relate the weather data to feather color in the following year when feathers were collected. The mean temperature and precipitation was quantified for each month based on the daily output data provided by NOAA. I standardized precipitation and temperature values by subtracting the overall mean and dividing by the standard deviation (Schielzeth 2010).

Statistical Analyses

All results were analyzed using SPSS v. 23 (IBM 2015). Data was split by sex (male and female) and by age (SY and ASY). I constructed a series of mixed models that related one weather variable (e.g. standardized mean monthly rainfall and mean monthly temperature for August-October in the year prior to feather collection; independent variable) to each plumage variable (e.g. UV-blue brightness, UV-chroma, chestnut brightness, and chestnut red-chroma; dependent variables) in separate models. For example, a full mixed model for the month of August would include one feather variable as the dependent variable (e.g. UV-blue brightness), year of feather collection as a random variable, and either weather (e.g. mean precipitation during Sept) as a covariate. For the total set of models, I included all combinations of previous variables for each month for a total of 48 mixed models per sex. I used Akaike Information Criterion (AIC) model fitting to determine the best models, models that did not differ by >4 AIC points were considered equal.

Results

Covariation between Precipitation and Temperature

In both August and September, there was a non-significant negative trend between temperature and precipitation, however, there was no trend in October (Table 1).

Structurally-based Rump Coloration

The best models explaining variation in UV-blue brightness for SY males and ASY females included effects of mean precipitation in August (Table 2, 3), such that birds were brighter following years of high precipitation. Among ASY males and SY females, UV-blue brightness was best predicted by both mean precipitation and mean temperature in August (Tables 2, 3; Figures 1, 2a, d), such that birds were brighter following years of high precipitation and low temperatures in August.

The best model of weather to explain variation in UV chroma of both ASY and SY males included September temperature, such that males were more colorful following cooler Septembers (Table 2, Figure 3), however, there were no significant temperature models to explain female UV chroma, regardless of age (Table 2). UV chroma of SY females was significantly positively related to precipitation in September (Table 3), but precipitation was not significantly related to ASY female or male color (Tables 2, 3).

Melanin-based Breast Coloration

The only model that significantly explained variation in breast brightness included October temperature for SY females, such that females were more ornamented following warmer

Octobers (Table 3). No other weather variables significantly predicted breast brightness or red chroma, regardless of sex or age (Tables 2, 3).

Discussion

Temperature and precipitation at time of molt were associated with structurally-based UV-blue, but not melanin-based chestnut, feather coloration of eastern bluebirds measured in the following spring. Temperature and precipitation per month were strongly negatively correlated in August and weakly so in September, such that in years of high temperatures, there was lower late summer precipitation. In general, greater precipitation during August was the best predictor of structural plumage ornamentation and most closely associated with rump brightness, regardless of age or sex. Birds had more-ornamented UV-blue coloration in years with wet Augusts. Moreover, lower mean temperature in August was significantly associated with an increase in rump brightness in both females and males, but the strength of the relationships varied with age class. Further, lower temperature in September was significantly associated with increased UV chroma of males, regardless of age. However, there were few weather models that predicted variation in melanin coloration, only one model of SY females showed a significant association with breast brightness and the trends were opposed of those predicted, as young females were less ornamented after cooler Octobers. Based upon the results, structural-based plumage appears to be 1) more affected by climate variation than melanin-based coloration and 2) more influenced by precipitation than temperature. Finally, brightness of structural coloration appears more sensitive than chroma, in general. However, precipitation and temperature cannot be fully separated, particularly for August, wetter years also tended to have lower mean temperature.

During the month of August, across the 12 years of this study, mean rainfall was especially variable, with August mean rainfall ranging from 0.0869 to 0.661 cm. Moreover, across age classes and sexes, precipitation was a better predictor than temperature of structural plumage coloration. This pattern is most likely the result of greater insect / arthropod abundance resulting from higher precipitation (reviewed in Williams 1961). Because precipitation causes a fluctuation of insect abundance, structural-based plumage could be negatively affected by lack of food due to lower precipitation levels, while melanin coloration was unaffected. In field studies, structurally-based plumage has been shown to be a condition-dependent, sexually selected signal of male quality (Doucet 2002; Siefferman and Hill 2005a). The honesty behind this type of plumage may be due to the costs associated with producing very precisely arranged keratin nanostructure (Shawkey et al. 2003). Conversely, it appears that melanin-based plumage is more regulated by hormones and physiology and less by the environment (reviewed in McGraw 2008). Finally, experimental work conducted with male brown-headed cowbirds (*Molothrus ater*) showed that their structurally-based but not melanin-based plumage coloration is adversely affected by nutritional stress during molt (McGraw et al. 2002). My correlative data corroborate those experimental studies, structurally-based coloration of eastern bluebirds appears to be more influenced by the environment during molt than does the melanin-based plumage.

For eastern bluebirds, brightness of structural coloration appears to be more sensitive to environmental variation during molt than UV-chroma, the measure of spectral purity. Regardless of age or sex, birds molting during cooler, wetter late summers had brighter structurally-based plumage; while only in males, was high temperature in September associated with lower UV chroma. The mechanism by which cool, wet late summer weather (and putatively greater access to arthropod prey) increases plumage brightness (but not UV chroma) remains unclear. Non-iridescent structurally-based colors are produced by coherent scattering of light particles from the precise arrangement of elements within the microstructure of a feather (Prum 2006). UV-blue structural barb feathers of eastern bluebirds have a keratin cortex and a spongy medullary layer with large central vacuoles surrounded by small granules of melanin (Shawkey et al. 2003). That structural colors are negatively affected by nutritional stress (Siefferman and Hill 2005a; McGraw et al. 2002) suggests that saturation of colors may depend on regularity of elements within the microstructure (Shawkey et al. 2003), but how nutritional condition changes structurally based coloration remains poorly understood (Prum 2006). The differential effects of weather on brightness and UV chroma in my study suggest that production of these two color descriptors have different mechanisms or pathways. It may be that food availability has a greater impact on keratin cortex thickness (associated with brightness) and has less effect on the precise arrangement of the medularly layer UV chroma (associated with UV chroma; Shawkey et al. 2003). Future work should lead to a better understanding of how food stress during feather development influences variation in non-iridescent plumage coloration.

Temperature, while negatively associated with precipitation in August and September, did not predict plumage coloration to the extent that precipitation did (across both sexes and age classes). In fact, temperature was only a good predictor of coloration of male UV chroma coloration. Indeed, UV-blue coloration has been shown to be more sensitive to environmental conditions among male than female bluebirds (Siefferman et al. 2005; Siefferman and Hill 2005c; Doyle and Siefferman 2014). Greater condition dependence of male color is logical as males have brighter and more chromatic UV-blue structural color than females (Shawkey et al. 2005) and thus creating more elaborate color maybe more costly to produce or involve more structural complexity. Because of this, male plumage may be more sensitive to changes in weather compared to females. Further, this may translate into greater impacts on male than female fitness, as color influences competition between males and thus likely influences access to females (Siefferman and Hill 2005c).

The plumage coloration of juveniles and adults appeared to be similarly influenced by weather during molt. Although birds in different age classes molt tail feathers at different times (Pitts 1985), the extent to which the ages classes differ in timing of body feathers is less clear and my study measured body feather coloration. Juveniles appear to molt from natal to SY plumage sometime in late summer, but often earlier than adult molt (L. Siefferman, personal communication). Because of this difference in timing of molt of rump and breast plumage, I thought it possible that weather would have age-class dependent effects on plumage coloration. However, the only weather variable that produced differences between the age classes was temperature in August, wherein only the rump brightness of SY female and ASY male were significantly negatively associated with temperature. For rump brightness, all age classes and sexes were positively associated with precipitation. It may be that more extreme differences would have been found if I had measured wing or tail feathers (retained from the natal plumage) as was done with the redstarts that Reudink et al. (2015) studied.

It is interesting that weather in August and September should be a better predictor of color than weather in October, because ASY bluebirds from this population, when brought into captivity, molted most contour (body) feathers in October (Siefferman and Hill 2005a). It may be that hot, dry weather 6-8 weeks earlier has the greatest influences on arthropods in October. Timing of molt for juveniles (that are SY in the following spring) is less clear, field observations suggest they may start molting contour feathers in summer and that hatching time should influence timing of molt (L. Siefferman, personal communication). Thus, hatching time could influence how in SY females cooler Octobers were found to increase brightness in melanin coloration (reduced ornamentation), as later hatching birds could delay molt. This one relationship with melanin coloration was opposite of my hypothesis, as I only expected UV-blue structural coloration to be effected. In other species, migratory birds are expected to face tradeoffs between investing in pre-migration fattening, migration and molt (Norris et al. 2004) and age can influence migration onset (Newton 2010). Because this population of bluebirds does not migrate, while other populations do, it would be interesting to compare how weather preceding and during molt influence plumage coloration in migratory and non-migratory populations of bluebirds. Indeed, current molt chronology studies have been conducted in many waterfowl species (e.g. Miller 1986). Among passerines, Cassin's Finches (*Carpodacus cassinii*) of different age classes vary in timing of molt in relation to fall migration departure (Samson 1976).

My results suggest that weather during molt can influence the development of sexually selected traits for following breeding season. Indeed, many studies have shown how plumage coloration, specifically UV-blue plumage, is associated with reproductive output (Siefferman and Hill 2003; Siefferman and Hill 2005a). My findings indicate that, in the future, as climate trends towards hotter temperatures (IPCC Synthesis Report 2014), bluebirds will display duller structurally based UV-blue coloration. In both sexes, brightness will likely decrease and, among males, UV chroma will decrease. However, precipitation was positively associated with UV-blue brightness but models that predict how precipitation will change in the future are more complex and varies considerably with region (IPCC Synthesis Report 2014). Thus, it may be difficult to project how plumage coloration with change in the future.

In conclusion, after following a population of eastern bluebirds for a 12-year period, my research demonstrates associations between UV-blue structural plumage coloration and weather during molt. Birds tended to display more ornamented UV-blue coloration following late summer-autumns of high precipitation and low temperature. These associations are likely due to higher arthropod prey abundance during wetter and cooler years. This is corroborated by studies demonstrating that structurally-based, but not melanin-based plumage, has been shown to be condition dependent. This work illustrates the importance of long-term biological data and sheds light on how climate change may influence sexually selected traits.

	August Precipitation	September Precipitation	October Precipitation
August Temperature	-0.511 (0.074)		
September Temperature		-0.261 (0.390)	
October Temperature			0.049 (0.874)

Table 1 Pearson's bivariate correlations for monthly precipitation and temperature all weather data are standardized (n = 13 years).

Model Category (Males)	AIC	ΔAIC	Df	F-value	p-value
ASY Rump Brightness					
Stand Mean Temp September	1410.812	0	1,10.510	0	0.987
Stand Mean Temp October	1410.567	0.245	1,12.036	0.061	0.809
Stand Mean Precipitation October	1410.389	0.423	1,9.318	0.312	0.59
Stand Mean Precipitation September	1409.952	0.86	1,11.053	0.608	0.452
Stand Mean Precipitation August	**1406.778**	**4.034**	**1,8.831**	**5.54**	**0.044**
Stand Mean Temp August	**1404.486**	**6.326**	**1,11.733**	**9.168**	**0.011**
SY Rump Brightness					
Stand Mean Precipitation September	771.144	0	1,3.880	0.231	0.657
Stand Mean Temp September	770.918	0.226	1,3.969	0.071	0.804
Stand Mean Precipitation October	770.419	0.725	1,3.912	0.605	0.481
Stand Mean Temp October	769.304	1.84	1,3.925	2.236	0.21
Stand Mean Temp August	767.867	3.277	1,4.091	4.248	0.107
Stand Mean Precipitation August	**765.421**	**5.723**	**1,3.902**	**11.783**	**0.028**
ASY Rump UV Chroma					
Stand Mean Temp August	-1123.629	0	1,10.185	0.907	0.363
Stand Mean Precipitation September	-1124.658	1.029	1,7.125	1.764	0.225
Stand Mean Precipitation August	-1124.794	1.165	1,7.80	2.244	0.173
Stand Mean Temp October	-1124.858	1.229	1,12.373	1.776	0.207
Stand Mean Precipitation October	-1126.71	3.081	1,3.450	6.956	0.067
Stand Mean Temp September	**-1131.29**	**7.661**	**1,14.661**	**11.11**	**0.005**
SY Rump UV Chroma					
Stand Mean Precipitation September	-678.458	0	1,3.758	0.006	0.941
Stand Mean Precipitation October	-678.972	0.514	1,3.832	0.134	0.734
Stand Mean Temp August	-679.069	0.611	1,3.885	0.045	0.842
Stand Mean Precipitation August	-679.875	1.417	1,4.013	1.01	0.372
Stand Mean Temp October	-681.292	2.834	1,3.679	2.585	0.137
Stand Mean Temp September	**-687.037**	**8.579**	**1,3.839**	**24.27**	**0.009**
ASY Breast Brightness					
Stand Mean Temp September	960.014	0	1,11.920	0.024	0.881
Stand Mean Precipitation October	959.93	0.084	1,10.082	0.025	0.876
Stand Mean Temp October	959.789	0.225	1,13.511	0.018	0.896
Stand Mean Temp August	959.498	0.516	1,11.101	0.688	0.424
Stand Mean Precipitation September	959.431	0.583	1,11.379	0.35	0.566
Stand Mean Precipitation August	958.434	1.58	1,9.476	1.925	0.197
SY Breast Brightness					
Stand Mean Precipitation October	546.508	0	1,3.939	0	0.99
Stand Mean Temp September	546.345	0.163	1,4.005	0.167	0.704
Stand Mean Temp August	545.567	0.941	1,4.158	0.821	0.414
Stand Mean Precipitation August	544.978	1.53	1,4.189	1.675	0.262
Stand Mean Precipitation September	544.878	1.63	1,3.741	2.647	0.184
Stand Mean Temp October	543.14	3.368	1,3.637	5.72	0.081
ASY Breast Red Chroma					
Stand Mean Precipitation September	-914.653	0	1,10.385	0.213	0.654
Stand Mean Temp September	-914.653	0	1,10.385	0.213	0.654
Stand Mean Precipitation August	-915.211	0.558	1,10.194	0.986	0.344
Stand Mean Temp August	-915.211	0.558	1,10.194	0.986	0.344
Stand Mean Precipitation October	-916.587	1.934	1,10.070	2.472	0.147
Stand Mean Temp October	-916.587	1.934	1,10.070	2.472	0.147
SY Breast Red Chroma					
Stand Mean Temp October	-562.665	0	1,3.988	0.177	0.696
Stand Mean Temp August	-563.048	0.383	1,3.989	0.284	0.622
Stand Mean Precipitation September	-563.284	0.619	1,3.952	1.226	0.331
Stand Mean Precipitation August	-563.473	0.808	1,4.034	0.839	0.411
Stand Mean Precipitation October	-563.599	0.934	1,3.979	1.135	0.347
Stand Mean Temp September	-567.12	4.455	1,4.046	1.854	0.244

Table 2 Summary of a models explaining variation in rump brightness, rump UV chroma, breast brightness, and breast red chroma for ASY and SY male Eastern Bluebirds.

Bolded numbers correspond to models with significant relationships. Year was included as a random effect in all models.

Model Category (Females)	AIC	ΔAIC	Df	F-value	p-value
ASY Rump Brightness					
Stand Mean Temp September	1792.322	0	1,12.195	0.004	0.952
Stand Mean Precipitation September	1792.07	0.252	1,11.502	0.302	0.593
Stand Mean Temp October	1792.061	0.261	1,13.323	0.229	0.64
Stand Mean Precipitation October	1791.947	0.375	1,10.312	0.482	0.503
Stand Mean Temp August	1789.977	2.345	1,10.381	2.878	0.12
Stand Mean Precipitation August	**1786.276**	**6.046**	**1,8.049**	**10.378**	**0.012**
SY Rump Brightness					
Stand Mean Precipitation October	649.171	0	1,5.214	0.172	0.695
Stand Mean Precipitation September	649.138	0.033	1,4.770	0.636	0.463
Stand Mean Temp September	648.555	0.616	1,5.064	0.856	0.397
Stand Mean Temp October	646.284	2.887	1,4.261	4.548	0.096
Stand Mean Precipitation August	**645.007**	**4.164**	**1,4.237**	**7.235**	**0.051**
Stand Mean Temp August	**644.884**	**4.287**	**1,4.854**	**6.518**	**0.053**
ASY Rump UV Chroma					
Stand Mean Temp August	-1442.964	0	1,12.359	0.008	0.93
Stand Mean Temp September	-1443.101	0.137	1,13.311	0.07	0.796
Stand Mean Temp October	-1443.138	0.174	1,14.535	0.043	0.839
Stand Mean Precipitation September	-1443.163	0.199	1,11.448	0.22	0.648
Stand Mean Precipitation August	-1443.269	0.305	1,11.359	0.367	0.557
Stand Mean Precipitation October	-1443.325	0.361	1,10.925	0.448	0.517
SY Rump UV Chroma					
Stand Mean Temp September	-512.23	0	1,5.541	0.022	0.888
Stand Mean Precipitation August	-512.445	0.215	1,5.439	0.033	0.861
Stand Mean Temp October	-512.563	0.333	1,4.996	0.428	0.542
Stand Mean Temp August	-512.912	0.682	1,4.998	0.521	0.503
Stand Mean Precipitation October	-514.434	2.204	1,5.021	3.248	0.131
Stand Mean Precipitation September	**-517.682**	**5.452**	**1,2.754**	**21.947**	**0.022**
ASY Breast Brightness					
Stand Mean Precipitation October	1668.044	0	1,10.612	0.026	0.876
Stand Mean Temp September	1667.95	0.094	1,12.452	0.023	0.881
Stand Mean Temp October	1667.924	0.12	1,13.313	0.008	0.928
Stand Mean Precipitation September	1667.33	0.714	1,11.562	0.711	0.416
Stand Mean Precipitation August	1665.474	2.57	1,10.031	3.115	0.108
Stand Mean Temp August	1665.093	2.951	1,11.025	3.647	0.083
SY Breast Brightness					
Stand Mean Precipitation October	600.565	0	1,5.234	0.026	0.877
Stand Mean Temp September	600.073	0.492	1,5.134	0.552	0.49
Stand Mean Precipitation September	599.553	1.012	1,4.987	1.668	0.253
Stand Mean Temp August	597.381	3.184	1,5.077	4	0.101
Stand Mean Precipitation August	597.236	3.329	1,4.916	4.532	0.087
Stand Mean Temp October	**596.163**	**4.402**	**1,4.551**	**7.465**	**0.046**
ASY Breast Red Chroma					
Stand Mean Precipitation August	-1227.955	0	1,11.015	0.005	0.944
Stand Mean Temp September	-1227.972	0.017	1,11.490	0.001	0.975
Stand Mean Temp October	-1228.061	0.106	1,11.667	0.08	0.782
Stand Mean Temp August	-1228.116	0.161	1,11.321	0.157	0.699
Stand Mean Precipitation October	-1228.617	0.662	1,11.023	0.69	0.424
Stand Mean Precipitation September	-1228.673	0.718	1,11.213	0.739	0.408
SY Breast Red Chroma					
Stand Mean Temp September	-438.053	0	1,4.903	0.001	0.974
Stand Mean Precipitation October	-438.066	0.013	1,4.916	0.015	0.908
Stand Mean Temp October	-438.791	0.738	1,4.680	0.814	0.411
Stand Mean Precipitation September	-438.804	0.751	1,4.539	1.31	0.309
Stand Mean Precipitation August	-438.934	0.881	1,4.767	0.726	0.435
Stand Mean Temp August	-441.384	3.331	1,4.697	4.247	0.098

Table 3 Summary of all models explaining variation in rump brightness, rump UV chroma, breast brightness, and breast red chroma for ASY and SY female Eastern Bluebirds.

Bolded numbers correspond to models with significant relationships. Year was included as a random effect in all models.

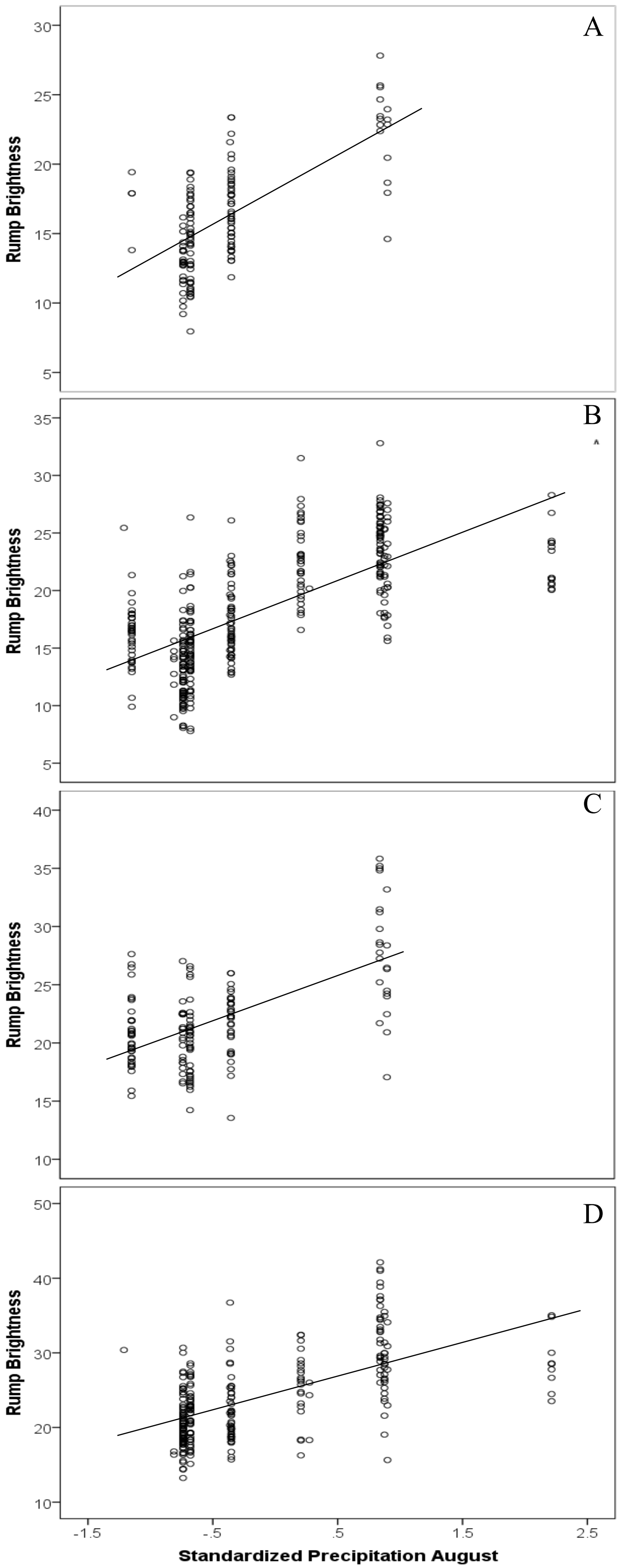

Figure 1 Relationships between mean rump brightness and August precipitation for a) female SY, b) female ASY, c) male SY and d) male ASY eastern bluebirds. Lines indicate significant relationships.

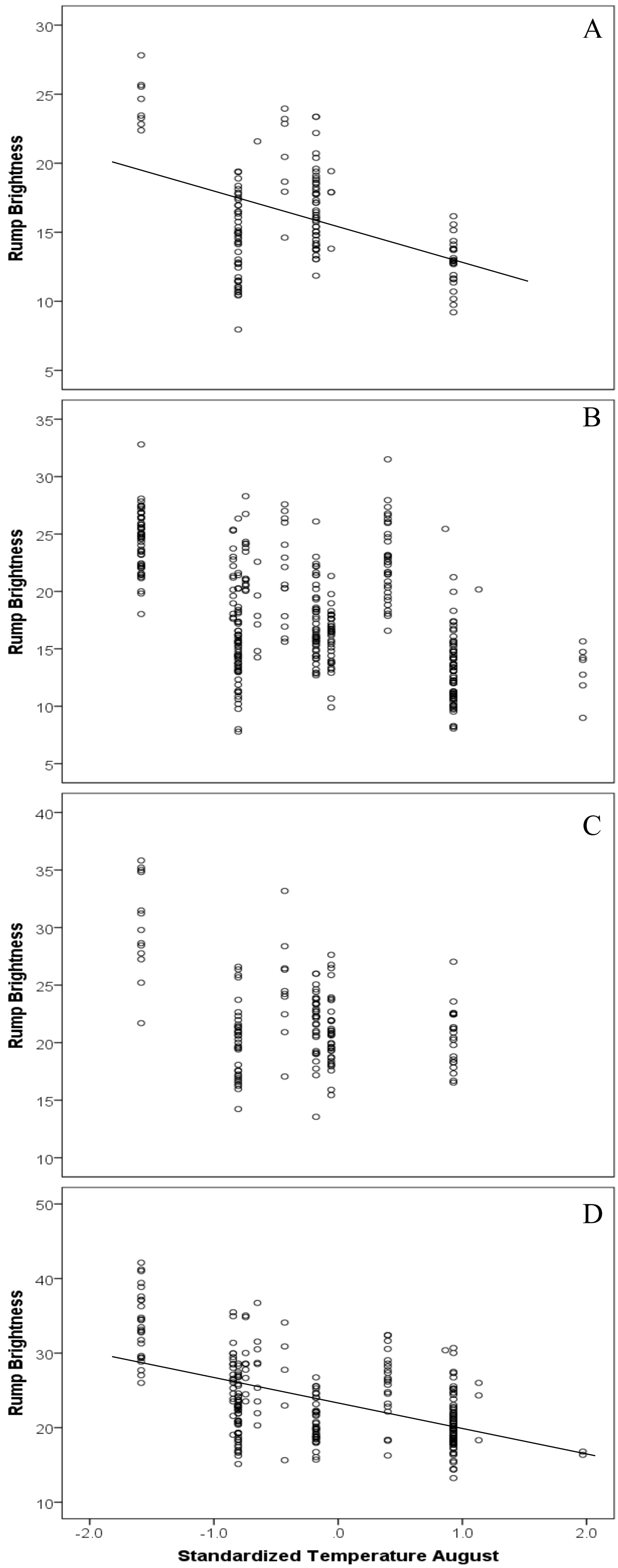

Figure 2 Relationship between mean rump brightness and August temperature for a) female SY, b) female ASY, c) male SY and d) male ASY eastern bluebirds. Lines indicate significant relationships.

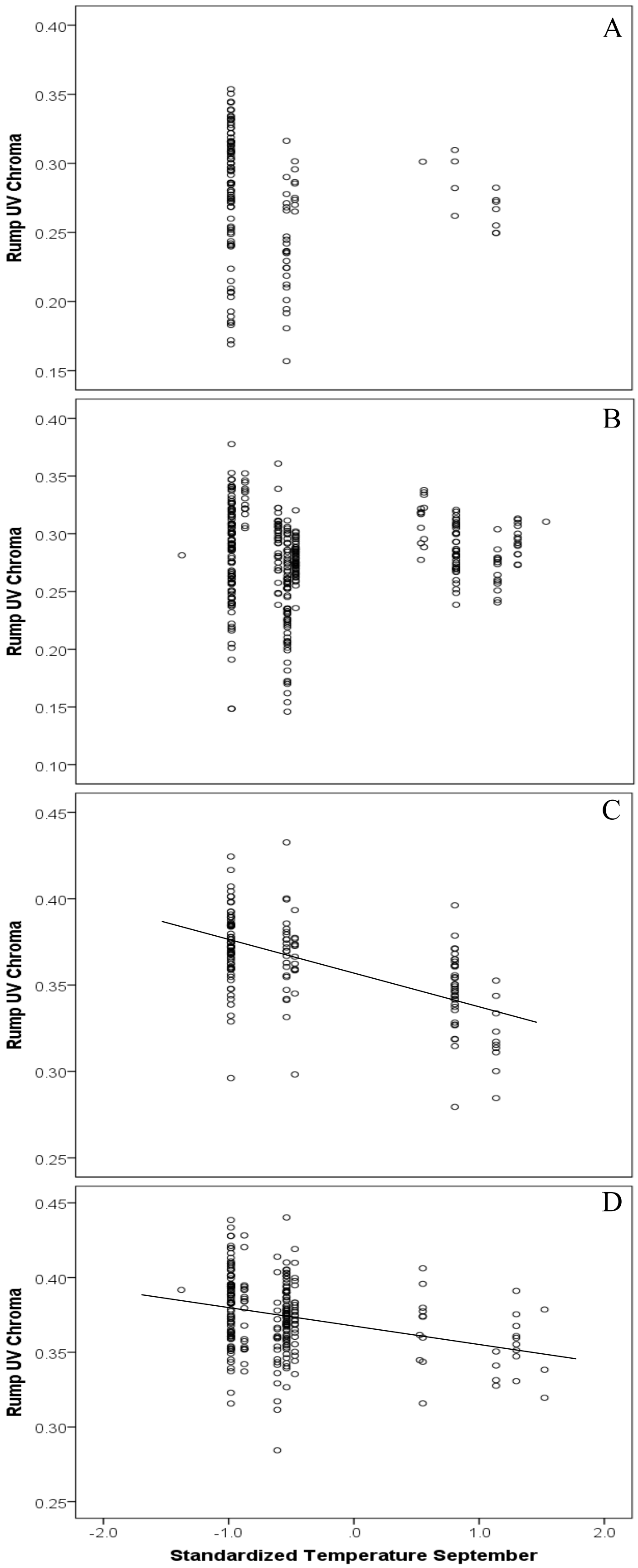

Figure 3 Relationship between mean rump UV chroma and September temperature for a) female SY, b) female ASY, c) male SY and d) male ASY eastern bluebirds. Lines indicate significant relationships.

Literature Cited

Andersson, M. B. 1994. Sexual selection. *Princeton University Press*.

Cronin, H. 1993. The ant and the peacock: Altruism and sexual selection from Darwin to today. *Cambridge University Press.*

Darwin, C. 1871. The descent of man.

Doucet, S. M. 2002. Structural plumage coloration, male body size, and condition in the blue-black grassquit. *The Condor*, **104**, 30-38.

Doyle, A. and Siefferman, L. 2014. Supplemental food increases ornamentation of male nestling Eastern Bluebirds. *Journal of Field Ornithology*, **85**, 31-39.

Gowaty, P. A. and Plissner J. H. 1998. Eastern Bluebird (*Sialia sialis*) In: The Birds of North America, No. 381 (A. Poole and F. Gill, eds.). *The Birds of North America*, Inc., Philadelphia, PA.

Hill, G. E. 2006. Environmental regulation of ornamental coloration. *Bird Coloration*, *Volume 1 Mechanisms and Measurements* (G. E. Hill and K. J. McGraw, eds.). Harvard University Press, Harvard, MA.

Hill, G. E., and Montgomerie, R. 1994. Plumage colour signals nutritional condition in the house finch. *Proceedings of the Royal Society of London B: Biological Sciences*, **258**, 47-52.

IPCC. 2014. IPCC Synthesis Report.

Keyser, A. J., and Hill, G. E. 1999. Condition–dependent variation in the blue–ultraviolet coloration of a structurally based plumage ornament. *Proceedings of the Royal Society of London B: Biological Sciences*, **266**, 771-777.

Keyser, A. J., and Hill, G. E. 2000. Structurally based plumage coloration is an honest signal of quality in male blue grosbeaks. *Behavioral Ecology*, **11**, 202-209.

Ligon, R. A. and Hill, G. E. 2009. Do adult eastern bluebird, *Sialia sialis*, males recognize juvenile-specific traits? *Animal Behaviour*, **77**, 1267-1272.

Ligon, R. A. and Hill, G. E. 2010a. Feeding decisions of eastern bluebirds are situationally influenced by fledgling plumage color. *Behavioral Ecology*, **21**, 456-464.

Ligon, R. A. and Hill, G. E. 2010b. Sex-biased parental investment is correlated with mate ornamentation in eastern bluebirds. *Animal Behaviour*, **79**, 727-734.

McGraw, K. J. 2006a. Mechanics of carotenoid-based coloration. *Bird Coloration*, *Volume 1 Mechanisms and Measurements* (G. E. Hill and K. J. McGraw, eds.). Harvard University Press, Harvard, MA.

McGraw, K. J. 2006b. Mechanics of melanin-based coloration. *Bird Coloration*, *Volume 1 Mechanisms and Measurements* (G. E. Hill and K. J. McGraw, eds.). Harvard University Press, Harvard, MA.

McGraw, K. J. 2008. An update on the honesty of melanin-based color signals in birds. *Pigment Cell & Melanoma Research*, **21.2**, 133-138.

McGraw, K. J., Mackillop, E. A., Dale, J. and Hauber, M. E. 2002. Different colors reveal different information: how nutritional stress affects the expression of melanin-and structurally based ornamental plumage. *Journal of Experimental Biology*, **205**, 3747-3755.

McGraw, K. J., Wakamatsu, K., Ito, S., Nolan, P. M., Jouventin, P., Dobson, F. S., Austic, R. E., Safran, R. J., Siefferman, L. M., Hill, G. E., and Parker, R. S. 2004. You can't judge a pigment by its color: carotenoid and melanin content of yellow and brown feathers in swallows, bluebirds, penguins, and domestic chickens. *The Condor*, **106**, 390-395.

Miller, M. R. 1986. Molt chronology of northern pintails in California. *The Journal of wildlife Management*, 57-64.

Newton, I. 2010. Sex and age differences in migration. *The Migration Ecology of Birds*. Academic Press.

Norris, D. R., Marra, P. P., Montgomerie, R., Kyser, T. K., and Ratcliffe, L. M. 2004. Reproductive effort, molting latitude, and feather color in a migratory songbird. *Science,* **306.5705**, 2249-2250.

Payne, R. B. 1972. Mechanisms and control of molt. *Avian Biology*, **2**, 103-155.

Pinkowski, B. C. 1976. Photoperiodic effects on the postjuvenal molt of the Eastern Bluebird. *The Ohio Journal of Science*, **76**, 268-273.

Pinkowski, B. C. 1977. Breeding adaptations in the Eastern Bluebird. *The Condor*, **79.3**, 289-302.

Pinkowski, B. C. 1978. Feeding of nestling and fledgling Eastern Bluebirds. *Wilson Bulletin*, **90**, 84-98.

Pitts, T. D. 1985. Identification of second-year and after-second-year eastern bluebirds. *Journal of Field Ornithology*, **56**, 422-424.

Prum, R. O. 2006. Anatomy, physics, and evolution of structural colors. *Bird Coloration, Volume 1 Mechanisms and Measurements* (G. E. Hill and K. J. McGraw, eds.). Harvard University Press, Harvard, MA.

Reudink, M. W., McKellar, A. E., Marini, K. L. D., McArthur, S. L., Marra, P. P., and Ratcliffe, L. M. 2015. Inter-annual variation in American redstart (*Setophaga ruticilla*) plumage colour is associated with rainfall and temperature during moult: an 11-year study. *Oecologia,* **178**, 161-173.

Samson, F. B. 1976. Pterylosis and molt in Cassin's Finch. *The Condor*, **78**, 505-511.

Schielzeth, H. 2010. Simple means to improve the interpretability of regression coefficients. *Methods Ecology Evolution*, **1**, 103-113.

Shawkey, M. D., Estes, A. M., Siefferman, L. M., and Hill, G.E. 2003. Nanostructure predicts intraspecific variation in ultraviolet–blue plumage colour. *Proceedings of the Royal Society of London B: Biological Sciences*, **270**, 1455-1460.

Shawkey, M. D., Estes, A. M., Siefferman, L., and Hill, G. E. 2005. The anatomical basis of sexual dichromatism in non-iridescent ultraviolet-blue structural coloration of feathers. *Biological Journal of the Linnean Society*, **84(2),** 259-271.

Siefferman L., and Hill, G. E. 2003. Structural and melanin plumage coloration indicate parental effort and reproductive success in male eastern bluebirds. *Behavioral Ecology*, **14**, 855–861.

Siefferman L., and Hill, G. E. 2005a. Evidence for sexual selection on structural plumage coloration in female eastern bluebirds (*Sialia sialis*). *Evolution*, **59**, 1819-1828.

Siefferman L., and Hill, G. E. 2005b. Blue structural coloration of male eastern bluebirds *Sialia sialis* predicts incubation provisioning to females. *Journal of Avian Biology,* **36**, 488-493.

Siefferman L., and Hill, G. E. 2005c. Male eastern bluebirds trade future ornamentation for current reproductive investment. *Biology Letters*, **1**, 208-211.

Siefferman L., and Hill, G. E. 2005d. UV-blue structural coloration and competition for nestboxes in male eastern bluebirds. *Animal Behaviour*, **69(1)**, 67-72.

Siefferman L., and Hill, G. E. 2007. The effect of rearing environment on blue structural coloration of eastern bluebirds (*Sialia sialis*). *Behavioral Ecology and Sociobiology*, **61**, 1839-1846.

Siefferman L, Hill G. E., and Dobson F. S. 2005. Ornamental plumage coloration and condition are dependent on age in eastern bluebirds *Sialia sialis*. *Journal of Avian Biology*, **36**, 428–435.

Williams, C. B. 1961. Studies in the effect of weather conditions on the activity and abundance of insect populations. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, **244**, 331-378.

Chapter 2: Museum Data Reveal Variation in Geography and Weather During Molt Influencing Plumage Coloration of Eastern Bluebirds (*Sialis sialis*)

Abstract

Museum specimens offer a unique opportunity to analyze phenotypic changes through time and over broad geographic ranges. Eastern bluebirds (*Sialis sialis*) are an insectivorous passerine that display ultraviolet (UV)-blue structural-based and chestnut melanin-based plumage. The UV-blue body coloration of eastern bluebirds is likely sexually selected as it has been shown to be sensitive to nutritional stress, indicates mate quality, influences male-male competitive interactions, and is correlated with reproductive success. The melanin coloration of the breasts of eastern bluebirds has been shown to be less sensitive to environmental conditions during molt and is likely an indicator of age but not necessarily mate quality. In my study, I examine whether geographic location, weather during the molt period (August to October), and year of specimen collection influenced male plumage coloration of eastern bluebirds using Smithsonian museum specimens dating back to 1895. Eastern bluebirds displayed brighter UV-blue plumage (more ornamented) and chestnut-plumage (less ornamented) when collected in more easterly locations and in locations with higher average temperature. Moreover, birds collected more recently display more-ornamented UV-blue coloration, suggesting that either color fades with time or birds are more ornamented in recent decades. The data suggest that structural-based plumage appears to be more affected by year of collection compared to melanin-based plumage, while both plumages appear to be affected by geographical location and climate. Overall, this study corroborates research suggesting that museum specimens could be an asset to large scale geographic studies and research on climate change.

Introduction

Plumage coloration is often considered a classic sexually selected trait and many species of birds have elaborate and dimorphic plumage (reviewed in Hill and McGraw 2006). Sexually selected traits are often physiologically costly and expected to be environmentally plastic (Andersson 1994). Because feather ornamentation is often energetically expensive (reviewed in Dale 2006), it is expected that the high-quality individuals will produce and maintain elaborate plumage and therefore can honestly signal male quality (Higham 2013; Biernaskie et al. 2014).

If plumage coloration is an honest signal of male quality, within-species plumage coloration may be expected to vary geographically and be associated with variation in climate- as climate should influence food availability. Geographical differences in phenotypic traits are expected across species' ranges of any given species, as habitats can change rapidly with latitude, longitude, and landscape (Allen 1877; Bergmann 1847) and because populations that are more distant geographically should share fewer genes (Huggett 2004). Climate is an important environmental variable that varies with latitude and longitude. Mean temperature is relatively stable between 25°S and 25°N, and decreases with increased northern latitude due to reduced solar radiation (reviewed in Stevens 1989; Gaston and Chown 1999). Precipitation also varies with latitude and tends to decrease when increasing towards northern latitudes (Gaston and Chown 1999).

Little research has focused on how sexually selected ornaments, especially plumage coloration, vary with geographic gradients (reviewed in Friedman and Remes 2016). Yet, theoretical models and empirical data suggest that there is a great diversity of variation in the strength of sexual selection within conspecifics or closely related species (reviewed in Wiens 2001). For example, barn swallows (*Hirundo rustica*) exhibit a great deal of geographic variation in body size, length of outer tails and ventral color (Dor et al. 2012; Hasegawa et al. 2013; Scordato and Safran 2014), and these two plumage traits are the subject of sexual selection research in several populations. Indeed, recent evidence suggests that localized sexual selection is the principal driver of phenotypic divergence between closely related swallow subspecies that vary geographically (Wilkins et al. 2016). In addition, melanin coloration in barn swallows appears to be, in-part, based on timing of molt; higher-quality individuals that delayed molting until after migrating towards their wintering grounds produced more-ornamented feathers (Norris et al. 2009). Moreover, American Redstarts (*Setophaga ruticilla*) with higher reproductive output tend to delay molting until the wintering grounds and produce less-ornamented orange color than birds that molted on the breeding grounds, likely due to lowered carotenoid availability (Norris et al. 2004). Together, these results suggest that geographic variation between populations, as well as resources available at the location of molt, play an important role in shaping color variation.

Museum specimens are particularly useful for investigations of how ornamental traits within species vary with geographic and climatic trends because they allow researchers to measure plumage coloration of many individuals over a large geographic range. However, museum skins vary tremendously in the date that the animals were collected and thus there may be changes in plumage over time that could be caused by multiple factors. First, it is possible that study skins have change in coloration while in the collection due to fading, degradation of feathers, or accumulation of dust or dirt (e.g. Doucet and Hill 2009). Second, it may be that species or populations of birds have change in ornamentation over time due to changes in strength of sexual selection (e.g. Galeotti et al. 2009). Finally, researchers need to address whether plumage variation of preserved skins mimics that of live birds (e.g. Doucet and Hill 2009).

Recent research has evaluated how well museum specimens represent plumage color variation measured from wild birds and found that they accurately represent plumage variation in wild birds (Doucet and Hill 2009). Using long-tailed manakins (*Chiroxiphia linearis*), which have structural-, melanin-, and carotenoid-based plumage, Doucet and Hill (2009) found that reflectance spectra collected from museum specimens accurately represents variation in wild bird coloration. Carotenoid-based plumage brightness appears to fade considerably with time as specimens collected in the most recent years have greater spectral reflectance (Doucet and Hill 2009). The brightness of the melanin- and structural-based plumage did not significantly change with specimen age; however, birds collected earlier had lower red chroma (melanin-based plumage) and lower UV chroma (structurally-based) reflected proportionately less at UV wavelengths (Doucet and Hill 2009). These authors suggested that some of the color degradation of the skins could be attributed to accumulation of chemicals, bacteria, or physical damage; however, differences aside, the authors were able to describe important seasonal and geographic variation trends in the long-tailed manakin coloration.

Although it is possible that plumage color of museum skins can degrade via fading, feather wear, or accumulation of debris, it is also possible that selective forces have changed plumage coloration through time. Galeotti et al. (2009) used 281 museum specimens of the scops owl (*Otus scops*) collected over 137 years to show positive trends where birds collected in past decades had pale-red coloration but more recent specimens tend to have dark-reddish/brown plumage. The trend in scops owl coloration was described as increasing density of both phaeomelanin and eumelanin pigments within feathers over time (Galeotti et al. 2009).

Moreover, the authors argue that this change in melanin coloration was not associated with museum fading, as researchers concluded the shift was most likely due to selective pressures (Galeotti et al. 2009). Indeed, museum specimen data suggest that melanin-based coloration is more stable than structurally- or carotenoid-based plumage (Doucet and Hill 2009; Galeotti et al. 2009).

In this study, my objective was to investigate associations between plumage coloration of male eastern bluebirds (*Sialis sialis*) and weather and geographic location during molt using museum specimens from a 100-year time span. I hypothesize that plumage coloration will be influenced by geography (latitude and longitude). I predict that birds breeding in more southern latitudes will be more colorful because greater temperature and precipitation may increase prey abundance and food quality during molt. Moreover, birds in the more southern latitudes do not migrate (Figure 1; National Gap Analysis Program) and thus should not be constrained by overlap between migration and molt (Norris et al. 2004; Reudink et al. 2008). I also hypothesize that plumage coloration may fade after collection and thus predict that specimens collected earlier may show reduced brightness and chroma values for both structurally and melanin-based plumage coloration.

Methods

Study Species

Eastern bluebirds are socially monogamous passerines that are partial migrants. Bluebirds occur year-round in the Southeast, south Midwest United States, and along the Atlantic and Gulf coasts (Figure 1; USGS National Gap Analysis Program). Eastern bluebirds are secondary cavity nesters that readily breed in nest-boxes when provided. Female eastern bluebirds build the nest, lay 3-6 eggs and incubate eggs for 12-14 days per nest, and brood the nestlings; however, both male and females feed the young (Pinkowski 1977). Bluebirds' diet consists of terrestrial arthropods in the orders Orthoptera, Araneae, and Lepidoptera (Pinkowski 1978).

Eastern bluebirds molt annually in the late summer and early fall following the spring-summer breeding season. Males and females are sexually dimorphic: male eastern bluebirds display brighter UV-blue structurally-based plumage on their heads, backs, rumps, wings, and tails and darker chestnut melanin-based plumage on their breasts compared to females (Gowaty and Plissner 1998). Plumage coloration is most likely driven by sexual selection for both male and female eastern bluebirds (Siefferman and Hill 2003; 2005a; b). The UV-blue body coloration of eastern bluebirds is likely sexually selected as it has been shown to be sensitive to nutritional stress (Siefferman and Hill 2005a; 2007), indicates mate quality (Siefferman and Hill 2003; 2005a) influences male-male competitive interactions (Siefferman and Hill 2005b), and is correlated with reproductive success (Siefferman and Hill 2003; 2005a). Structural coloration in eastern bluebirds may be affected by inadequate nutrients because feathers are built from molecules derived from food (Hill 2006; Shawkey et al. 2003). The melanin coloration of the breasts of eastern bluebirds has been shown to be less sensitive to environmental conditions during molt (Author, Chapter 1) and is likely an indicator of age (Siefferman et al. 2005) but not necessarily mate quality.

Color Analysis

One researcher, Dr. Lynn Siefferman, visited the Smithsonian Museum (DC, USA) and measured specimens ranging from 1822 to 1996. Museum specimen data included: sex, location of collection (county and state), date of collection (Figure 1). Latitude and longitude data based on locality were determined post-collection.

Plumage coloration was measured via an Ocean Optics S2000 spectrometer (range 250-880nm: Dunedin, Florida, USA) using a micron fibre-optic probe at a 90-degree angle to the feather surface (see detailed methods in Siefferman and Hill 2003). Spectral data measurements were taken from each museum specimen in the same location on the bird. For each individual, she recorded plumage coloration of the melanin pigment-based breast feathers and the UV-blue structural coloration from the rump feathers five times and averaged the measurement per body region.

Reflectance data were summarized by calculating two standard descriptors of reflectance spectra: chroma and brightness. For the UV-blue rump feathers, UV-chroma, a measure of spectral purity, was calculated as the ratio of the UV reflectance (300-400 nm) to the total reflectance (300-700 nm). For the chestnut breast feathers, red-chroma was calculated as the ratio of the total reflectance in the orange-red range (500-700 nm). Higher chroma scores for each body region is considered more-ornamented (Siefferman and Hill 2003). Brightness, or total amount of light reflected by the feather, is the summed reflectance from 300 to 700 nm. For UV-blue plumage, a *higher* brightness value is considered more-ornamented while, for chestnut plumage, a *lower* brightness score is considered more-ornamented (Siefferman and Hill 2003).

ESRI ArcMap GIS

In ESRI ArcMap GIS Desktop, distribution data was obtained from the USGS National Gap Analysis Program for the eastern bluebird. A vector polygon Shape file, datum NAD83, of the United States (States.shp) was used as a base map (obtained from Appalachian State Department of Geography and Planning). I added in the coordinates for each museum specimen in the study to display where they were collected. I used these data to determine whether each specimen was a from a migratory or non-migratory population (Figure 1).

Weather Data

I collected precipitation and temperature data per climate division (Figure 2) for the continental U.S. from the National Oceanic and Atmospheric Administration (NOAA) Earth System Research Laboratory (ESRL): Physical Science Division. I obtained weather data from August-October 1895-2000, the earliest recorded weather data. The mean temperature and precipitation for each month were included in the output from NOAA ESRL. Decadal averages (1900-1909, 1910-1919, 1920-1929, 1930-1939, 1940-1949, 1950-1959, 1960-1969, 1980-1989, and 1990-1999) were obtained by taking the average value for the decade for each month (excluding 1895-1899 as no data exists prior to 1895). Decadal averages were excluded from 1970-1979 due to lack of color data for the decade.

Statistical Analyses

All results were analyzed using SPSS v. 23 (IBM 2015). I used Pearson's correlations to compare how plumage traits varied with year of collection. I used Pearson's correlations to compare how plumage traits varied with decadal temperature and precipitation (regardless of season). However, because both years of collection and temperature were significantly related to most measures of color, I used standardized residuals of regression to control for 1) year of collection when investigating how geography and climate are associated with plumage and 2) temperature when investigating how year of collection is associated with plumage color variables.

Results

Latitude and temperature and latitude and precipitation were significantly negatively correlated (Table 1). Longitude and temperature were significantly negatively correlated (Table 1), but longitude was not significantly associated with precipitation (Table 1). Further, temperature and precipitation were highly positively correlated (Table 1). Thus, for future analyses, I will use temperature, precipitation and longitude only.

Structural UV-Blue Coloration

When standardized by year, structural coloration brightness was significantly positively correlated with temperature (Figure 3, Table 2); such that males were more ornamented in warmer regions. However, when standardized by year, brightness was significantly negatively associated with precipitation and longitude (Table 2), suggesting that males were more ornamented in regions with low rainfall as well as in more easterly locations.

When standardized by temperature, brightness was significantly positively associated with year of specimen collection (Figure 4, Table 3); such that males were more ornamented when collected in more recent times.

When standardized by year, UV-chroma was significantly positively correlated with temperature (Figure 3, Table 2), such that males were more ornamented in warmer regions. Also, when standardized by year, UV-chroma was significantly negatively associated with longitude, suggesting that birds had more-ornamented plumage in more easterly regions (Table 2). When standardized by year, UV-chroma was significantly related to neither precipitation nor latitude (Table 2).

When standardized by temperature, UV-chroma was significantly positively associated with year of collection (Figure 4, Table 3); such that specimens collected in more recent times displayed more-ornamented UV-chroma.

Melanin-Based Coloration

When standardized by year, chestnut brightness was significantly positively associated with temperature (Figure 3, Table 2), suggesting that males were *less* ornamented in warmer regions (Table 2). However, there was not significant relationship between chestnut brightness and precipitation (Table 2). When standardized by year, chestnut brightness was significantly negatively correlated with longitude (Table 2), such that males were *more* ornamented in more easterly locations.

When standardized by temperature, brightness was not significantly correlated with year of collection (Figure 4, Table 3).

When standardized by year, chestnut red chroma, was not significantly associated with average decadal temperature (Figure 3, Table 2), decadal precipitation nor longitude (Table 2).

When standardized by temperature, chestnut red chroma was not significantly associated with year of specimen collection (Figure 4, Table 3).

Discussion

Year of specimen collection appeared to have strong influences on structural-based plumage but melanin-based plumage coloration was less effected; birds collected in more recent times displayed brighter and more UV chromatic structural plumage coloration. These results suggest either feather degradation or accumulation of dust/dirt has occurred during museum storage or that wild birds are more colorful in recent compared to past decades. Further, even when controlling for the effects of time of feather collection, birds display more ornamented UV-blue coloration in warmer and wetter climates (more southern latitudes, eastern longitudes, and in locations in which birds are non-migratory). Although chestnut melanin-based coloration appears to be less influenced by geography, climate, and museum wear, males displayed less ornamented (lighter/brighter) chestnut (melanin-based) coloration when collected in warmer climates and in more westerly locations, suggesting that birds display less ornamented chestnut coloration in the same regions where they display more-ornamented UV-blue (structurally-based) plumage. Further, it appears that structural-based plumage is more influenced by year of collection than is melanin-based plumage, while both plumages appear to be affected by geographical location and climate. Moreover, 1) temperature appears to have more of an effect than precipitation on color variables and 2) brightness of both plumage types appear to be the most sensitive to climate and geographical influences than does chroma (spectral purity).

It is impossible to decouple temperature and precipitation, as these weather variables were significantly positively associated. However, color was statistically more closely tied to temperature than precipitation. In fact, precipitation was only a good predictor of the brightness of the UV-blue structural plumage, while temperature was a good predictor of brightness and UV chroma of the UV-blue structural plumage and the brightness of the chestnut melanin plumage. These results are perhaps not surprising as structural plumage in male eastern bluebirds has been shown to be sensitive to environmental conditions (Siefferman and Hill 2007; Doyle and Siefferman 2014). Condition dependence of structural coloration in male bluebirds should have a strong influence on male fitness (Siefferman and Hill 2005b). Further, melanin colors have been shown to be less sensitive to environmental stress than structural coloration (McGraw et al. 2002). Finally, these data corroborate research on single population of bluebirds followed for 13 years, wherein structural coloration is more influenced than melanin coloration by weather conditions during molt (Author, Chapter 1). In Alabama, bluebirds tend to show more-ornamented UV-blue structural coloration following years of high precipitation and lower temperature during molt (Author, Chapter 1).

I hypothesized that both plumage types would be subject to fading due to year of collection; yet, only structural coloration appeared to be more influenced by collection date than melanin coloration. Structural UV-blue coloration, when standardized for climatic and geographical trends, was still strongly correlated with year of collection, while melanin-based coloration was not. Indeed, both brightness and UV-chroma of structural coloration were positively associated with year of collection; males had more-ornamented structural plumage when collected in more recent times. In a study that used 24 eastern bluebird museum specimens over a time range from 1892 to 2003 to test for fading, significant interactions were found between year of collection and the UV spectrum, indicating that fading occurred more so in the UV regions (Armenta et al. 2008). Structural-based plumage has been shown to be highly subject to dust, oils, and feather-degrading bacteria (reviewed in Doucet and Hill 2009). UV chroma may be especially affected by the oils and dirt found on hands from handling the specimens, as they have absorption peaks in the UV (Andersson and Prager 2006). Further, structural-based plumage has been shown to be affected by feather degrading bacteria, as experimentally induced degradation of the barb cortex and keratin matrix increased brightness but decreased UV chroma of the UV-blue structural feathers in eastern bluebirds (Shawkey et al. 2007).

However, if it is assumed that specimen fading was not the cause of the strong positive trend with year of collection, it is possible that birds are more ornamented in recent times because sexual selection is stronger now than in past decades. This change could be due to stronger selection or because structural coloration is influenced by diet, by changes in food availability over time. Indeed, weather has been shown to affect abundance of arthropods (reviewed in Williams 1961), and, Alabama bluebirds tend to show more-ornamented UV-blue structural coloration following years of high precipitation and lower temperature during molt (Author, Chapter 1). A similar long-term study of the carotenoid pigmentation of American Redstarts (*Setophaga ruticilla*) found that ornamentation tracked weather during molt and the authors attributed this to associations between weather during molt and insect abundance (Reudink et al. 2015). Climate change, in general, has been shown to influence reproductive output, and timing of migration and breeding in birds. Charmantier et al. (2008) show that mean laying date for great tits (*Parus major*) has advanced about 14 days in the time span from 1961-2007. Further, the timing of arrival on breeding territories and over wintering grounds have advanced in 17 species of birds that breed in the United Kingdom in response to climate changes and changes in food abundance (Cotton 2003). Change in structural coloration over time towards brighter UV chroma and brightness could be a response to increases in food availability during molt.

Melanin-based plumage, however, is less sensitive than structural and carotenoid plumage coloration to environmental variation during molt (McGraw et al. 2002; McGraw 2006). Moreover, melanin-based coloration is less susceptible to feather degrading bacteria (Goldstein et al. 2004) and physical damage (McGraw 2006) than is structural coloration. Further, studies of museum-based fading of the melanin-based coloration of eastern bluebird museum specimens showed no effect of specimen age on brightness (Armenta et al. 2008). Chroma of melanin plumage, however, was found to be higher in live birds compared to museum specimens, and this trend was especially pronounced within older specimens (Doucet and Hill 2009). My results, however, suggest that red chroma of chestnut melanin is not susceptible to changes due to specimen age. Overall, it seems that melanin coloration overall more stable than structural-based plumage, leading me to believe that the positive trend associated with breast brightness and temperature is a valid trend not driven by museum fading or damage.

Other biogeographical studies have found geographical trends in phenotypic traits of conspecifics. For example, brightness of melanin coloration is associated with climate (e.g. rainfall) and capture date in Kentish plovers (*Charadrius alexandrinus*) from five geographically distinct populations (Arguelles-Tico et al. 2015). Further, brightness of structural coloration of Eurasian teals (*Anas crecca*) is associated with breeding location longitude (Legagneux et al. 2012).

In conclusion, for eastern bluebirds, it is not possible to fully untangle whether structural color fades with time or whether birds are more ornamented in recent decades. My data support the idea melanin-based plumage data are more accurate than structurally-based plumage when using long-term museum data to investigate how traits change over time geography or climates. Overall, museum data are valuable in understanding how plumage might have changed in climate over time. The relationships between structural coloration and weather and geography suggest that the environment during molt likely allows for greater ornamentation in populations that do not migrate and that experience warmer and wetter conditions during molt. This work demonstrates the importance of long-term biological data and sheds light on how a changing climate can influence sexually selected traits in animals across geographic regions.

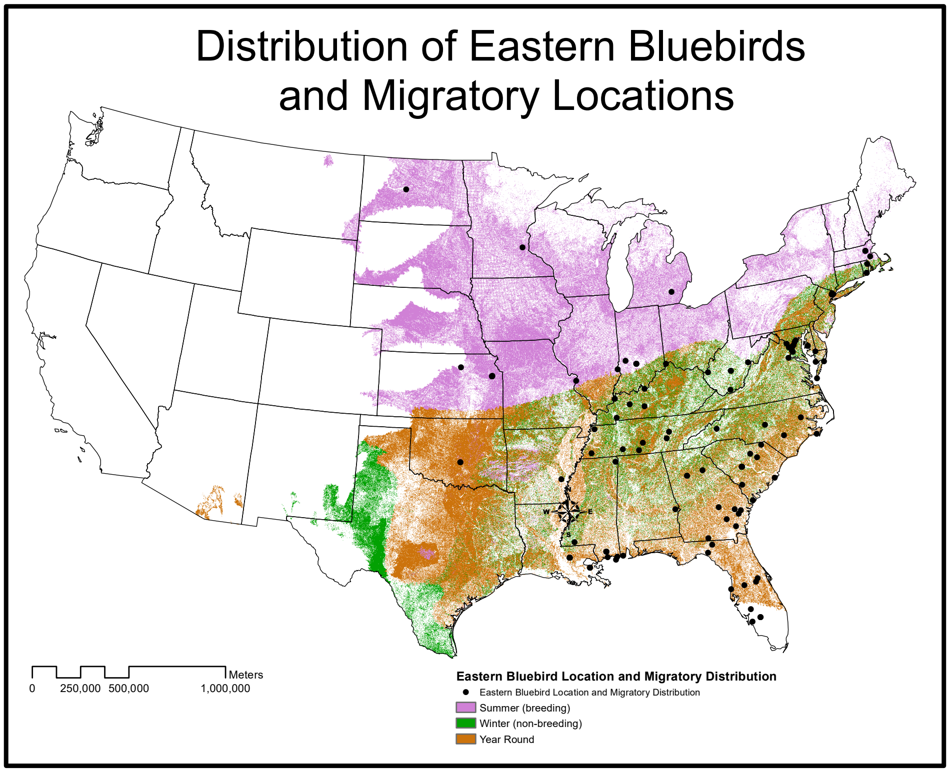

Figure 1 Migratory status map created in ESRI ArcMap Desktop. Migratory patterns obtained from USGS National Gap Analysis Program, <https://gapanalysis.usgs.gov/species/data/download/#forest>

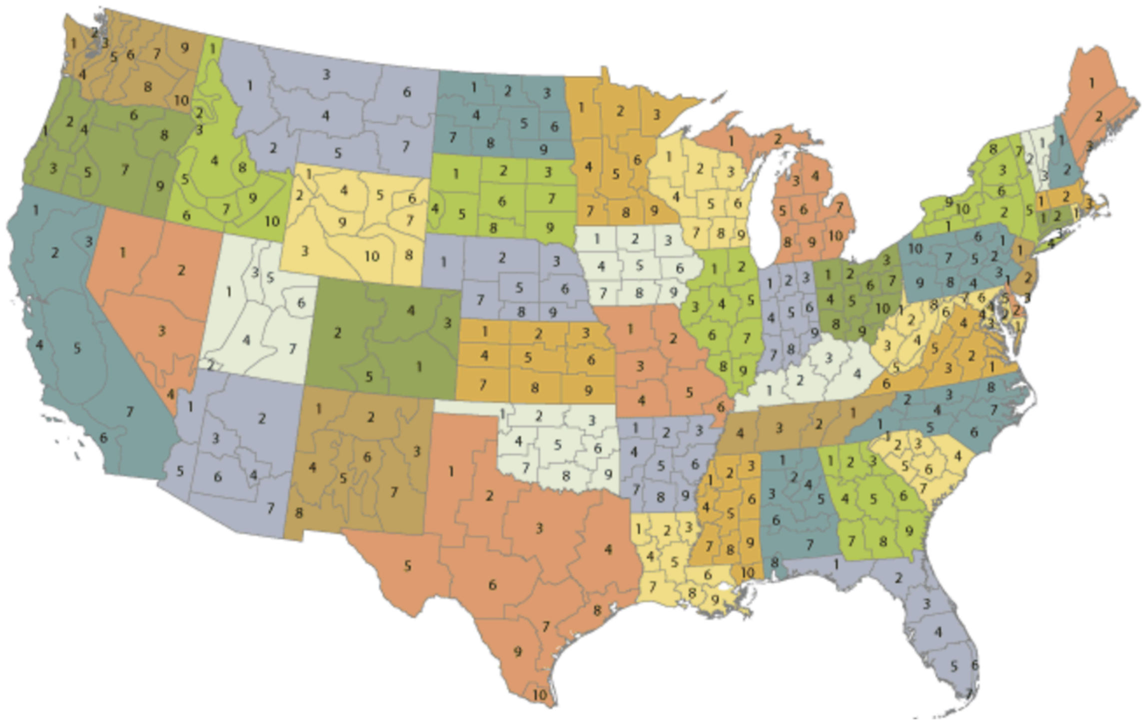

Figure 2 U.S. Climate Division Map. Image obtained from NOAA National Centers for Environmental Information, < https://www.ncdc.noaa.gov/monitoring-references/maps/us-climate-divisions.php>.

	Temperature Decade Average	Precipitation Decade Average
Latitude	-0.963 ($p < 0.0001$)	-0.671 ($p < 0.0001$)
Longitude	-0.495 ($p < 0.0001$)	0.067 ($p = 0.412$)
Precipitation Decade Average	0.592 ($p < 0.0001$)	

Table 1 Pearson's Correlations between geography and weather ($n = 152$) with significance values in parentheses.

	Temperature	Precipitation	Latitude	Longitude
Rump Brightness	0.285 (< 0.001)	0.165 (0.042)	-0.305 (< 0.001)	-0.232 (0.004)
Rump UV Chroma	0.182 (0.025)	0.057 (0.484)	-0.154 (0.059)	-0.217 (0.007)
Breast Brightness	0.252 (0.002)	0.135 (0.097)	-0.247 (0.002)	-0.204 (0.012)
Breast Red Chroma	-0.042 (0.605)	-0.002 (0.985)	0.061 (0.458)	-0.050 (0.544)

Table 2 Pearson's correlations of plumage characteristics of male eastern bluebirds and decadal mean temperature, decadal mean precipitation, latitude and longitude (n = 152) with significance values (P) in parentheses. All color variables are standardized by year.

	Year
Rump Brightness	$r = 0.191$, $p = 0.018$
Rump UV Chroma	$r = 0.506$, $p < 0.001$
Breast Brightness	$r = 0.124$, $p = 0.129$
Breast Red Chroma	$r = 0.129$, $p = 0.11$

Table 3 Pearson's correlations between plumage traits standardized by temperature and year of collection for male eastern bluebirds ($n = 152$).

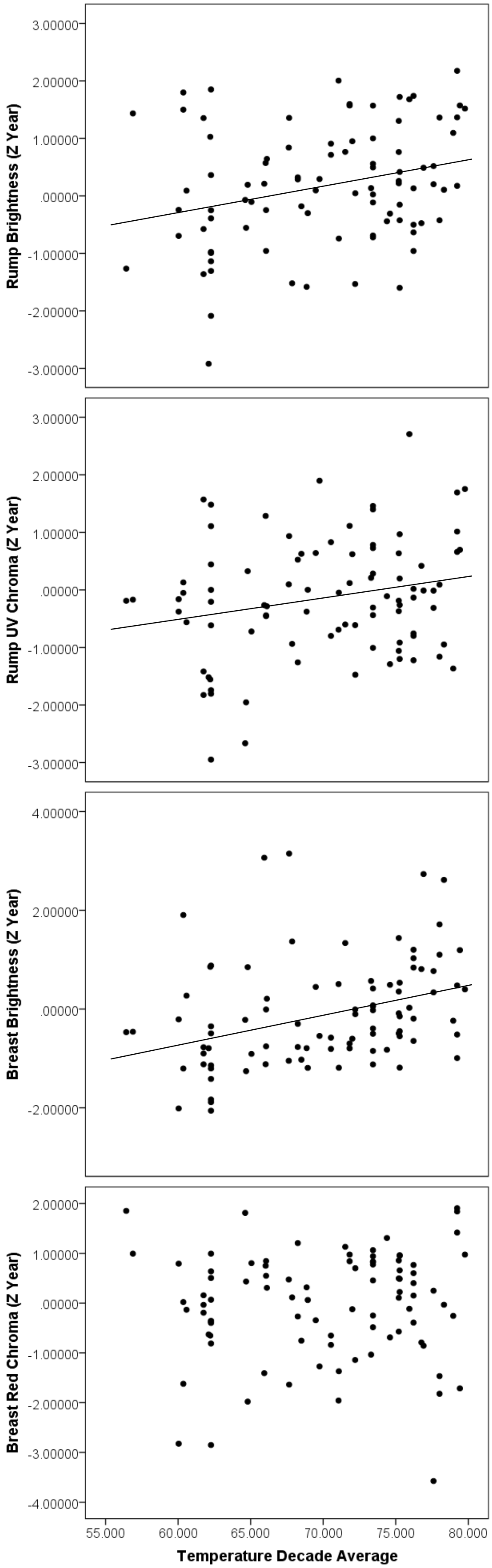

Figure 3 Relationships between color variables (standardized by year) and temperature. Lines indicate significant relationships.

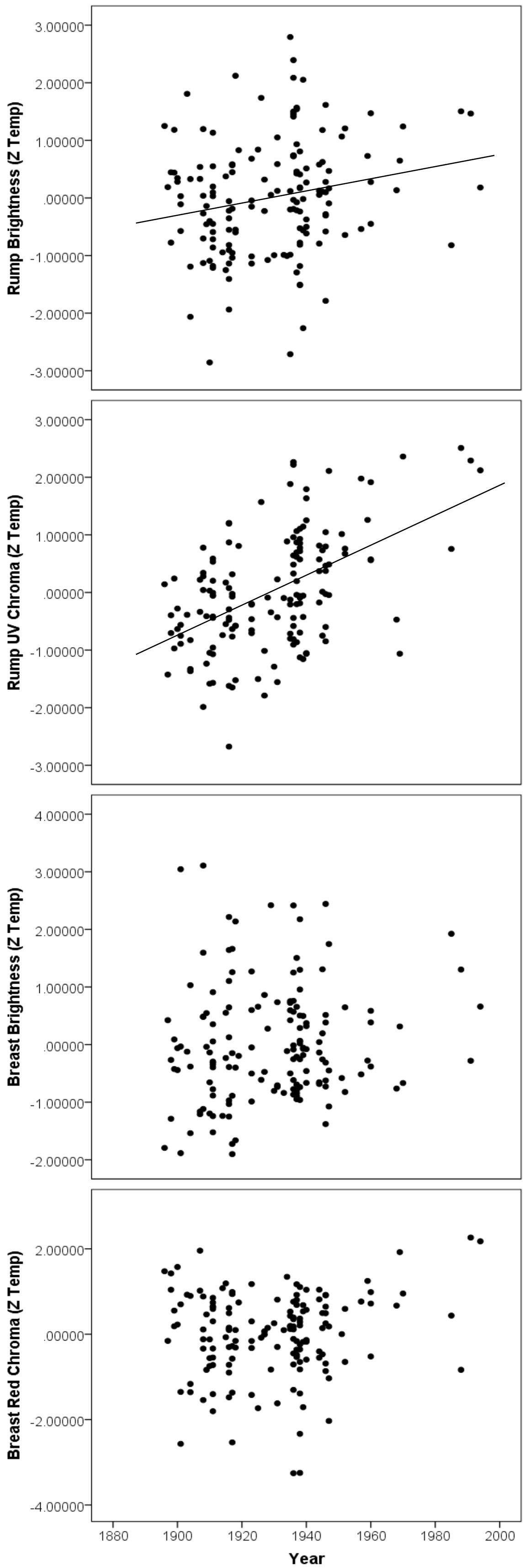

Figure 4 Relationships between color variables (standardized by temperature) and year of collection. Lines indicate significant relationships.

Literature Cited

Allen, J. A. 1877. The influence of physical conditions in the genesis of species. *Radical Review*, **1**, 108-140.

Andersson, M. B. 1994. Sexual selection. *Princeton University Press*.

Anderson, S., and Prager, M. 2006. Quantifying colors. *Bird Coloration*, *Volume 1 Mechanisms and Measurements* (G. E. Hill and K. J. McGraw, eds.). Harvard University Press, Harvard, MA.

Argüelles-Ticó, A., Kupper, C., Kelsh, R. N., Kosztolanyi, A., Szekely, T., and van Dijk, R. E. 2016. Geographic variation in breeding system and environment predicts melanin-based plumage ornamentation of male and female Kentish plovers. *Behavioral Ecology and Sociobiology*, **70(1)**, 49-60.

Armenta, J. K., Dunn, P. O., and Whittingham, L. A. 2008. Effects of specimen age on plumage color. *The Auk*, **125(4)**, 803-808.

Bergmann, K. G. L. C. (1847) Ueber die Verhaltnisse der Warmeokonomie der Thiere zu ihrer Grosse. Gottinger Studien, **3**, 595-708.

Biernaskie, J. M., Grafen, A., and Perry, J. C. 2014. The evolution of index signals to avoid the cost of dishonesty. *Proceedings of the Royal Society B*, **281 (1790)**, 20140876.

Charmantier, A., McCleery, R. H., Cole, L. R., Perrins, C., Kruuk, L. E. B., and Sheldon, B. C. 2008. Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science*, **320(5877)**, 800-803.

Cotton, P. A. 2003. Avian migration phenology and global climate change. *Proceedings of the National Academy of Sciences*, **100(21)**, 12219-12222.

Dale, J. 2006. Intraspecific variation in coloration. *Bird Coloration*, *Volume 2 Mechanisms and Measurements* (G. E. Hill and K. J. McGraw, eds.). Harvard University Press, Harvard, MA.

Dor, R., Safran, R. J., Vortman, Y., Lotem, A., McGowan, A., Evans, M. R., and Lovette, I. J. 2012. Population genetics and morphological comparisons of migratory European (*Hirundo rustica rustica*) and sedentary East-Mediterranean (*Hirundo rustica transitiva*) barn swallows. *Journal of Heredity*, **103(1)**, 55-63.

Doucet, S. M. and Hill, G. E. 2009. Do museum specimens accurately represent wild birds? A case study of carotenoid, melanin, and structural colours in long-tailed manakins *Chiroxiphia linearis*. *Journal of Avian Biology*, **40(2)**, 146-156.

Doyle, A. and Siefferman, L. 2014. Supplemental food increases ornamentation of male nestling Eastern Bluebirds. *Journal of Field Ornithology*, **85**, 31-39.

Friedman, N. R. and Remeš, V. 2016. Ecogeographical gradients in plumage coloration among Australasian songbird clades. *Global Ecology and Biogeography*, **26(3)**, 261-274.

Galeotti, P., Rubolini, D., Sacchi, R., and Fasola, M. 2009. Global changes and animal phenotypic responses: melanin-based plumage redness of scops owls increased with temperature and rainfall during the last century. *Biology Letters*, **5**, 532-534.

Gaston, K. J., and Chown, S. L. 1999. Why Rapoport's rule does not generalise. *Oikos*, **1**, 309-312.

Goldstein, G., Flory, K. R., Browne, B. A., Majid, S., Ichida, J. M., and Burtt Jr., E. H. 2004. Bacterial degradation of black and white feathers. *The Auk*, **121(3)**, 656-659.

Gowaty, P. A. and Plissner J. H. 1998. Eastern Bluebird (*Sialia sialis*) In: The Birds of North America, No. 381 (A. Poole and F. Gill, eds.). *The Birds of North America*, Inc., Philadelphia, PA.

Hasegawa, M., and Arai, E. 2013. Divergent tail and throat ornamentation in the barn swallow across the Japanese islands. *Journal of Ethology*, **31(1)**, 79-83.

Higham, J. P. 2013. How does honest costly signaling work? *Behavioral Ecology,* **25**, 8–11.

Hill, G. E. 2006. Environmental regulation of ornamental coloration. *Bird Coloration*, *Volume 1 Mechanisms and Measurements* (G. E. Hill and K. J. McGraw, eds.). Harvard University Press, Harvard, MA.

Huggett, R. J. 2004. Fundamentals of biogeography. *Routledge Fundamentals of Physical Geography*, New York.

Legagneux, P., Clark, R. G., Guillemain, M., Eraud, C., Thery, M., and Bretagnolle, V. 2012. Large-scale geographic variation in iridescent structural ornaments of a long-distance migratory bird. *Journal of Avian Biology*, **43(4)**, 355-361.

McGraw, K. J. 2006. Mechanics of melanin-based coloration. *Bird Coloration*, *Volume 1 Mechanisms and Measurements* (G. E. Hill and K. J. McGraw, eds.). Harvard University Press, Harvard, MA.

Norris, D. R., Marra, P. P., Montgomerie, R., Kyser, T. K., and Ratcliffe, L. M. 2004. Reproductive effort, molting latitude, and feather color in a migratory songbird. *Science*, **306(5705)**, 2249-2250.

Norris, R. D., Kleven, O., Johnsen, A., and Kyser, T. K. 2009. Melanin-based feather colour and moulting latitude in a migratory songbird. *Ethology*, **115(11)**, 1009-1014.

Pinkowski, B. C. 1977. Breeding adaptations in the Eastern Bluebird. *The Condor*, **79.3**, 289-302.

Pinkowski, B. C. 1978. Feeding of nestling and fledgling Eastern Bluebirds. *Wilson Bulletin*, **90**, 84-98.

Reudink, M. W., McKellar, A. E., Marini, K. L. D., McArthur, S. L., Marra, P. P., and Ratcliffe, L. M. 2015. Inter-annual variation in American redstart (*Setophaga ruticilla*) plumage colour is associated with rainfall and temperature during moult: an 11-year study. *Oecologia,* **178**, 161-173.

Scordato, E. S. C. and Safran, R. J. 2014. Geographic variation in sexual selection and implications for speciation in the Barn Swallow. *Avian Research*, **5(8)**.

Shawkey, M. D., Estes, A. M., Siefferman, L., and Hill, G. E. 2005. The anatomical basis of sexual dichromatism in non-iridescent ultraviolet-blue structural coloration of feathers. *Biological Journal of the Linnean Society*, **84(2),** 259-271.

Shawkey, M. D., Shreekumar, R. P., Hill, G. E., Siefferman, L. and Roberts, S. R. 2007. Bacteria as an agent for change in structural plumage color: correlational and experimental evidence. *The American Naturalist*, **169**, S112-S121.

Siefferman L., and Hill, G. E. 2003. Structural and melanin plumage coloration indicate parental effort and reproductive success in male eastern bluebirds. *Behavioral Ecology*, **14**, 855–861.

Siefferman L., and Hill, G. E. 2005a. Evidence for sexual selection on structural plumage coloration in female eastern bluebirds (*Sialia sialis*). *Evolution*, **59**, 1819-1828.

Siefferman L., and Hill, G. E. 2005b. UV-blue structural coloration and competition for nestboxes in male eastern bluebirds. *Animal Behaviour*, **69(1)**, 67-72.

Siefferman, L., Hill G. E., and Dobson F. S. 2005. Ornamental plumage coloration and condition are dependent on age in eastern bluebirds *Sialia sialis*. *Journal of Avian Biology*, **36(5)**, 428-435.

Stevens, G. C. 1989. The latitudinal gradient in geographical range: how so many species coexist in the tropics. *The American Naturalist*, **133(2)**, 893-911.

USGS National Gap Analysis Program. 2016. Eastern Bluebirds GIS Distribution and Range Raster Files.

Wiens, J. J. 2001. Widespread loss of sexually selected traits: how the peacock lost its spots. *Trends in Ecology & Evolution*, **16(9)**, 517-523.

Wilkins, M. R., Karaardıç, H., Vortman, Y., Parchman, T. L., Albrecht, T., Petrželková, A., Özkan L3,10, Pap P. L., Hubbard, J. K., Hund, A. K., and Safran, R. J. 2016. Phenotypic differentiation is associated with divergent sexual selection among closely related barn swallow populations. *Journal of Evolutionary Biology*, **29(12)**, 2410-2421.

Williams, C. B. 1961. Studies in the effect of weather conditions on the activity and abundance of insect populations. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, **244**, 331-378.