EFFECTS OF WEATHER AND AGE ON FEATHER COLOURATION IN MOUNTAIN BLUEBIRDS

by

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

BACHELOR OF SCIENCE (HONS.)
in the
DEPARTMENTS OF BIOLOGICAL AND PHYSICAL SCIENCES
(Chemical Biology)

THOMPSON RIVERS UNIVERSITY

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Dated this 22nd day of May, 2020, in Kamloops, British Columbia, Canada

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ABSTRACT

Birds exhibit a vast array of colours and ornaments and while much work has focused on understanding the function and evolution of carotenoid-based colours (red, orange, yellow), structural colouration (blue, green, purple, iridescent) can also play a key role in sexual signaling by acting as an indicator of individual quality. While several studies have examined how factors such as diet and age may influence structural colour, few studies have looked at how structural colour may be influenced by variation in weather conditions experienced during the annual moult. In this study, we examine variation in structural colour expression in relation to age as well as rainfall and temperature during moult for a population of Mountain Bluebirds (*Sialia currucoides*) breeding in Western Canada. We looked at feather colouration over a period of nine years by using reflectance spectrometry of rump and tail feathers from male and female Mountain Bluebirds and examined the relationship between feather colour and temperature and rainfall from the previous breeding season as well as age-related colour changes. At a population level, we found that feathers from males and females were more colourful (higher brightness and chroma, hue values shifted more towards UV) as birds aged from juveniles to adults. Within individuals, after they reached adulthood (second breeding season and beyond), male bluebird rump and tail feathers became less colourful. At a population level, male Mountain Bluebirds exhibited more colourful feathers following years with higher August rainfall, while female Mountain Bluebirds expressed more colourful tail feathers after years with higher July and August rainfall. However, these effects were dependent on age. Young female Mountain Bluebirds were most affected by weather conditions, exhibiting more colourful structural plumage after years with higher rainfall and higher August temperatures. We suggest that higher rainfall may increase insect abundance and thus improve food intake and overall condition of Mountain Bluebirds, and we suggest that males and females may be affected differently due to differences in behavior during the breeding and post breeding periods. This is one of the first studies to show how both age and weather conditions may affect structural colours in birds.

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ACKNOWLEDGEMENTS

I would like to thank Dr. Matthew Reudink for his teaching and support throughout this project, and I would like to thank Dr. Sean Mahoney for all his help during the project. I also want to thank Stephen Joly for his guidance in the field, and Dr. Kingsley Donkor for his role as co-supervisor. Thank you to the Natural Sciences and Engineering Research Council of Canada for providing funding for this project through my Undergraduate Student Research Award as well as Dr. Reudink's Discovery Grant. Finally, thank you to my parents, Phil and Barb, for their unwavering help and support throughout this and every other project I attempt.

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INTRODUCTION

Scientists and naturalists alike have long been fascinated by birds, due in part to the incredible diversity of their colours and ornaments and many have spent decades unravelling the evolution of such vibrant plumages. Sex-specific differences in colour are often driven by sexual selection and are important signals for mate choice (see Hill 2006a, Robinson et al. 2012). Females are typically the choosy sex and more colorful males tend to experience greater reproductive success (see Hill 2006a, Siefferman et al. 2005). However, bright colours also come with costs. Greater ornamentation is typically associated with higher risks, as it is physiologically more challenging to produce (see Hill 2006b) and may increase the risk of predation (see Zuk and Kolluru 1998; Promislow 1992). As a result, brighter colours tend to act as honest indicators of health/condition and overall individual quality (see Hill 2006a). A greater degree of ornamentation has been associated with more parental efforts in female birds (Siefferman and Hill 2005), success in male-male competition (Robinson et al. 2012), better individual health (Siefferman et al. 2005), and ultimately greater breeding success (Hill 2006b). While many studies focus on the impacts of male coloration on breeding success, female ornamentations can also act as important inter- and intrasexual signals (reviewed in Hill 2006a, Santos et al. 2011).

In birds with carotenoid-based plumage, feather colouration is closely linked to nutrient access, as ingested carotenoids can be directly deposited into feathers or metabolically converted into orange or red pigments and subsequently deposited into growing feathers (Hill and Montgomerie 1994). Birds are incapable of producing carotenoid pigments themselves; instead, they must ingest carotenoids by consuming plants or insects that have consumed carotenoid-containing plants (reviewed in McGraw 2006, and see Eeva et al. 2010). Carotenoid-based pigmentation is thus dependent on the individual bird's access to nutrients (reviewed in Hill and

McGraw 2006). However, because carotenoids also have other important physiological roles as antioxidants and immunostimulants birds face a trade-off between utilizing carotenoids for self-maintenance and ornamentation (McGraw 2006). Furthermore, the metabolic conversion of dietary carotenoids to orange/red pigments appears to incur further costs, indicating that the display of pigments from derived carotenoids may be the best honest indicators of individual condition (Weaver et al. 2018).

In birds with structurally based plumage (e.g., blue, purple, iridescent colours) a clear link between diet and feather colouration is not apparent because structural colours do not come directly from ingested nutrients (see Hill and McGraw 2006). Whereas carotenoid-based colours are dependent on the intake of carotenoids, structural colours such as UV-blue colouration are produced by the arrangement of melanosomes (produced *in vivo*) within the feather microstructure and the scattering of light as it passes through nanoscale variations in the feather structure (Prum 2006). At the nanoscale level, feather barbules can contain a matrix of air and biological structures, such as proteins and pigment granules, like melanin (Prum 2006). As light strikes the feather and passes through these biological structures, it encounters different refractive indices, causing some of the light waves to scatter in different directions (Prum 2006). Thus, the presence and spatial distribution of miniscule biological structures, such as melanosomes, proteins, and pigment granules, and their respective refractive indices in the feather determine how light waves are scattered and what colour results (Prum 2006).

Despite the lack of a direct mechanism linking nutrient ingestion and structural colours, numerous studies have found evidence that structural colours are linked to individual condition and quality: a study by Siefferman and Hill (2005) found that female Eastern Bluebirds (*Sialia sialis*) on a food-restricted diet displayed less-colourful rump feathers than females that were given free access to food, and a study by Doyle and Siefferman (2014) found that male Eastern Bluebird nestlings that were fed supplemental mealworms exhibited brighter plumage. While they did not directly manipulate diet, a study by Jacot and Kempenaers (2007) on Blue Tits (*Cyanistes caeruleus*) found that nestlings raised in larger broods developed duller feathers than males raised in smaller broods. There is also evidence that aspects other than diet might influence structural colour. Harper (1999) found a correlation between lower plumage brightness and higher mite load in Blue Tits, while Doucet and Montgomerie (2003) found that brighter Male Bowerbirds (*Ptilonorhynchus uiolaceus*) had fewer blood parasites.

Besides variation that may occur due to individual quality, other factors like aging may affect feather colouration. In species that experience delayed plumage maturation, a significant shift in colour occurs after the birds have undergone their first moult as an adult, typically during their second year of life (see Lyon and Montgomerie 1986; Hawkins et al. 2012; Lyu et al. 2015). Even species with a less-stark transition to adulthood show changes as they age, however, and colour may continue to change after they reach maturity (see Siefferman et al. 2005; Budden and Dickinson 2009; Delhey and Kempenaers 2006). A study on male Western Bluebirds (*Sialia mexicana*) found that UV-blue plumage patches were larger and brighter in older males (Budden and Dickinson 2009), while a study examining both male and female Blue Tits showed increased structural chroma and brightness values as they aged (Delhey and Kempenaers 2006). In American Redstarts (*Setophaga ruticilla*), Marini et al. (2015) found that the orange plumage exhibited by adult males became more yellow-shifted as birds aged, which may have indicated a shift in how carotenoids were metabolized and deposited in older male birds.

For both carotenoid- and structural-based colouration, variation can also be influenced by geography and weather. A study by Tisdale et al. (2018) found latitudinal differences in ornamentation in Golden-Winged Warblers (*Vermivora chrysoptera*), with more southern birds showing less colourful crown and throat feathers, while Gamero et al. (2015) found that feather brightness in Great Tits (*Parus major*) was lower in populations that lived at higher elevations. Even minor geographic variation can have a major impact; Hill (1993) found that male House Finches (*Haemorhous mexicanus*) sampled only 12 km apart showed significant variation in average male colouration. Possible explanations for geographic variation in colouration are diverse: Hill (1994) suggested genetic drift as possible mechanism, while Gamero et al. (2015) pointed to higher population density in the southern Great Tit population potentially causing stronger sexual selection to explain the population differences. Finally, another possible explanation could be nutrient access: for birds with carotenoid-based feathers, carotenoid availability within habitats could influence population colouration (Tisdale et al. 2018), as could nutrient access.

Weather can also play a major role in plumage variation from year to year. Migratory birds moult annually, usually in late summer, on or near the breeding grounds at the end of the breeding season (Pyle 1997). Reudink et al. (2015) found that American Redstarts exhibited more colourful carotenoid-based plumage when moult occurred during years of higher rainfall and temperatures in July, and lower temperatures in August; they suggested that the most likely mechanism behind this was variation in insect abundance, as higher levels of rainfall would produce a more abundant insect population, thus increasing the redstarts' access to nutrients (and specifically to carotenoids). A similar mechanism could be at work in birds with structurally based colouration, as a more abundant insect population could provide more nutrients and improve individual feather quality during moulting by altering feather nanostructure. While the specific effects of nutrition on microscopic feather structure are not known, changes in diet could potentially influence the layout and expression of proteins and other biological structures that feather barbules are composed of, thus changing the feather's appearance. A study by Shawkey et al. (2003) found that UV-violet chroma in feathers was associated with a higher number of circular keratin rods, and suggested that at least some colour variation could be explained by feather nanostructure.

Mountain Bluebirds (*Sialia currucoides*) possess brilliant UV-blue structural colouration, and there is evidence that females prefer more colourful males as social and extra-pair partners (Balenger et al. 2009). While colour seems to be an important factor in mate selection (Balenger et al. 2009; but see Liu et al. 2007; Liu et al. 2009), little is known about how specific factors like aging or weather conditions during moult affect feather colouration. And, while male and female Mountain Bluebirds undergo a significant shift in colouration as they transition from their first breeding season (second year, SY) to subsequent breeding seasons (after second year, ASY), how their colour changes after reaching their first season as an ASY (ASY1) remains largely unknown. While studies have looked at possible effects of aging (Siefferman et al. 2005) and weather conditions (Warnock 2017) on structurally based plumage colouration separately, few studies have offered a concurrent examination of both effects. Our goal was to examine Mountain Bluebird feathers grown over 9 seasons to determine the effects of aging and weather on the expression of UV-blue structural colouration. We predicted that aging would be correlated with an increase in ornamentation (higher brightness, greater UV-blue chroma, and more UV-shifted hue) and higher temperatures and less rainfall would be associated with lower ornamentation in male and female Mountain Bluebirds.

MATERIALS AND METHODS

Field methods

Field work was conducted during breeding seasons (May-July) from 2011-2019 in Kamloops, British Columbia, Canada (885–1116 m asl; 50°40.23′ N, 120°23.86′ W). Adult male and female Mountain Bluebirds were captured and banded at nest boxes along routes maintained by the Kamloops Naturalist Club. Birds were banded with a Canadian Wildlife Service (CWS) aluminum band and a unique combination of three colour bands. Adults were classified as either second-year (SY) or after-second-year (ASY) based on moult limits. A bird was classified as ASY1 if it was captured as an SY in the previous season or was captured for the first time as an adult. Birds that were initially banded as ASY1 and recaptured the subsequent season were classified as ASY2, and so on. This approach assumes that birds captured for the first time as an ASY were actually ASY1 and thus it is likely that some of the bird classified as ASY1 were in fact older. However, breeding site fidelity tends to be high with Mountain Bluebirds after they reach ASY and the effects of misclassification, if any, would weaken any observed effects of age and weather (see also Marini et al. 2015).

Between five and ten rump feathers were collected from each adult, and a single tail feather (R3) was plucked for colour analysis. Feathers were stored in manila coin envelopes prior to analysis. Our analysis involved a total of 363 tail feathers collected from 308 individuals, as some birds were caught more than once. All work was approved by the Thompson Rivers University Animal Care and Use Committee and was conducted under a Canadian Federal Master Banding Permit (#10834) and Scientific Collection Permit.

Feather colour analysis

Feathers were mounted on low-reflectance black paper and scanned using a JAZ spectrometer and a PX-2 xenon light source from Ocean Optics (Dunedin, FL). Rump feathers were placed with an overlapping pattern, mimicking the way the feathers would lie on a bird, while tail feathers were mounted individually. Ten readings were taken for each plumage area (rump and tail), haphazardly across the feathers. The probe was held in a non-reflective probe holder at a 90° angle at a set distance of 5.9 mm. Measurements between each successive tail feather or group of rump feathers were standardized using an Ocean Optics WS-1 white standard, and a non-reflective dark standard.

RCLR v.28, an R-based colour analysis program, was used to analyze reflectance measurements (Montgomerie 2008). A smoothing function was performed on all curves to eliminate noise (to reduce random effects from other sources of colour that may have been present when scanning). Using RCLR v.28, brightness, chroma, and hue were calculated for each feather. Brightness was calculated as the mean amount of light reflected across the mean visual spectrum (in other words, the area under the reflectance peak). Chroma was calculated as ($R_{300\text{-}510}/R_{300\text{-}700}$), or the area under the peak between wavelengths of 300-500 nm divided by the area under the peak between wavelengths of 300 and 700 nm. Hue was calculated as the wavelength at maximum reflectance. The ten readings taken for each plumage area were averaged to produce a single value for hue, chroma, and brightness for each feather. Due to high collinearity among the three colour variables, we used principal component analysis to collapse the three variables into a single factor, and we used the first principal component (PC1) to represent overall plumage colour variation, as it explained most of the variance among birds with respect to each of the plumage areas (Table 1).

A greater PC1 value corresponded to increased brightness and chroma, but a decrease in hue (meaning maximum reflectance shifted more towards the UV portion of the spectrum).

Table 1. Results from a principal components analysis of three measures of rump and tail plumage colouration (brightness, hue and chroma).

	Eigenvalue	Proportion of variance	Colour variable	Factor loading
Male tail PC1	1.53	0.51	Brightness	0.61
			UV + blue chroma	0.57
			Hue	-0.54
Female tail PC1	1.84	0.61	Brightness	0.54
			UV + blue chroma	0.58
			Hue	-0.60
Male rump PC1	1.88	0.63	Brightness	0.47
			UV + blue chroma	0.66
			Hue	-0.59
Female rump PC1	2.11	0.71	Brightness	0.47
			UV + blue chroma	0.64
			Hue	-0.60

Weather Data

Rainfall and temperature data were obtained from the Government of Canada website (https://climate.weather.gc.ca/historical_data/search_historic_data_e.html), which provided data collected at a weather station in Kamloops. Weather data for the years 2010-2018 were obtained from Environment and Climate Change Canada for the Kamloops A station, located at the Kamloops Airport (50.70°N, 120.44°W). In some months, weather data for the station was not available; instead, data from the Pratt Road weather station, located approximately 20 km SE (50.60°N, 120.20°W) was used.

Statistical Analysis

In our analyses of whether older individuals differed from younger individuals in plumage coloration at a population level, we analyzed tail and rump PC1 values separately. Males and females were also analyzed separately, due to the differences in male and female plumage colouration. We compared SY and ASY age classes for both male and female Mountain Bluebirds with linear mixed models that included individual as a random effect, using data standardized by year to account for yearly variation. Data were standardized separately for males and females by setting the mean to 0 and standard deviation to 1 within each year. This was done to account for the significant variation among years and allowed us to ask whether different age classes showed, on average, different plumage colouration, while controlling for environmental effects during feather growth (Marini et al. 2015).

We next wanted to examine within-individual changes as bluebirds aged. To determine whether individuals changed over time, we performed paired *t*-tests on unstandardized PC1 values for both rump and tail colouration using birds that were caught more than once.

To assess how colour variation between sexes and among age classes was explained by weather during moulting periods, we related rump PC1 and tail PC1 separately to rainfall and temperature during June, July, and August from the previous year using mixed effects models. In each model, we set rump or tail PC1 as the response variable, age, sex, rainfall and temperature as fixed effects, and individual as a random effect. We built separate models for each month to avoid problems associated with multiple correlation among months (Reudink et al. 2015). To help with interpretation, we only allowed two-way interactions between all fixed effects. We then undertook model reduction for all mixed models based on the change in Akaike information criterion (AIC Burnham and Anderson 2003) between the full model and each reduced model. We chose our final model based on no significant change in AIC. Using the final model, we then assessed the relationship between weather (either rainfall or temperature) and colour (rump or tail PC1) using the R package *emmeans*, an R-based software package that can generate least-squares means for linear and mixed models (Lenth 2019). Analyses were performed in R v.3.6.3 (R Development Core Team 2014).

RESULTS

Population-level changes over time: SY vs. ASY

At a population level, when we examined all individuals captured from 2011-2019 using a linear mixed model with individual as a random effect, SY birds had tail feathers with significantly lower tail PC1 values than ASY birds for both males and females (male: $F_{1,173} = 22.07$, $p < 0.0001$, Figure 2; female: $F_{1,183.4} = 11.20$, $p = 0.001$, Figure 1). However, neither males nor females differed in rump PC1 between SY and ASY age classes (male: $F_{1,175.1} = 2.5864$, $p = 0.1096$; female: $F_{1,182.2} = 0.2192$, $p = 0.6402$).

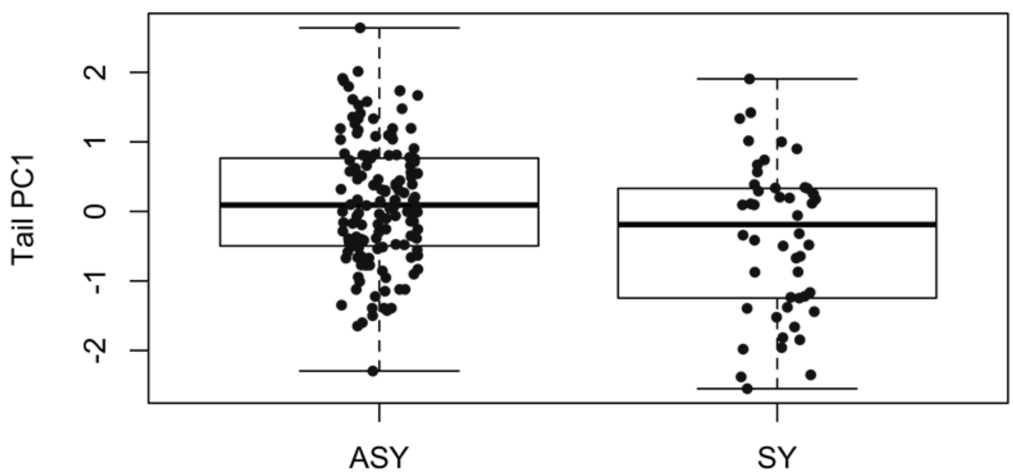

Figure 1. ASY females had greater tail PC1 values than SY females

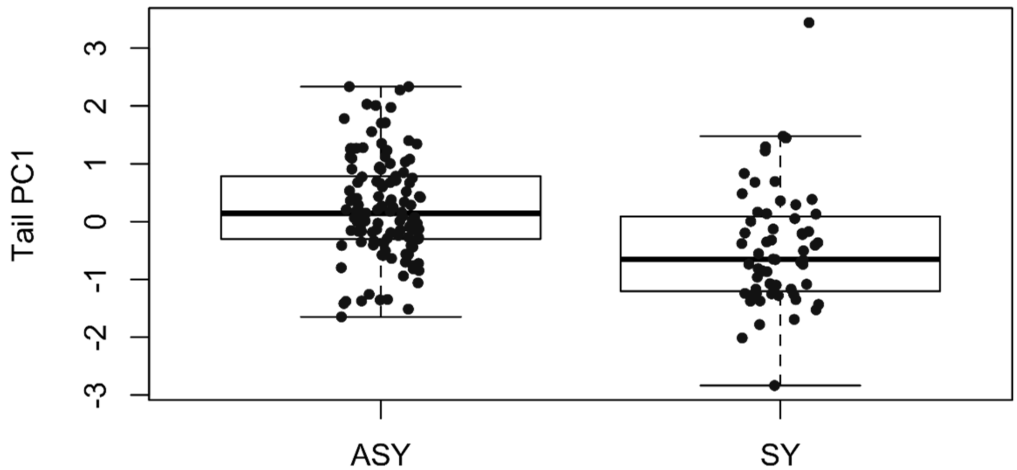

Figure 2. ASY males had greater tail PC1 values than SY males

Within-individual changes over time

When we examined within-individual changes in tail PC1 values, we found no difference between individuals in year ASY1 and year ASY2 and this pattern held true examining both standardized (female: $t_{1,16} = -0.36$, $p = 0.72$; male: $t_{1,14} = -0.41$, $p = 0.69$) and non-standardized colour values (female: $t_{1,16} = 0.14$, $p = 0.89$; male: $t_{1,14} = -0.27$, $p = 0.79$, Figure 3). There were too few recaptures of individuals first captured as an SY and subsequently recaptured as an ASY to perform statistical analyses within individuals (female n = 3, male n = 5).

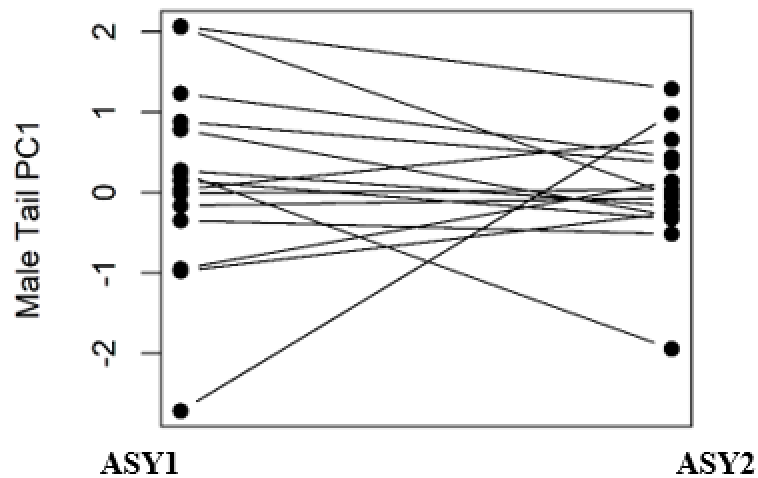

Figure 3. Within-individuals, there was no significant difference in PC1 tail values for ASY1 and ASY2 males.

Sample sizes were too small (n = 4 males, 4 females) to examine changes from ASY2 to ASY3. However, when we examined changes from ASY 1 to ASY 3 using non-standardized values, we found differences in tail colours in males ($t_{1,7}$ = 2.53, p = 0.04) and in rump colour values ($t_{1,7}$ = -3.18, p = 0.02). We found no difference when we examined standardized values for rump colour in males ($t_{1,7}$ = -1.42, p = 0.20), however, we still found a difference between the tails of ASY1 and ASY3 birds using standardized values for colour in males ($t_{1,7}$ = -2.60, p = 0.04, Figure 4). Sample size was too small to examine changes from ASY1 to ASY3 in females (n = 5).

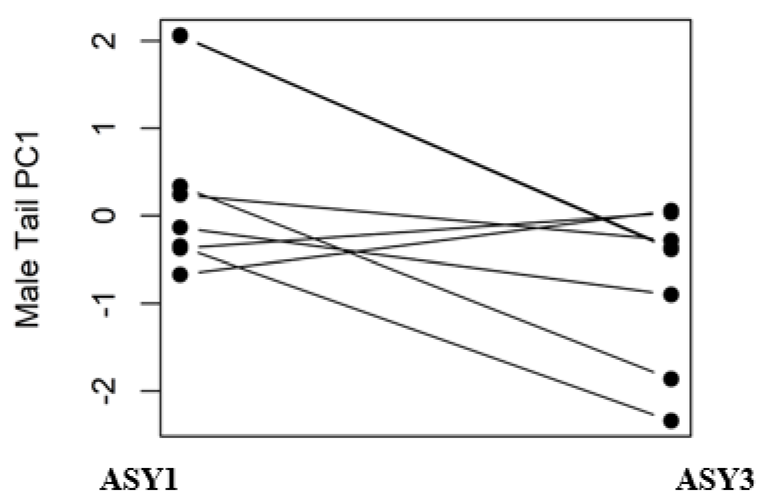

Figure 4. Within-individuals, ASY3 males had lower tail PC1 values than ASY1 males.

Yearly Variation

When we examined yearly variation in plumage colouration (unstandardized colour values), all age and sex classes varied significantly across years (Table 2, Figures 5 and 6). In Table 2, df stands for degrees of freedom, F is the F-statistic, and p is the probability value.

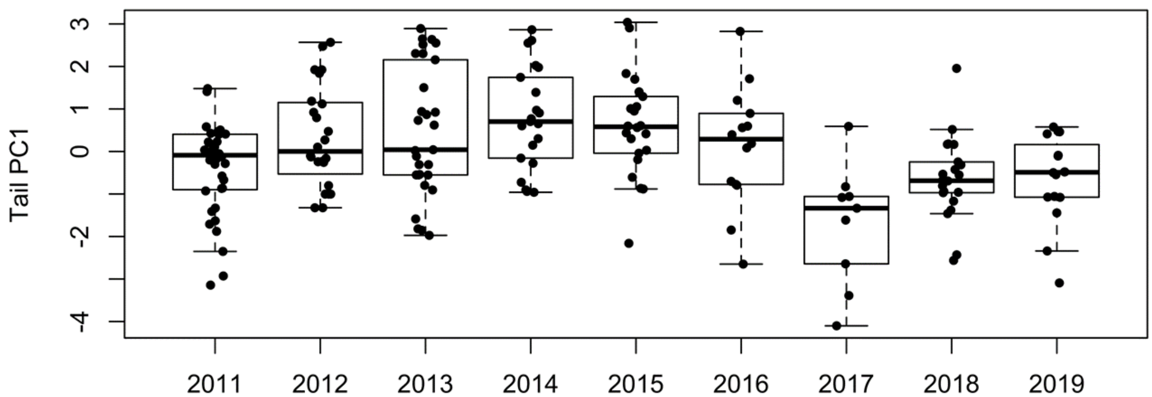

Figure 5. Yearly colour variation in female Mountain Bluebirds.

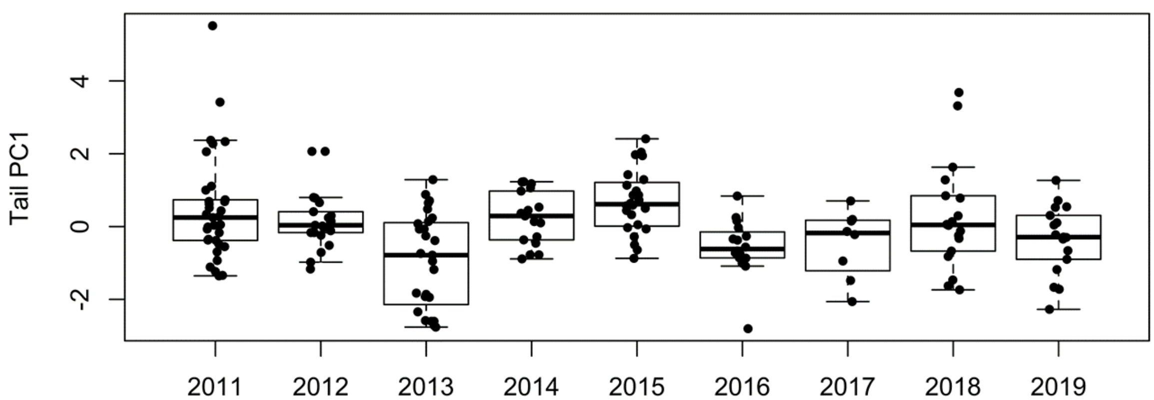

Figure 6. Yearly colour variation in male Mountain Bluebirds.

Table 2. Tail and Rump PC1 value variation across years.

	Tail PC1			**Rump PC1**		
Age/Sex Class	**df**	**F**	**p**	**df**	**F**	**p**
ASY Female	8, 114.5	3.14	0.003	8, 126.4	8.27	<0.0001
SY Female	8, 41	5.75	<0.0001	8, 41	5.6	<0.0001
ASY Male	8, 101.8	3.57	0.001	8, 101.2	6.12	<0.0001
SY Male	8, 511	2.93	0.009	8,51	4.69	0.0002

Weather effects

To test the combined effects of weather and age on colouration, we conducted models separately for males and females and conducted one analysis in which birds were classified only as SY/ASY and a second analysis in which birds were classified by age class (e.g., ASY1, ASY2). For our first analysis using only SY/ASY classification, we found different effects for both males and females and significant interactions between weather and age. For males, more colourful tail feathers were correlated with higher levels of rainfall in August, but only for ASY males (Table 3). For females, higher rainfall in July was associated with more colourful rump feathers for both SY and ASY females, but the strength of this effect was much stronger in SY females (Table 4).

For our second analysis that included all age classes (ASY1, ASY2, etc.), we observed no associations between age class or feather colour in males. In females, higher rainfall in July positively influenced tail feather colour for both ASY1 and SY birds, but not older age classes (Table 5, Table 6). Higher July temperature and rainfall were also associated with brighter rump plumage in SY females (Table 5). Higher August temperature was associated with brighter tail plumage for both SY and ASY3 females (Table 5, Table 6, although the small sample size for

ASY3 females (n=5) should be noted. In all tables, SE stands for standard error, t is the t-statistic, and p is the p-value (probability value).

Table 3. Final best fit models examining the effects of weather and age (SY/ASY) on male colouration.

				SY				ASY			
Patch	**Month**	**Final Model**	**Variable**	**Slope**	**SE**	**t**	**p**	**Slope**	**SE**	**t**	**p**
Rump PC1	June		Temp								
			Rain								
	July		Temp								
			Rain								
	August*		Temp								
			Rain								
R3 PC1	June		Temp								
			Rain								
	July		Temp								
			Rain								
	August	Age*AuRn+(1|Band.Number)	Temp								
			Rain	0.002	0.007	0.27	0.8	0.02	0.007	3.4	**0.0009**

Table 4. Final best fit models showing the effects of weather and age (SY/ASY) on female colouration.

				SY				ASY			
Patch	**Month**	**Final Model**	**Variable**	**Slope**	**SE**	**t**	**p**	**Slope**	**SE**	**t**	**p**
Rump PC1	June	Age*JlRn+(1|Band. Number)	Temp								
			Rain								
	July		Temp								
			Rain	0.032	0.009	3.71	**0.0003**	0.02	0.006	2.62	**0.01**
	August		Temp								
			Rain								
R3 PC1	June		Temp								
			Rain								
	July		Temp								
			Rain								
	August		Temp								
			Rain								

Table 5. Final best fit models showing the effects of weather and age class (SY and ASY1) on female feather colouration.

Females				SY				ASY 1			
Patch	**Month**	**Final Model**	**Variable**	**Slope**	**SE**	**t**	**p**	**Slope**	**SE**	**t**	**p**
Rump PC1	June		Temp								
			Rain								
	July	Age Class*JlRn+(1|Band.Number)	Temp								
			Rain	0.04	0.01	3.7	**0.0003**	0.02	0.008	2.3	**0.03**
	Aug		Temp								
			Rain								
R3 PC1	June		Temp								
			Rain								
	July	Age Class*JlTp+Age*JlRn+(1|Band.Number)	Temp	0.76	0.24	3.2	**0.002**	0.002	0.13	0.01	0.99
			Rain	0.031	0.01	2.8	**0.006**	0.007	0.008	0.85	0.4
	Aug	Age Class*AuTp+(1|Band.Number)	Temp	0.6	0.21	2.9	**0.005**	0.18	0.14	1.34	0.18
			Rain								

Table 6. Final best fit models showing the effects of weather and age class (ASY2, ASY3, ASY4) on female feather colouration.

<table>
<tr><th colspan="4">Females</th><th colspan="4">ASY2</th><th colspan="4">ASY3</th><th colspan="4">ASY4</th></tr>
<tr><th>Patch</th><th>Month</th><th>Final Model</th><th>Variable</th><th>Slope</th><th>SE</th><th>t</th><th>p</th><th>Slope</th><th>SE</th><th>t</th><th>p</th><th>Slope</th><th>SE</th><th>t</th><th>p</th></tr>
<tr><td>Rump PC1</td><td>June</td><td></td><td>Temp</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
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<tr><td></td><td>July</td><td>Age Class*JlTp+Age*JlRn+(1|Band. Number)</td><td>Temp</td><td>-0.07</td><td>0.21</td><td>-0.33</td><td>0.74</td><td>-2.2</td><td>1.4</td><td>-1.6</td><td>0.13</td><td>NA</td><td>NA</td><td>NA</td><td>NA</td></tr>
<tr><td></td><td></td><td></td><td>Rain</td><td>-0.02</td><td>0.014</td><td>-1.13</td><td>0.27</td><td>-0.06</td><td>0.02</td><td>-2.85</td><td>0.007</td><td>NA</td><td>NA</td><td>NA</td><td>NA</td></tr>
<tr><td></td><td></td><td></td><td>Temp</td><td>-0.17</td><td>0.29</td><td>-0.61</td><td>0.55</td><td>10.8</td><td>2.9</td><td>3.7</td><td>0.0009</td><td>5.02</td><td>3.8</td><td>1.3</td><td>0.2</td></tr>
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DISCUSSION

Plumage ornamentation serves a variety of functions in birds, but the mechanisms that lead to variation in structural colours are not well understood. In this study, we found that for both male and female Mountain Bluebirds, SY tail feathers, but not rump feathers, were less colourful than those of ASY birds. We also found some evidence of colour change among age classes of ASY birds, with ASY birds appearing to become less colourful in later years. Finally, weather conditions during moult were associated with differences in colour for both males and females, but these effects were dependent on age.

At a population level, both male and female SY birds had less colourful tail feathers (i.e., feathers with lower brightness, with hue shifted towards longer wavelengths and lower chroma values) than ASY birds. Siefferman et al. (2005) found similar results in Eastern Bluebirds, with older males expressing more colourful structural plumage for both rump and tail feathers. In contrast to what was found in Eastern Bluebirds, however, we found no differences in rump colouration between SY and ASY birds. In Bluebirds, the duller colour of tail feathers in SY birds may result from tail feathers being grown in the nest under different conditions than those of adults, which are grown later during the late summer post-breeding period. Because SY feathers are grown earlier, these birds have also maintained their tail feathers for a longer period of time. Maintaining tail feathers longer could in turn contribute towards decreased brightness: a study by Surmacki et al. (2011) found that male Eastern Bluebirds captured twice within a breeding season showed a decrease in UV chroma and brightness of feathers later in the season. Similarly, Örnborg et al. (2002) found that the UV/blue crowns in both male and female Blue Tits varied in hue, chroma, and brightness throughout the year. In our study, the lack of a difference in rump feather colouration between SY and ASY birds was likely because both juveniles and adults moult body feathers either during the pre-supplemental (juvenile) or pre-basic (adult) moult in late summer.

Within individuals, there was no difference in plumage between ASY1 and ASY2 males and females. Male ASY3 birds, however, exhibited less-colourful feathers than male ASY1 birds, in both rump and tail feathers. This appears to contrast with past studies: Siefferman et al. (2005) found that male Eastern Bluebird rump feathers increased in brightness and tail feathers increased in hue and chroma between first year (SY) and subsequent years. Similarly, Budden and Dickinson (2009) found that older male Western Bluebirds had brighter blue head plumage, although rump feathers did not show a difference with aging. Budden and Dickinson (2009) did find that the size of the blue plumage head patches increased on male Western Bluebirds with age, while the size of rufous back patches decreased with age. Budden and Dickinson (2009) suggested that the quantity of blue plumage, rather than the quality, could vary with age; while our study did not examine the size of blue plumage patches, it does provide support for the idea that colour quality may not be a good indicator of age once bluebirds have reached adulthood.

In addition to age-based variation, we also observed high variation in plumage colour across years for all age classes, suggesting that large-scale factors are likely influencing population-level variation in colouration. Previous work on American Redstarts demonstrated that population-level variation may be driven by differences in temperature and rainfall across years (Reudink et al. 2009). The authors suggested that temperature and rainfall could influence the abundance of the carotenoid-rich insects necessary for producing colourful orange plumage. Though structural plumage colouration would not be directly influenced by dietary pigments, temperature and rainfall could still influence colouration directly or indirectly through exposure to weather-induced stress or diet-mediated condition, by affecting feather nanostructure.

Males and females appeared to be affected differently by weather conditions. In our first analysis, we included age in our models with individuals classified only as SY or ASY. ASY males had more colourful tail feathers after years with higher August rainfall, while female rump colour for both SY and ASY birds was higher after years with more July rainfall. In previous studies examining the effects of rainfall on birds with carotenoid colours, results have been mixed: Reudink et al. (2015) found that American redstarts expressed plumage with higher red chroma and lower brightness after years following high July rainfall, but there were no significant effects from August rainfall. Laczi et al. (2020) found that Great Tits developed breast feathers with lower brightness and UV chroma and higher yellow chroma after drier and warmer August weather. Reudink et al. (2015) suggested that rainfall could result in higher insect abundance, while Laczi et al. (2020) suggested that weather conditions could influence insect populations by reducing the activities of arthropods and that the birds might shelter under wetter conditions rather than foraging, thus reducing overall food intake bye Great Tits. Laczi et al. (2020) also suggested that wetter weather conditions may adversely affect foraging by making flight more difficult and increasing energy expenditure for foraging birds, which provides a contrast to the presumed benefit of increased insect populations.

In birds with structural colours, Warnock (2017) found that Eastern Bluebirds expressed brighter and more saturated UV-blue plumage after cooler and wetter moulting seasons, and that greater precipitation during August in particular had the strongest positive correlation with structural plumage ornamentation. Again, insect abundance was suggested as a possible cause for changes in structural colours. Given that higher levels of precipitation are typically associated with greater insect abundance (Moser 1967; Boomsma and Leusink 1981), wetter summers could potentially increase food intake and improve structural colours for Mountain Bluebirds.

Differences between the sexes could be due in part to breeding behaviours: in July, re-nesting female bluebirds or those with a second clutch may be spending more time in the nest. Those females would then be less affected by adverse effects of weather (such as increased energy expenditure to forage in the rain), while still benefitting from increased insect abundance. Alternatively, females may be moulting slightly earlier than males, though we currently do not have the data to test that hypothesis.

In our second analysis examining the effects of weather on colouration, we used birds classified with respect to different age classes (ASY1, ASY2, etc.). When comparing weather effects across age classes, we found no effects of weather or age on male birds. We did find weather effects on female birds, however the small sample sizes (n = 17 for ASY2, n = 5 for ASY3, and n = 2 for ASY4) should be taken into consideration. For female birds, higher July rainfall was correlated with more colourful rump feathers in ASY1 and SY birds, and in SY and ASY3 tail feathers. The increased effects on female rather than male birds contrast with past studies, as most found that UV-blue colour in males was more sensitive to environmental conditions than UV-blue colour in females (Siefferman et al. 2005; Doyle and Siefferman 2014). Warnock (2017) did find effects from temperature and precipitation on rump feather brightness in both male and female Eastern Bluebirds, however, while another study by Siefferman and Hill (2005) found that female Eastern Bluebirds on restricted diets exhibited duller structural coloration than female Eastern Bluebirds on unrestricted diets, suggesting that condition influences structural colouration in both male and female bluebirds.

Overall, weather effects were most pronounced in SY birds, and precipitation had a stronger effect than temperature on structural colouration. Given that SY birds moult earlier in the season than ASY birds, the stronger effects of July conditions make sense. SY birds typically grow their first set of adult feathers in June or July, so wetter conditions in July may contribute towards higher insect abundance and thus overall food intake for younger birds, resulting in more colourful plumage. For SY birds that have already grown their adult feathers by August, temperature effects on feathers may be unrelated to moult conditions and may instead be dependent on variation in conditions throughout the season. Warnock (2017) found hotter temperatures in August were associated with decreased rump brightness in SY females, although tail feathers were not examined. The contrasting temperature effect could be due to differences in local climates: hotter temperatures in August could have different effects on insect populations in more northern climates. Our study was conducted at (50.70°N), compared to Warnock's (2017), which occurred at 32.5889° N. Alternatively, factors other than insect abundance could be contributing towards temperature effects on feather colour: Delhey et al. (2010) found an increase in the brightness of structurally based crown plumage of Blue Tits after moult and continuing throughout the year, and suggested that keratinolytic bacteria could alter the feathers' nanostructures throughout the year, thus altering brightness, while Gunderson et al. (2009) found that female Eastern Bluebirds were particularly sensitive to changes in plumage colouration after exposure to feather-degrading bacteria.

In conclusion, we found evidence of combined effects of individual- (age) and population-level (weather) processes influencing the expression of structural colouration in Mountain Bluebirds. The effects of weather on feather colouration were more pronounced on female birds, but in both sexes any effects of weather were dependent on age. Overall, rainfall appeared to have more of an effect on feather colour than temperature, which we suggest is mostly due to an increase in insect abundance during wetter summers influencing body condition during moult. Overall, structural colours appear to be influenced by both age and weather conditions, and future studies on the relationship between condition and structural colour as well as the effects of rainfall and temperature on feather growth and microstructure could provide more insight into the factors that can affect structural plumage ornamentation.

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