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Effects of age and weather during moult on mountain bluebird *Sialia currucoides* structural colouration

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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. Several studies have examined how factors such age may influence structural colour, however few studies have looked at how structural colour may be influenced by environmental conditions such as variation in weather conditions experienced during moult. In this study, we examined variation in structural colour expression in relation to age as well as rainfall and temperature during post-breeding moult for a population of mountain bluebirds *Sialia currucoides* in western Canada over nine breeding seasons. Overall, we found structural colouration was explained by sex, age and weather patterns during moult. At a population level, tail and rump feathers from males were more colourful (higher brightness and chroma, hue values shifted more towards UV) than females, and adults were more colourful than juvenals. Male and female rump feathers generally became less colourful with age. Noise-receptor colour models revealed colour differences were discernible among sexes, suggesting bluebird colouration is an important sexual signal. Tail and rump plumage variation was associated with weather during moulting periods, though the effects were sex- and age-dependent. Female plumage was generally more colourful following wetter and warmer early summers, while males were more colourful following warmer late summers, and plumage of older birds was more resilient to colour variation due to weather patterns. We suggest that more rainfall may increase insect abundance and thus improve food intake and overall condition of mountain bluebirds. This is one of the first studies to examine how both age and weather conditions concurrently influence the expression of structural colours in birds.

Keywords: climate, long-term, moult, plumage, structural colour, ultraviolet

Introduction

Colour is an important signaling modality among animals and is used in inter- and intrasexual communication (Bradbury and Vehrencamp 1998). Vibrant colours appear to be driven by sexual selection pressures which can result in conspicuous colouration (Hill 2006a, Robinson et al. 2012); however, a greater degree of ornamentation is often associated with higher physiological costs (Hill 2006b) and may increase the risk of predation (Promislow 1992, Zuk and Kolluru 1998). As a result, exaggerated colouration often acts as an honest indicator of health/condition and overall individual mate quality (Hill 2006a, Weaver et al. 2018, White 2020).

Much research has focused on understanding the factors mediating carotenoid-based plumage colour (Hill and McGraw 2006). Carotenoid feather colouration is closely linked to nutrient access, as ingested carotenoids are directly deposited into feathers or metabolically converted into orange or red pigments and subsequently deposited into growing feathers (Hill and Montgomerie 1994). Less understood however, are factors explaining variation in structurally based plumage (e.g. blue, purple, iridescent colours) because there is not a clear direct link to diet (Hill and McGraw 2006). Structural colours, such as UV-blue coloration, are produced by the arrangement of melanosomes (produced de novo) within the feather microstructure which causes the scattering of light as it passes through nanoscale-level variations in the feather structure (Prum 2006). Feather barbules can contain a matrix of air and biological structures, such as proteins and pigment granules, like melanin, and as light strikes the feather and passes through these biological structures, it encounters different refractive indices, causing light scattering. 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).

Numerous studies have linked structural colours to individual condition (but see exceptions in White 2020), including to diet (Siefferman and Hill 2005, Jacot and Kempenaers 2007, Doyle and Siefferman 2014), immune function (Harper 1999, Doucet and Montgomerie 2003) and reproductive performance (as estimated by moult speed, Griggio et al. 2009). Additionally, structural plumage colour may be influenced by ontogeny, particularly in species that experience delayed plumage maturation, where a significant shift in colour occurs after the birds have undergone their first moult as an adult, typically during their second year of life (Lyon and Montgomerie 1986, Hawkins et al. 2012, Lyu et al. 2015). Even species with a less-stark transition show changes as they age, however, and colour may continue to change after they reach maturity. For example, western bluebird *Sialia mexicana* UV-blue plumage patches were larger and brighter in older males

(Budden and Dickinson 2009) and similarly, both male and female blue tits *Parus major* showed increased structural chroma and brightness values as they aged (Delhey and Kempenaers 2006).

Plumage colour may be influenced by environmental variables such as geography and elevation as well (Hill 1994, Norris et al. 2007, Tisdale et al. 2018). A non-exclusive alternative predicts changes in insect abundance or availability due to weather patterns during moulting periods affects plumage colours (Reudink et al. 2015, Warnock 2017, Laczi et al. 2020). In a study assessing the influence of weather on carotenoid-based plumage in American redstarts *Setophaga ruticulla*, more colourful redstarts were associated with wetter moulting periods (Reudink et al. 2015), while a study of carotenoid plumage in great tits found the opposite effect (Laczi et al. 2020). Eastern bluebirds *Sialia sialis* exhibit more colourful UV-blue plumage following wetter and cooler periods (Warnock 2017). The hypothesized mechanism behind these patterns is variation in insect abundance, as more rainfall would produce higher insect abundances (Studds and Marra 2007), thus increasing the access to nutrients. Alternatively, insects may reduce their activity during cooler periods with more rain, restricting resource availability despite no change in resource abundance for insectivorous birds (Williams 1951, Taylor 1963, Gruebler et al. 2007) or alter foraging behavior in birds (Kennedy 1970, Grubb 1975). Whichever the direction, rainfall and temperature may modulate insect populations (Williams 1951, Taylor 1963, Gruebler et al. 2007, Studds and Marra 2007) thus mediating food resources and therefore individual feather quality during moult by altering feather nanostructure.

Although a recent meta-analysis provides compelling evidence that structural plumage colouration signals individual quality (White 2020), the mechanisms mediating individual variation in structural colouration are poorly resolved. Here, we leverage a long-term dataset to investigate the effect of ontogeny and environmental factors (i.e. weather during moult) on structural plumage colouration using insectivorous mountain bluebirds *Sialia currucoides*. Mountain bluebirds are particularly useful in elucidating the ontogenetic and environmental effects in structural plumage for several reasons: First, mountain bluebirds exhibit UV-blue structural colouration (Johnson and Dawson 2020). Second, mountain bluebird plumage colour appears to be used in mate selection (Balenger et al. 2009; but see Liu et al. 2007, 2009) and subject to sexual selection pressure (Balenger et al. 2009, O'Brien and Dawson 2011, Bonderud et al. 2016), so plumage may signal foraging ability and metabolic performance, which are aspects of avian biology potentially influenced by weather patterns during moult (Kennedy 1970, Grubb 1975, Laczi et al. 2020). Lastly, mountain bluebirds exhibit some age-related plumage variation between younger and older individuals (Morrison et al. 2014), so age may predict yearly colour variation. The main goals of our study were to assess mountain

bluebird plumage differences 1) between sexes, 2) between younger and older individuals, 3) among individuals of different ages and 4) as a function of the combined effect of age, sex and weather patterns during moult. Within these objectives, we predicted 1) males and older individuals would exhibit more ornate plumage (sensu Budden and Dickinson 2009) and 2) more rainfall and cooler temperatures during moult would be associated with increased ornamentation in mountain bluebirds as these condition would be associated with higher insect abundance and activity (Williams 1961).

Material and methods

Study species

Mountain bluebirds are insectivorous birds native to North America that occur in open woodland habitat that nest in secondary cavities and readily make use of nest boxes. Mountain bluebird display sexually dichromatic UV-blue plumage (Johnson and Dawson 2020) on the tail and rump (Fig. 1) and females undergo a shift in colouration as they
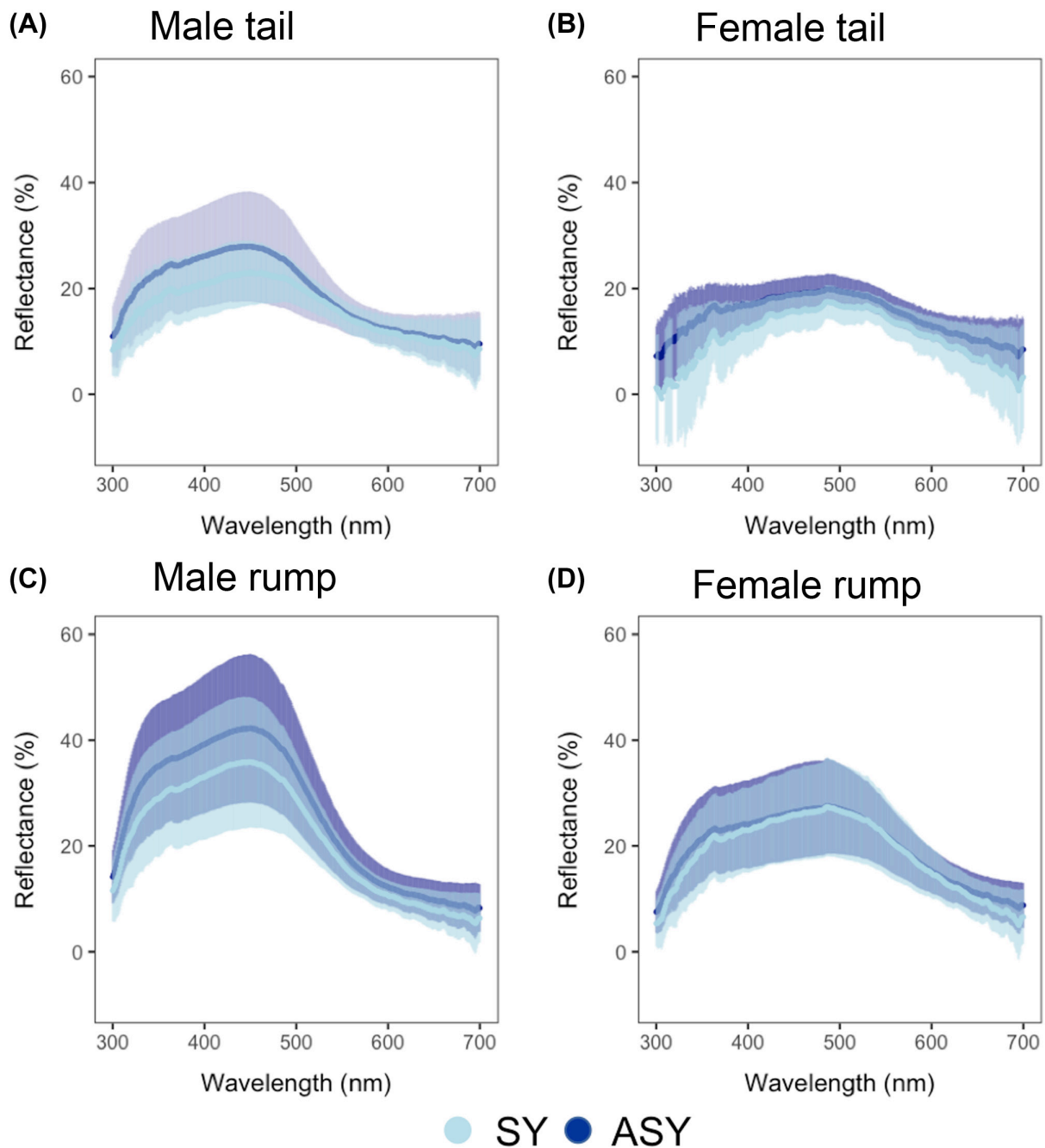

Figure 1. Mean (± SE) reflectance plots of tail feathers (R3) from male (A) and female (B) mountain bluebird *Sialia currucoides* tails (R3) and of rumps from male (C) and female (D) individuals of different ages: second year (SY) and after second year (ASY).

transition from their first breeding season (second year, SY) to subsequent breeding seasons (after second year, ASY; Morrison et al. 2014). Second year (SY) and after second year (ASY) feathers are grown under different developmental conditions: SY tail feathers are grown during a prejuvenal moult (beginning in the nest in late spring/early summer) as opposed to ASY birds who complete a prebasic moult during the post-breeding period in late summer; growth of rump feathers in SY and ASY birds corresponds more closely in time but constitutes the preformative moult for SY birds and the prebasic moult in ASY birds (Johnson and Dawson 2020). Mountain bluebirds are socially monogamous (Morrison et al. 2014), but extra-pair paternity rates are high (72%, Balenger et al. 2009) and males that sire extra-pair young have brighter UV-blue plumage than males with only within-pair young (Balenger et al. 2009, O'Brien and Dawson 2011).

Field methods

Field work was conducted during breeding seasons (May–July) from 2011 to 2019 in Kamloops, British Columbia, Canada (885–1116 m a.s.l.; 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 (Pyle 1997). A bird was classified as ASY1 if it was captured as an SY in the previous season or was captured for the first time in ASY plumage (similar to Marini et al. 2015). 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 relatively high with mountain bluebirds (Johnson and Dawson 2020) and the effects of misclassification (e.g. classifying an ASY2 bird as an ASY1), if any, would weaken any observed effects of age and weather (Marini et al. 2015).

We sampled rump (n = 367) and tail feathers (n = 367) on 306 individuals (n$_{female}$ = 161, n$_{male}$ = 145), as some birds were caught more than once. A rump sample comprised of between five and ten rump feathers collected from each adult, and tail samples comprised of a single tail feather (R3). Feathers were stored in manila coin envelopes prior to analysis. These two feather patches were chosen based on previous work in our population indicating the potential for these patches to convey information on individuals (Bonderud et al. 2016, Morrison et al. 2014).

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 ($R_{300-510}/R_{300-700}$), 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 (following Balenger et al. 2009). 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 a principal component analysis of females and males combined, 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). PC1 for both the tail (67% of variation, eigenvalue = 2.01) and rump (73% of variation, eigenvalue = 2.20) corresponded to increased brightness and chroma, but a decrease in hue (meaning maximum reflectance shifted more towards the UV portion of the spectrum, Fig. 1). Therefore, enhanced or more ornate plumage colouration was defined as brighter, more saturated and more UV-shifted.

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
Tail PC1	2.01	0.67	Brightness	0.52
			UV+blue chroma	0.60
			Hue	−0.61
Rump PC1	2.20	0.73	Brightness	0.47
			UV+blue chroma	0.64
			Hue	−0.61
Tail PC2	0.63	0.21	Brightness	0.85
			UV+blue chroma	−0.42
			Hue	0.31
Rump PC2	0.67	0.22	Brightness	0.87
			UV+blue chroma	−0.23
			Hue	0.44

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 southeast (50.60°N, 120.20°W) was used. We used monthly total rainfall and mean air temperature from June to August (the pre-moulting and moulting period, Pyle 1997) to characterize moulting period weather and standardized weather data using the *scale* function in R prior to analyses (Reudink et al. 2015), which subtracts all values by the mean and then divides by the standard deviation.

Statistical analysis

To test for general ontogenetic effects on structural plumage, we first tested if younger (those classified as SY) and older birds (those classified as ASY) differed in plumage coloration by analyzing tail and rump PC1 values separately using linear mixed effects models in R (<www.r-project.org>). In our models, we included rump or tail PC1 as the response variable and sex, age (SY: $n_{female} = 50$, $n_{male} = 61$ and ASY: $n_{female} = 139$, $n_{male} = 117$), and their interaction as fixed effects. Individual and year were included as random effects. We assessed assumptions of linearity and homoscedasticity by visual inspections of QQ and residual plots.

We then tested for ontogenetic effects on structural plumage within individuals by assessing color changes as individuals aged. Similar to the above analysis, we constructed a linear mixed model using individuals classified by age class (SY, ASY1, ASY2, ASY3, ASY4), with tail PC1 or rump PC1 as a response variable, sex, age class and their interaction as fixed effects and individual and year as random effects.

We assessed visual discrimination of statistical differences using models that correct for avian visual systems (Vorobyev et al. 1998) and by calculating 'just noticeable differences' (JNDs) in the R package *pavo2* (Maia et al. 2019). A JND > 1 indicates that a comparison is distinguishable by birds when viewed under optimal viewing conditions. We first constructed a tetrahedral colourspace model using the *vismodel* function. Although the visual system of mountain bluebirds is unknown, most passerines are ultraviolet sensitive (Ödeen and Håstad 2003), so our models use this visual system. We then used a receptor-noise discrimination model to calculate the photon catch of each cone type used in colour vision for avian vision. Based on a recent review of noise receptor estimates (Olsson et al. 2018), we used a value of 0.1 for colour (chromatic) and luminance (achromatic) discrimination and set the photoreceptor ratios to 1:2:2:4 (ultraviolet, shortwave, mediumwave, longwave) in our models (Maier and Bowmaker 1993). We then calculated bootstrapped (n = 10 000) JNDs and 95% confidence intervals for both chromatic and achromatic contrasts.

To assess how colour variation between sexes and among age classes was explained by weather during moulting periods when feathers were produced, we related rump PC1 and tail PC1 values separately to rainfall and temperature during June, July and August from the previous year using mixed effects models. In each model, we set tail or rump PC1 as the response variable, age, sex, rainfall and temperature as fixed effects, and individual and year random effects. 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 fixed effects (Zuur et al. 2009, Bates et al. 2015). We then undertook model reduction for all mixed models based on the change in Akaike information criterion corrected for small sample sizes (AICc, Burnham and Anderson 2003) between the full model and each reduced model. We considered ΔAIC values within 4 to be competitive and chose our final model based on the lowest AIC (Burnham and Anderson 2003).

Results

Population-level age changes over time (SY versus ASY)

Plumage colouration varied significantly across years in all age and sex classes (Supporting information). Consistent with our predictions, we found males and older birds were generally more colourful than females and younger birds (Table 2). Males expressed higher tail PC1 values relative to females ($F_{1,298.1} = 172.0$, $p < 0.0001$) and SY birds exhibited lower tail PC1 values ($F_{1,345.9} = 28.1$, $p < 0.0001$; Fig. 2). Males also expressed higher rump PC1 values ($F_{1,311.6} = 327.4$, $p < 0.0001$), but there was no difference between SY and ASY

Table 2. Mean and standard deviation (SD) PC1 values for tail and rump colouration on female and male mountain bluebird *Sialia currucoides* summarized by age (SY versus ASY) and by age class (SY, ASY1–ASY4). Sample sizes are indicated.

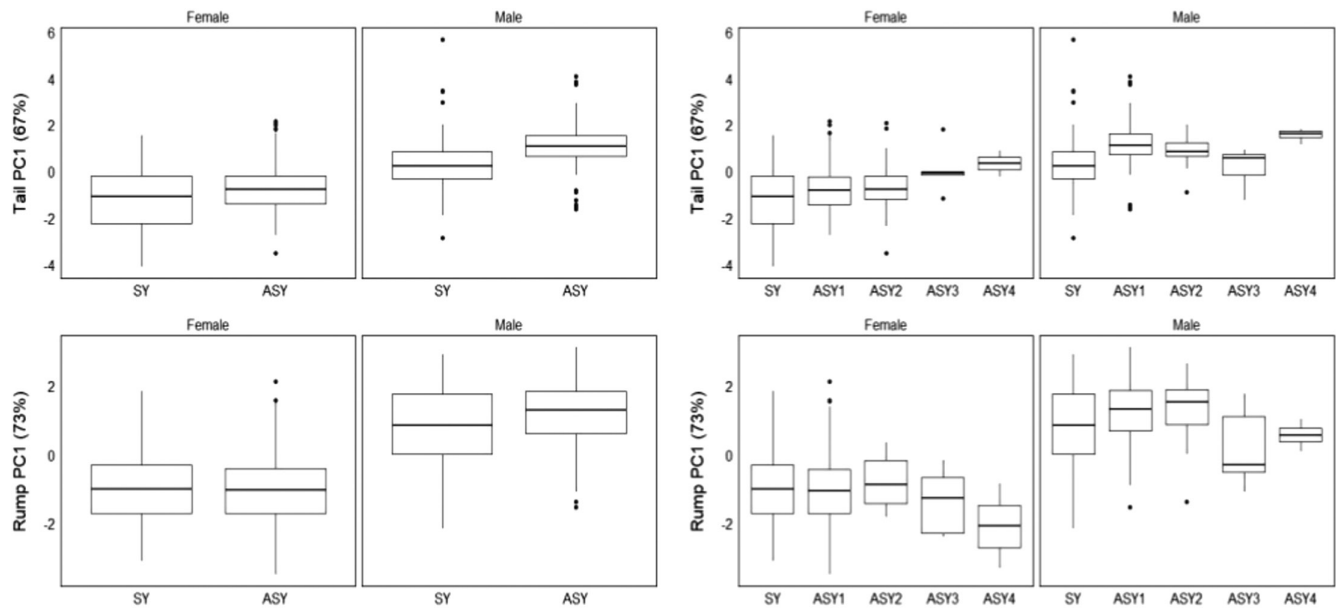|	Sex	Age	Tail PC1	Tail SD	Rump PC1	Rump SD	n
Age	F	SY	−1.20	1.36	−0.90	1.22	50
	F	ASY	−0.65	1.07	−1.00	1.08	139
	M	SY	0.35	1.32	0.76	1.17	61
	M	ASY	1.10	0.94	1.18	0.99	117
Age class	F	SY	−1.20	1.36	−0.90	1.22	50
	F	ASY1	−0.70	1.01	−0.98	1.12	115
	F	ASY2	−0.64	1.39	−0.84	0.71	17
	F	ASY3	0.07	1.09	−1.37	0.98	5
	F	ASY4	0.33	0.79	−2.11	1.75	2
	M	SY	0.35	1.32	0.76	1.17	61
	M	ASY1	1.21	0.96	1.27	0.95	89
	M	ASY2	0.85	0.67	1.29	0.98	16
	M	ASY3	0.19	0.81	0.16	1.08	8
	M	ASY4	1.54	0.29	0.56	0.39	4

Figure 2. Left panels: Boxplots showing mountain bluebird *Sialia currucoides* tail and rump colouration (PC1) for second year (SY) and after second year (ASY) females and males. Right panels: Boxplots showing mountain bluebird tail and rump colouration (PC1) for females and males among age classes (SY, ASY1, ASY2, ASY3, ASY4). Samples sizes are indicated in Table 2. Higher PC1 values represent more colourful tail or rump plumage (i.e. brighter, more saturated and more UV-blue shifted hue).

birds ($F_{1,348.0} = 1.9$, $p = 0.17$; Fig. 2). There was no age × sex interaction on either the tail ($F_{1,347.7} = 0.50$, $p = 0.48$) or the rump ($F_{1,346.6} = 2.0$, $p = 0.16$), indicating the direction of the age effect was similar between males and females (Fig. 2).

Population-level age class changes over time (SY, ASY1, ASY2, ASY3, ASY4)

In support for our predictions, we found bluebird color varied predictably as individuals aged and between sexes (Table 2). On the tail, PC1 values increased with age class ($F_{4,197.1} = 7.9$, $p < 0.0001$) and males averaged higher PC1 values in each age class ($F_{1,348.7} = 28.6$, $p < 0.0001$, Fig. 2). In contrast, rump PC1 decreased as individuals aged ($F_{4,350.9} = 2.8$, $p = 0.03$), but males were again more colourful than females in each age class ($F_{1,350.5} = 101.6$, $p < 0.0001$, Fig. 2). There was no age × sex interaction on either the tail ($F_{4,186.5} = 1.8$, $p = 0.13$) or the rump ($F_{4,350.1} = 1.2$, $p = 0.33$), indicating the direction of the age class effect was similar between males and females (Fig. 2).

Colour models and just noticeable differences (JNDs)

The results from our colour models and JND analyses revealed within-sex differences among ages (e.g. female SY versus ASY and male SY versus ASY) on the tail and rump were likely indistinguishable by birds, as all chromatic and achromatic JNDs were < 1 or confidence intervals overlapped 1 (Supporting information). Because of the weak effects of age, we did not assess JNDs among age classes (SY, ASY1, ASY2, etc.).

In contrast, colour differences among sexes and ages (female SY versus male SY and female ASY versus male ASY) on the tail and rump were likely discernible by birds as all chromatic JNDs were > 1 (Supporting information). However achromatic differences were only discernible between ASY female and males (Supporting information). All chromatic JNDs among ages and sexes (e.g. female SY versus male ASY and male SY versus female ASY) were > 1 on both the tail and rump, but only achromatic comparisons were > 1 between SY females and ASY males on the rump (Supporting information).

Weather effects

To test the combined effects of weather and age on colouration, we first conducted an analysis using models in which birds were classified only as SY or ASY (Supporting information). We found several lines of evidence that weather during moult influenced bluebird colour, but this effect was age- and sex-dependent (Table 3). During all months, tail and rump PC1 was generally higher in males and older individuals than in females and younger individuals (Table 3). Warmer early summers resulted in higher tail PC1 in males and lower tail PC1 in females (Fig. 3; June temperature × sex interaction: $F_{2,15.6} = 5.2$, $p < 0.02$), but warmer late summers resulted in higher female PC1 and lower male PC1 values (Fig. 3; August temperature × sex interaction: $F_{2,11.5} = 5.9$, $p < 0.02$). Early and mid-summer periods that were warmer and wetter, increased younger tail PC1 values (Fig. 3; June rain × age interaction: $F_{2,16.1} = 5.4$, $p < 0.02$; July rain × age interaction: $F_{1,350.0} = 7.50$, $p < 0.006$, July temperature × age interaction: $F_{1,365.6} = 5.59$, $p < 0.02$). Female rump PC1 generally

Table 3. Model results demonstrating the effect of AICc selected fixed effects of age and weather on male and female rump and tail (R3) mountain bluebird *Sialia currucoides* colouration, and variances of the random effects of individual and year. Significant effects are in bold.

	Fixed effect	df	F	p	Individual σ^2	Year σ^2
Tail	Sex	1,249.02	218.41	**< 0.0001**	0.24	0.06
	Age	1,349.24	33.52	**< 0.0001**		
	Sex × June Temp	2,16.07	4.11	**0.04**		
	Sex × June Rain	2,16.91	5.43	**0.02**		
	Age × June Rain	1,360.30	5.66	**0.02**		
	Sex	1,259.69	207.97	**< 0.0001**	0.21	0.06
	Age	1,349.79	30.92	**< 0.0001**		
	July Temp	1,9.76	5.37	**0.04**		
	Age × July Temp	1,365.55	5.59	**0.02**		
	Age × July Rain	1,349.96	7.5	**0.006**		
	Sex	1,242.05	216.37	**< 0.0001**	0.18	0.07
	Age	1,348.55	29.48	**< 0.0001**		
	Sex × August Temp	2,11.9	6.07	**0.02**		
Rump	Sex	1,358.20	430.45	**< 0.0001**	<0.3	<0.8
	Sex × June Rain	2,15.97	7.01	**0.007**		
	Sex	1,248.23	437.47	**< 0.0001**	0.007	0.27
	Sex × July Temp	2,15.08	5.51	**0.02**		
	Sex × July Rain	2,15.7	9.85	**0.002**		
	Age × July Rain	1,357.03	4.14	**0.04**		
	Sex	1,316.92	328.55	**< 0.0001**	0.04	0.25
	Age	1,350.24	3.04	0.08		
	August Temp	1,9.12	0.06	0.8		
	August Rain	1,9.04	0.57	0.5		
	Sex × Age	1,350.05	3.31	0.07		
	Sex × August Temp	1,358.07	11.27	**0.0009**		
	Sex × August Rain	1,353.03	5.08	**0.03**		
	Age × August Temp	1,355.62	0.03	0.87		
	Age × August Rain	1,358.72	3.19	0.07		

increased relative to males during wet summers (Fig. 4; June rain × sex interaction: $F_{2,15.9}=7.3$, p < 0.006; July rain × sex interaction: $F_{2,16.5}=9.3$, p < 0.003; August rain × sex interaction: $F_{1,353.0}=11.3$, p < 0.03). Male rump PC1 generally decreased during warmer summers, while female rump PC1 remained relatively unaffected (Fig. 4; July temperature × sex interaction: $F_{2,15.1}=5.5$, p < 0.02; August temperature × sex interaction: $F_{1,358.1}=11.3$, p=0.0009).

In our second analysis, we used models that assessed the effect of weather among individuals classified in age classes (SY, ASY1, ASY2, ASY3, ASY4, Supporting information). We found further evidence that weather during moult influenced bluebird colour, but this effect was again age class and sex dependent (Table 4). During warmer early summers, female tails across all age classes were less colorful, while male color remained relatively unaffected (Table 4, June temperature × sex interaction: $F_{2,15.6}=5.2$, p < 0.02). In contrast, warmer late summers resulted in higher female tail PC1 values while again male color remained relatively unaffected across all age classes (Table 4, August temperature × sex interaction: $F_{2,11.5}=5.9$, p < 0.02). Early and mid-summer rain generally increased female tail PC1 and decreased male tail PC1 (Table 4, June rain × sex interaction: $F_{2,14.1}=4.2$, p < 0.04; July rain × sex interaction: $F_{2,55.0}=4.5$, p < 0.02), but the direction of this effect varied by age class (Table 4, July rain × age class interaction: $F_{4,212.5}=2.6$, p < 0.04). Early summer rains generally increased female rump PC1 across all age classes but

there was no effect on male rumps (Table 4, June rain × sex interaction: $F_{2,15.9}=7.3$, p < 0.006). Mid-summer rains also affected female but not male rumps (Table 4, July rain × sex interaction: $F_{2,55.0}=4.5$, p < 0.02) but the direction of this effect varied among age classes (Table 4, July rain × age class interaction: $F_{4,212.5}=2.6$, p < 0.04). Male rump PC1 generally decreased across age classes during warmer mid- and late summer periods, while female rump PC1 were unaffected (Table 4, July temperature × sex interaction: $F_{2,15.1}=5.5$, vp < 0.02; August temperature × sex interaction: $F_{2,15.5}=4.9$, p < 0.03).

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 general, we found both ontogeny and weather patterns during moult affected plumage colouration, based on a long-term dataset of structural UV-blue colouration in mountain bluebirds. Males exhibited more colourful tail and rump plumage than female mountain bluebirds, and tail feathers of SY individuals were less colourful than those of ASY birds. Male and female tails generally became more colourful, while rumps became less colourful with age (SY, ASY1, ASY2, etc.), but the direction of the effect varied among age classes. From noise-receptor colour
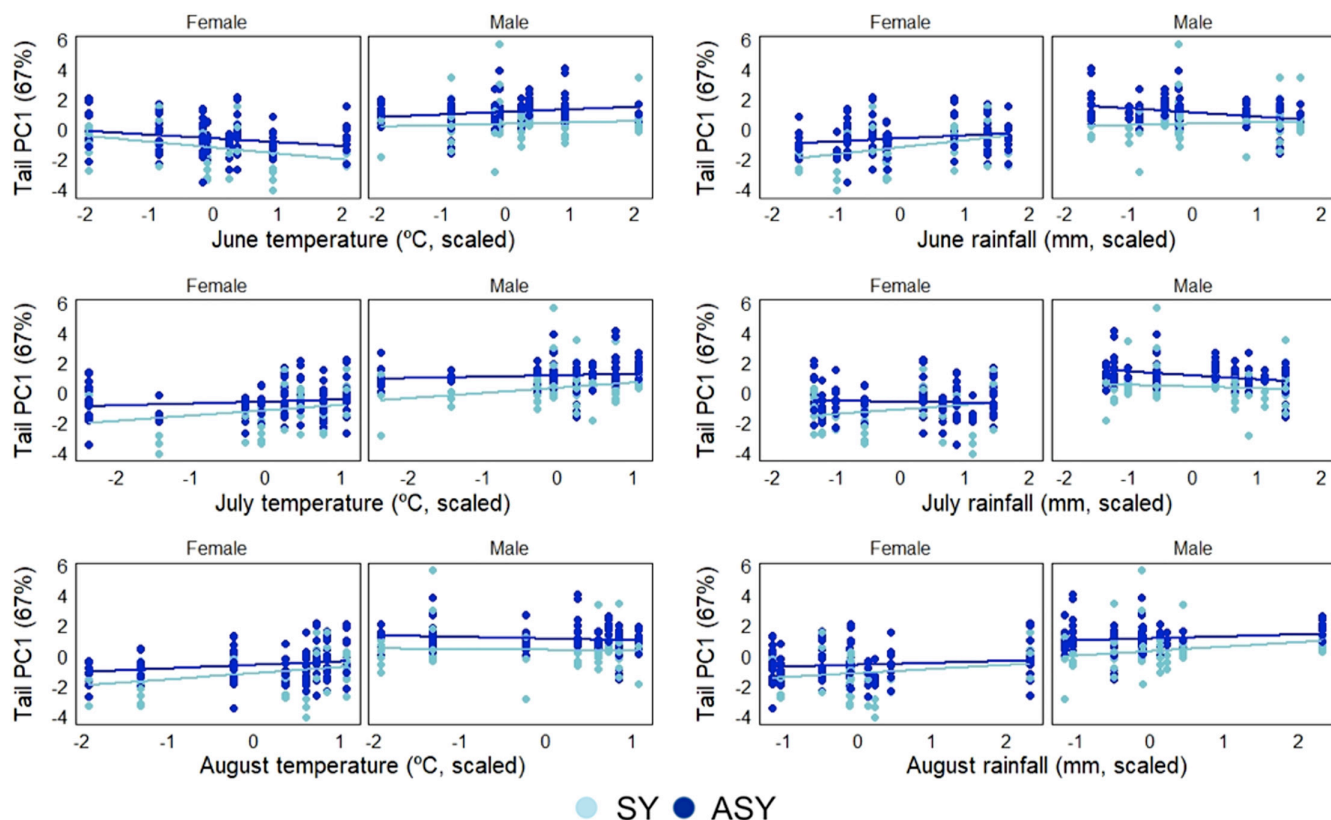
Figure 3. The effect of weather (June–August temperature (°C) and rainfall (mm)) on male and female mountain bluebirds *Sialia currucoides* tail colouration (tail PC1, 67% of variation) in second year (SY) and after second year (ASY) individuals. Samples sizes are indicated in Table 2. Higher PC1 values represent more colourful tail or rump plumage (i.e. brighter, more saturated and more UV-blue shifted hue). Weather data are scaled by subtracting all values by the mean and dividing by the standard deviation.

models, plumage differences among ages were generally discernible between sexes. Finally, we found weather conditions during moult were associated with differences in colour in males and females but these effects were dependent on sex and 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 away from the ultraviolet part of the spectrum, 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 individuals grew their feathers at an earlier time of year, these birds 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 were most UV-shifted just after moult and moved towards longer wavelengths as the year progressed. In our study, the lack of a difference in rump feather colouration between SY and ASY birds was likely because both juvenals and adults moult body feathers in late summer either during the pre-formative (juvenal) or pre-basic (adult) moult. That statistical differences of colour were discernible between sexes, lends support for the hypothesis that colouration is an important sexual signal used in mate selection in mountain bluebirds (Bonderud et al. 2016), however behavioral experiments will be needed to confirm this.

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. 2015). The authors suggested that temperature and rainfall could influence the abundance of the carotenoid-rich insects necessary for producing colourful orange plumage (Reudink et al. 2015). Though structural plumage colouration would not be directly influenced by dietary pigments, temperature and rainfall could still
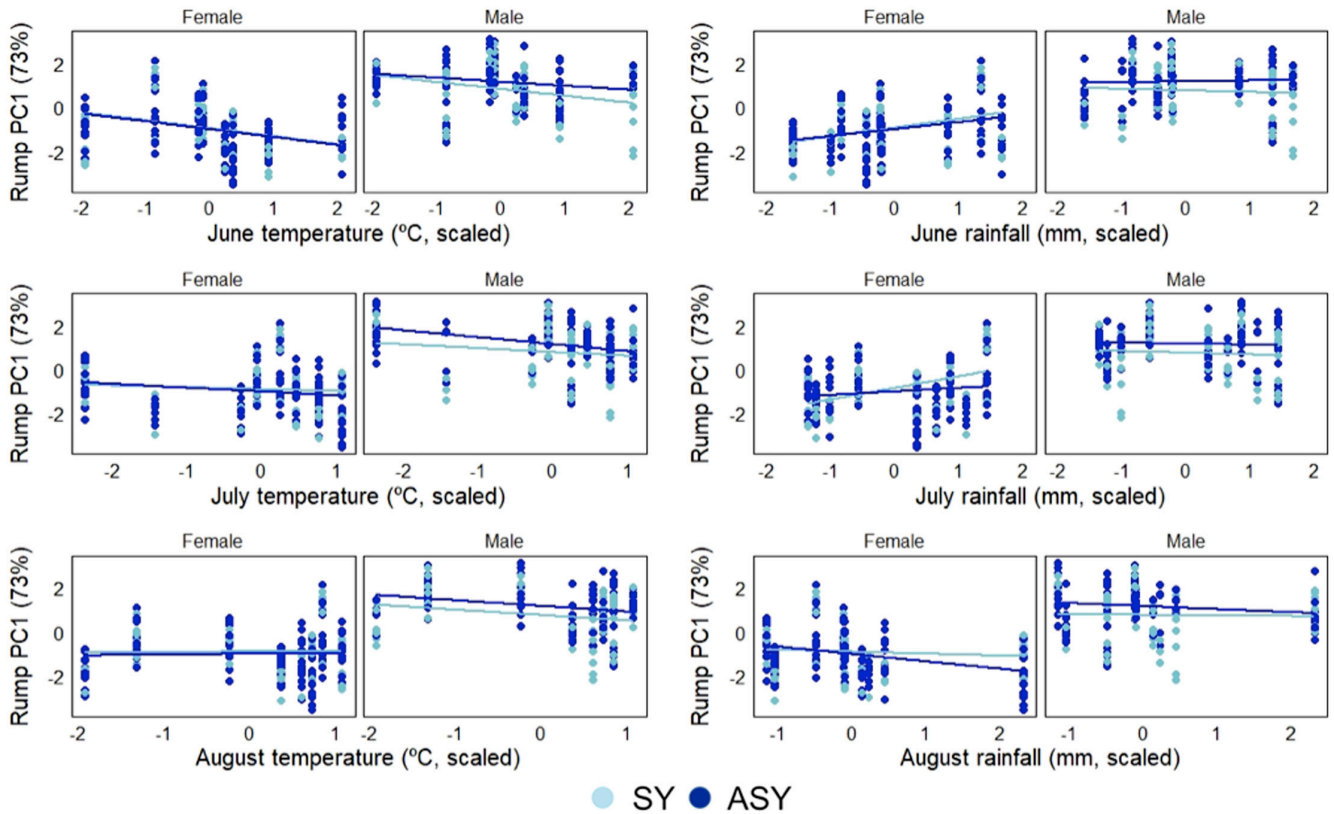
Figure 4. The effect of weather (June–August temperature (°C) and rainfall (mm)) on male and female mountain bluebirds *Sialia currucoides* rump colouration (tail PC1, 73% of variation) in second year (SY) and after second year (ASY) individuals. Samples sizes are indicated in Table 2. Higher PC1 values represent more colourful tail or rump plumage (i.e. brighter, more saturated and more UV-blue shifted hue). Weather data are scaled by subtracting all values by the mean and dividing by the standard deviation.

Table 4. Model results demonstrating the effect of AICc selected fixed effects of age class and weather on male and female rump and tail (R3) mountain bluebird *Sialia currucoides* colouration, and variances of the random effects of individual and year. Significant effects are in bold.

	Fixed effects	df	F	p	Individual σ^2	Year σ^2
Tail	Sex	1,252.37	219.24	**< 0.0001**	0.28	0.06
	Age class	4,162.90	9.11	**< 0.0001**		
	Sex × June Temp	2,15.55	5.15	**0.02**		
	Sex × June Rain	2,14.07	4.22	**0.04**		
	Sex	1,261.97	216.12	**< 0.0001**	0.28	0.07
	Age class	4,177.67	8.47	**< 0.0001**		
	Sex × July Rain	2,54.97	4.54	**0.01**		
	Age class × July Rain	4,212.46	2.64	**0.04**		
	Sex	1,247.10	217.21	**< 0.0001**	0.21	0.07
	Age class	4,195.34	8.23	**< 0.0001**		
	Sex × August Temp	2,11.53	5.86	**0.02**		
Rump	Sex	1,358.18	453.66	**< 0.0001**	0	0.25
	Age class	4,360.14	3.02	**0.02**		
	Sex × June Rain	2,15.92	7.26	**0.006**		
	Sex	1,258.49	438.92	**< 0.0001**	0.1	0.27
	Sex × July Temp	2,15.07	5.46	**0.02**		
	Sex × July Rain	2,16.54	9.23	**0.002**		
	Sex	1,358.10	450.22	**< 0.0001**	0	0.28
	Age class	4,359.92	3.18	**0.01**		
	Sex × August Temp	2,15.52	4.95	**0.02**		

influence nanostructure-based feather colouration directly or indirectly through their effect on food supply and/or physiological stress.

Weather influenced tail and rump colouration, but the overall effects were dependent upon sex and age. In our first analysis, we included age in our models with individuals classified only as SY or ASY. Males generally expressed enhanced tail colour during warmer early summers, while female tails were more colourful during warmer late summers. Younger male birds exhibited enhanced tail plumage following warmer and wetter summers, suggesting plumage colour on older birds may be more resilient to fluctuations in weather patterns. On the rump, female colour increased during wetter summers and male colour increased during warmer summers. This work is generally consistent with previous work examining the effects of rainfall on birds with carotenoid colours: Whereas Reudink et al. (2015) found American redstarts expressed plumage with higher red chroma and lower brightness after years following high July rainfall, Laczi et al. (2020) found 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 during wetter conditions rather than foraging, thus reducing overall food intake by 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 abundance.

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. Although the diet-mediated effects of weather on plumage depend greatly on the foraging ecology of the bird and the effects of weather on insect availability and abundance on specific taxa (Williams 1961, 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 benefiting from increased insect abundance.

In our second analysis examining the effects of weather on colouration, we used birds classified with respect to different age classes (SY, ASY1, ASY2, etc.). We found evidence of weather influencing plumage colour across age classes, but the effects were again sex and age dependent, and our small sample sizes should be taken into consideration. Generally, female tails were less colourful during warmer early summers, while wetter and warmer late summers enhanced female colour but decreased male tail colouration. On the rump, summer rains increased female rump colour, while male rump colour decreased during warmer summers. The apparent effects of weather on male and female plumage colour contrasts with past studies, as most found that UV-blue colour in males was more sensitive to environmental conditions than females (Siefferman et al. 2005, Doyle and Siefferman 2014). Warnock (2017) did find cooler and wetter weather increased rump feather brightness in both male and female eastern bluebirds, however, Siefferman and Hill (2005) found that female eastern bluebirds on restricted diets exhibited duller structural coloration than females on unrestricted diets, suggesting that condition influences structural colouration in both male and female bluebirds. 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 the activity of keratinolytic bacteria could degrade the feathers' nanostructures, thus reducing brightness over the course of the year. In addition, 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 ontogenetic and environmental (weather) processes influencing the expression of structural colouration in mountain bluebirds. Structural colours in mountain bluebirds appear to be strongly influenced by sex and age, and the interaction with weather conditions. The effects of weather on feather colouration were present in both sexes and were dependent on age. Overall, temperature and rainfall appeared to influence feather colour, and we hypothesize insect abundance, mediated by weather during moult, influences body condition. 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.

Author contributions

Genevieve Ward: Formal analysis (equal); Investigation (equal); Methodology (equal); Visualization (lead); Writing – original draft (lead); Writing – review and editing (equal). **Sean Mahoney**: Conceptualization (supporting); Formal analysis (lead); Investigation (equal); Methodology (equal); Project administration (equal); Supervision (supporting); Writing – original draft (supporting); Writing – review and editing (lead). **Steve Joly**: Investigation (lead); Methodology (lead); Project administration (supporting); Writing – original draft (supporting); Writing – review and editing (supporting). **Matthew Reudink**: Conceptualization (lead); Data curation (lead); Formal analysis (supporting); Funding acquisition (lead); Investigation (equal); Methodology (equal); Project administration (lead); Resources (lead); Supervision (lead); Validation (lead); Visualization (supporting); Writing – original draft (equal); Writing – review and editing (equal).

Transparent Peer Review

Data availability statement

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