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Sexual dichromatism, size dimorphism, and microscale anatomy of white wing stripe in blue tits

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Abstract

Achromatic patches are a common element of plumage patterns in many bird species and there is growing body of evidence that in many avian taxa they can play a signaling role in mate choice. Although the blue tit *Cyanistes caeruleus* is a well-established model species in the studies on coloration, its white wing patch has never been examined in the context of sex-specific trait expression. In this exploratory study, we examined sexual size dimorphism and dichromatism of greater covert's dots creating white wing patch and analyzed its correlations with current body condition and crown coloration—a trait with established role in sexual selection. Further, we qualitatively analyzed microstructural barb morphology underlying covert's coloration. We found significant sexual dimorphism in the dot size independent of covert size and sexual dichromatism in both white dot and blue outer covert's vane spectral characteristics. Internal structure of covert barbs within the white dot was similar to the one found in barbs from the blue part that is, with a medullary area consisting of dead keratinocytes containing channel-type ß-keratin spongy nanostructure and centrally located air cavities. However, it lacked melanosomes which was the main observed difference. Importantly, UV chroma of covert's blue vane was positively correlated with crown UV chroma and current condition (the latter only in males), which should be a premise for further research on the signal function of the wing stripe.

Key words: achromatic coloration, blue tit, dichromatism, dimorphism, structural coloration, wing stripe

Achromatic plumage patches are widespread in birds (Hegyi et al. 2008). Usually, by developing against much darker or brightly colored patches, they create contrasting, conspicuous patterns. Although being quite common, white plumage, in comparison to structural or pigment-based types of feather coloration, received so far considerably less attention. One reason may be a relatively simpler mechanism of color production, based on incoherent scattering of incident light on unspecialized unpigmented keratin filaments and air-filled cavities in keratinocytes (Prum 2006). Thus, it is believed that the maintenance of white plumage patches is more costly than

its production. It has been suggested that potential costs of bearing achromatic patches might be associated with its higher sensitivity to abrasion, caused by lack of melanin, which has properties enhancing mechanical rigidity and resistance of the feather (Bonser 1995), and with higher maintenance costs due-to more time-consuming preening (Roulin 2007). In a number of taxa, white ornaments are located at tips of wing or tail feathers, which further expose them to mechanical damage. White patches were also shown to be more susceptible to chewing lice or feather degrading bacteria like *Bacillus licheniformes* (Moreno-Rueda and Hoi 2012). Moreover, bearing bright contrasting patches may be associated with higher detectability and, therefore, an increased risk of predation (Götmark and Hohlfält 1995). Thus, the size of white patches has all properties of an honest signal of individual quality and resistance to ectoparasites (Kose et al. 1999).

In many bird species, the size of achromatic elements has been shown to be under sexual selection (Hill 2006). A prime example might be the pied and collared flycatcher (*Ficedula hypoleuca* and *Ficedula albicollis*), where the size of a white forehead patch constitutes a secondary sexual character in males (Potti and Montalvo 1991; Gustafsson et al. 1995; Robinson et al. 2012). Other species with reported female mate choice based on the white structural ornaments are (reviewed in Hill 2006): the barn swallow (*Hirundo rustica*), black-capped chickadee *Poecile atricapillus*, dark-eyed junco *Junco hyemalis*, and great snipe *Gallinago media*. Sexual selection based on white wing patch was reported by Moreno-Rueda and Hoi (2012) in the house sparrow *Passer domesticus*, and by Hegyi et al. (2008) in the duck's subfamily (*Anatinae*).

Some studies suggest that not only the size of the achromatic ornament but also its spectral characteristics might matter in female mate choice, as keeping feathers clean and in good condition also requires an effort (Hill 2006). Male black-capped chickadees *P. atricapillus* with brighter white cheeks were reported to have higher reproductive success, and higher proportion of within-pair offspring in their nests (Doucet et al. 2005) compared with duller ones. Brightness of breast plumage, ranked by human observers, was described to be a trait of female preference in Northern Pintails *Anas acuta* in the study of Sorenson and Derrickson (1994). However, not only brightness, but also UV-chroma of achromatic patches might be correlated with reproductive success. In pied flycatcher, *F. hypoleuca* adult males exhibited higher UV reflectance of a white breast than females and yearling males (Siitari and Huhta 2002). Moreover, males with higher UV reflectance of the white forehead and mantle were reported to arrive earlier at the breeding sites, which is a good predictor of their breeding success (Siitari and Huhta 2002; Lehtonen et al. 2009; Sirkia and Laaksonen 2009).

The blue tit *Cyanistes caeruleus*, thanks to its conspicuous, vivid coloration, is an important model species in the studies on bird coloration. However, majority of research so far was devoted to carotenoid-based and structural coloration, usually of yellow breast and blue crown feathers, respectively, whereas neglecting achromatic patches. The notable exception is study of Griggio et al. (2009), where white cheeks of adult blue tits were shown to be sexually dichromatic. The quality of cheek feather coloration was recently investigated in the context of carry-over effects (in the study of Badás et al. 2018), and extra-pair paternity (Badás et al. 2020). Besides the cheeks, other white patches in blue tit plumage are: forehead, wing stripe on greater coverts, tips of tertials, and nape patch, among which only the forehead patch was reported to be sexually dimorphic (Hunt et al. 1998). It is surprising that other achromatic patches did not receive much attention so far, especially taken their

contrasting appearance and possible importance during courtship display (Stokes 1960). The wing stripe is formed by white dots on the tips of greater coverts and creates a very conspicuous patch against cobalt-blue feathers. During courtship, a male blue tit performs so-called "moth flights" and dance that consist spreading and shaking its wings to attract a female, making the white stripe clearly visible (Stokes 1960). Based on such observations, we presume that this patch may potentially have a signaling function.

In this explorative study, we investigate the presence of sexual dichromatism and size dimorphism in white dots forming the wing stripe in adult blue tits (Figure 1A,B). According to the redundant signal hypothesis (Møller and Pomiankowski 1993), a combination of several patches, each correlated with individual condition, can provide a female with a more complete evaluation of a male's
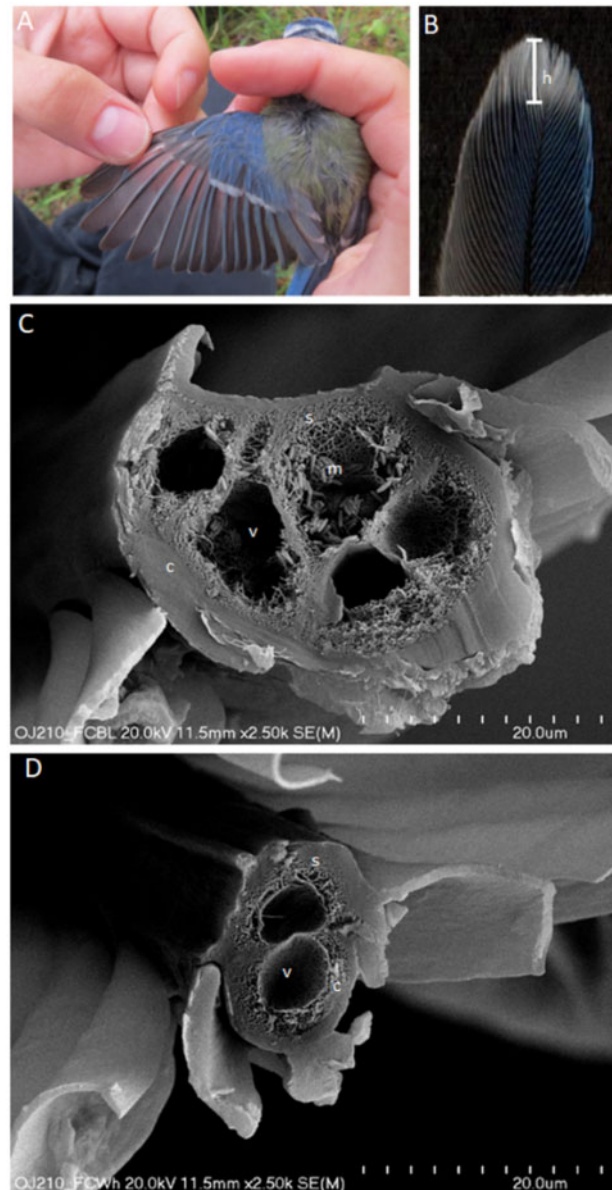

Figure 1. (A) Adult blue tit wing patch (fot. D. Lutyk). (B) A close-up of the upper part of the covert, with dot height marked. (C and D) Scanning electron micrographs of the greater coverts barb's cross-section, from the blue part of the feather (C) and from the white dot (D), showing keratin cortex (c), spongy structure (s), air vacuoles (v), and melanosomes (m).

quality. Thus, we examine interrelations between the reflectance properties and size of covert dots and traits considered to signal individual quality in the blue tit: residual body mass as a proxy of current condition (Hegyi et al. 2019) and the coloration of crown feathers. Although still debated (see critical meta-analysis of Parker 2013), among all blue tit ornaments, the largest number of premises as to the role in sexual selection was gathered for crown feathers (e.g., Andersson et al. 1998; Hunt et al. 1998; Sheldon et al. 1999; Griffith et al. 2003). In many passerine species, there is a tendency for older birds, especially males, to produce more elaborate plumage (Delhey and Kempenaers 2006). Thus, we also verify the presence of age-related differences in dot size parameters.

On the mechanistic level, considering the process of color production, there are known cases of white ornaments evolved from feathers with plesiomorphic non-iridesent UV-blue coloration. In such cases, (e.g., in the snowy-capped manakin *Lepidothrix nattereri* and the white-fronted manakin *Lepidothrix serena*) the quasi-ordered spongy keratin structure is present in the medullary part of the feather barbs, but is devoid of melanin granules, which results in white coloration (Prum 2006). As a second goal of our study, to verify whether achromatic blue tit covert dots share structural properties with the chromatic feather parts, we qualitatively compare microscale barb morphology from the area of white dots with the structure of the blue part of covert feathers, using scanning electron microscopy (SEM).

Materials and Methods

Feather samples collection and measurements

We used feather samples collected in 2015 and 2016, from adult blue tits of the nest-box population inhabiting Swedish island of Gotland (57°01′N, 18°16′E). Adult birds were caught using mist nets, at the end of the nesting period, not earlier than 14 days after hatching of the chicks. Sex and age of individuals were assigned based on the presence of a brood patch and the molt limit present in yearlings between the primary and greater coverts, respectively (Svensson 1992), and body mass, tarsus length, and wing length measurements were also taken. About a dozen of crown feathers and a single second greater covert on the right wing were plucked from each individual. Covert feathers were preserved in parchment envelopes, whereas crown feathers were placed on black paper with transparent double-sided adhesive tape. The feathers were stuck to the tape in 1 bundle, so that their arrangement maximally resembled that on a bird's body, and the background did not show through from underneath. In total, we collected samples from 271 females and 248 males (307 birds in 2015 and 212 in 2016, respectively). Among birds caught in 2016, 45 were re-traps from the previous year.

The length of greater coverts was measured with a digital calliper to the nearest 0.1 mm (Table 1). Subsequently, feathers were placed on black cardboard and scanned to 300 dpi JPG files together with pieces of graphing paper for scale. Height of white dots was measured in freeware ImageJ (11.52a). Reflectance of coverts and crown feathers in the 300–700 nm range was measured with an Ocean Optics JAZ Spectrophotometer, coupled with a xenon pulsed light source and bifurcated probe with $6 \times 400\,\mu m$ illuminating fibers and 1 read fiber held perpendicularly to the sample (Ocean Optics, Dunedin, FL). We took 5 measurements of the white covert's dot and 5 measurement of the blue part of outer vfane. Each crown feathers' sample was measured 10 times (for more details on measurement see Janas et al. 2018). The spectra were further processed,

smoothed, and averaged, using the *pavo* package (Maia et al. 2019). To quantify coverts' and crown feathers' coloration, "brightness" and "UV chroma" were calculated as sum of reflectance values over all wavelengths, and total reflectance in the region between 320 and 400 nm divided by brightness, respectively. Following Mennill et al. (2003), we calculated "achromatic contrast" between white covert's dot and blue part of the feather, as the absolute value of the difference between the brightness of both spots.

To estimate the overlap between males and females' covert colors we applied the avian tetrahedral color space model (Stoddard and Prum 2008; Maia et al. 2019), by using the *vismodel* function, that allows for including sensory phenotype of the blue tit (argument *visual* set to "bt"). This model allows for representing each reflectance measurement as a point in tetrahedral space, whose vertices correspond to 4 types of cones in avian retina (for more detailed description see Stoddard and Prum 2008). Using the *voloverlap* function we calculated the volumes occupied by each sex, separately for white dots and blue outer coverts' vanes (Maia et al. 2019). The percentage of the volume overlap was calculated in relation to the convex hull of lower volume (males in both analyzed patches).

SEM

To characterize the covert's barbs microscale morphology, we applied SEM. For this analysis, we used feather samples from 10 individuals (5 males and 5 females), randomly drawn by a person not involved in the research from among samples from both studied seasons. Feather cross-sections were made under binocular; a cut within the white dot was made perpendicular to the rachis ∼0.5 mm from the feather's tip; a cut within the blue part of the outer vane was made perpendicular to the barbs, starting at ∼3 mm from the feather's tip. Cropped fragments were placed on a graphite block covered with carbon adhesive tape and double-coated with gold. The micrographs were made on a cold field emission Scanning Electron Microscope HITACHI S-4700 at magnification of 2,500×. We have chosen 2 micrographs from each patch sample, counted the number of air vacuoles and measured the diameter and area of cross-section in the ImageJ software (Rasband 2004; Schneider et al. 2012).

Table 1. (A) Sexual size dimorphism in covert's morphological parameters and (B) white dot and blue vane barb's microstructure basic parameters The table shows mean and standard deviations of measurement within sex

Trait	Mean ± SD	
	Males	Females
A.		
Covert length (mm)	19.81 ± 1.00	18.89 ± 0.98
Dot height (mm)	1.98 ± 0.43	1.8 ± 0.44
B.		
White dot barb cross-section		
diameter (μm)	24.07 ± 46.28	23.80 ± 2.39
area (μm²)	267.02 ± 46.28	262.66 ± 47.95
number of vacuoles	2.95 ± 0.60	3 ± 0.63
Blue vane barb cross-section		
diameter (μm)	40.40 ± 4.23	40.32 ± 4.24
area (μm²)	727.06 ± 70.96	712.32 ± 71.92
number of vacuoles	4.56 ± 0.86	4.39 ± 0.92

Statistical analysis

All covert's parameters, as well as body mass, tarsus length, wing length, and crown color metrics were normally distributed. Residual body mass, that can be treated as a proxy of current condition (Hegyi et al. 2019), was calculated as residuals from body mass regressed against the tarsus length. In 2016, we caught 45 birds, that were "re-traps" from the previous season, thus we intended to include the ring number as a random term in the models. However, low proportion of re-traps in our dataset caused problems with model convergence. For this reason, we removed records from "re-trapped" birds from the 2016 data set, which—given that they constituted 8.67% of total sample size—should not affect the results. To test for sex differences in the dot height and color metrics, general linear models were applied. Before the analysis, all models were inspected for normality and homoscedasticity of residuals. The model analyzing dimorphism of dot size (height) included fixed factor of sex, individual age (second calendar year or older), and the year of study as categorical predictors and covert's length as a continuous predictor (the latter to account for potential influence of feather size). Initial model also tested for interaction between covert's length and sex, but it was not significant and, therefore, removed from the model. To aid in interpretation of effect sizes between different response variables color metrics in this and all further statistical analysis were scaled to zero mean and unit standard deviation (mostly due to very high values for brightness, which is measured on strikingly different scale than other variables). The models testing for differences in covert's and crown color metrics (with the following metrics as dependent variables: dot brightness, dot UV chroma, blue vane brightness, blue vane UV chroma, crown brightness, and crown UV chroma) included sex, age, and year of study as categorical predictors and dot height to account for relation between patch size and its reflectance properties. Initial models also tested for an interaction between dot height and sex, which occurred to be significant only in one of the models, whereas it was removed from the others.

To explore the network of relationships between the color variables, dot size, and the index of current condition, we applied the structural equation modeling (a type of path analysis), using the *lavaan* package (Rosseel 2012). The path analysis allows for exploring causal relationships and covariances between analyzed variables; it also enables 1 variable to simultaneously serve as an explanatory or a response variable, depending on its relationship with other variables. In the first step of the analysis, we built 2 analogous sets of 6 models (Supplementary Tables S2 and S3; Supplementary Figures S1 and S2), separately for brightness and UV chroma metrics. In each model, we included the index of current condition as the main predictor for color variables. In most models (apart from the first and the third one), we also included dot height as a predictor of its color metrics. The models also included the color metric of the blue part of the covert's vane as a predictor of the respective dot color metric. In Model 5, we included the covariance between blue vane and crown color metric, whereas in all remaining models, the required covariances were automatically generated by the package *lavaan*. To select the best fitting models, we compared them using a set of goodness-of-fit indices including: the χ^2 statistic, the comparative fit index (CFI), the Akaike information criterion (AIC), and the root mean square error of approximation (RMSEA). A non-significant value of χ^2 is generally considered to signal a good fit of the model, yet the large sample size is known to considerably reduce the credibility of this indicator (Tabachnick and Fidell 2007; Roff and Fairbairn 2007). For this reason, as the main criterion for

Table 2. Results of linear models analyzing dimorphism in dot height (A) and dichromatism of dot and blue part of covert vane and crown feathers (B). (A) The model included dot height as a dependent variable, covert length as a continuous variable and sex, age (second calendar year or older), and year of study as a categorical predictor. (B) The models included color metric: brightness or UV chroma of dot or blue part of the vane as a dependent variable. Dot height was treated as a continuous predictor (in part B of the table), and sex, age (second calendar year or older), and year of study as a fixed categorical predictor. Color metrics were scaled to zero mean and unit standard deviation. Significant values are marked with the asterisks (*$P < 0.05$, **$P < 0.01$, *** $P < 0.001$).

	Estimate	SE	t	P
A.				
Dot height				
Intercept	1.335	0.427	3.131	0.002**
Covert length	0.028	0.023	1.247	0.213
Sex	0.146	0.043	3.414	< 0.001***
Age	0.052	0.039	1.330	0.184
Year	−0.211	0.039	−5.356	<0.001***
B.				
Dot brightness				
Intercept	−2.313	0.308	−7.510	<0.001***
Dot height	1.116	0.162	6.909	< 0.001***
Sex	1.205	0.407	2.962	0.003**
Age	0.057	0.089	0.635	0.526
Year	0.316	0.094	3.350	<0.001***
Dot height*sex	−0.547	0.210	−2.610	0.009**
Dot UV chroma				
Intercept	0.296	0.193	1.533	0.126
Dot height	−0.468	0.096	−4.851	< 0.001***
Sex	1.078	0.079	13.595	< 0.001***
Age	0.001	0.001	0.009	0.993
Year	0.221	0.085	2.605	0.009**
Blue vane brightness				
Intercept	0.218	0.230	0.948	0.343
Dot height	−0.049	0.115	−0.424	0.672
Sex	−0.281	0.094	−2.977	0.003**
Age	−0.009	0.095	−0.099	0.921
Year	0.037	0.101	0.367	0.714
Blue vane UV chroma				
Intercept	−0.826	0.132	−6.250	< 0.001***
Dot height	−0.048	0.066	−0.729	0.466
Sex	1.630	0.054	30.079	< 0.001***
Age	0.182	0.055	3.330	< 0.001***
Year	0.183	0.058	3.162	0.002**
Achromatic contrast				
Intercept	−1.957	0.244	−8.009	< 0.001***
Dot height	0.850	0.122	6.971	< 0.001***
Sex	0.449	0.100	4.480	< 0.001***
Age	0.074	0.101	0.733	0.464
Year	0.268	0.107	2.502	0.013*
Crown brightness				
Intercept	−0.342	0.216	0.092	0.115
Dot height	−0.031	0.110	−0.287	0.775
Sex	0.838	0.089	9.440	< 0.001***
Age	0.161	0.089	1.804	0.072
Year	−0.175	0.092	−1.892	0.059
Crown UV chroma				
Intercept	−0.762	0.170	−4.476	0.000***
Dot height	−0.084	0.086	−0.969	0.333
Sex	−0.084	0.070	19.118	< 0.001***
Age	0.425	0.070	6.048	0.000***
Year	0.293	0.073	4.041	0.000***

selecting the best models, we adopted AIC values, with the help of CFI (that is supposed to signal an acceptably good fit if tis value is >0.95) and RMSEA (with values <0.06 considered as a good fit). Fit indices for all tested models are presented in Supplementary Tables S2 and S3 in the Supplementary Materials. In the second step of analysis, to verify whether the obtained pattern of relationships is sex-specific, we run the best-fitted model for each color metric, separately on the subset of males ($N = 210$) and females ($N = 214$). Path diagrams were prepared in the package *lavaanPlot* and further processed using the Adobe

Illustrator CC version 6.0 software. All statistical analyses and graphs were done in R (version 3.6.0, R Core Team 2020).

Results

Covert's dot size dimorphism and dichromatism

The height of covert's white dot was significantly larger in males (Table 2A and Figure 2A,B), which (taken the arrangement of dots
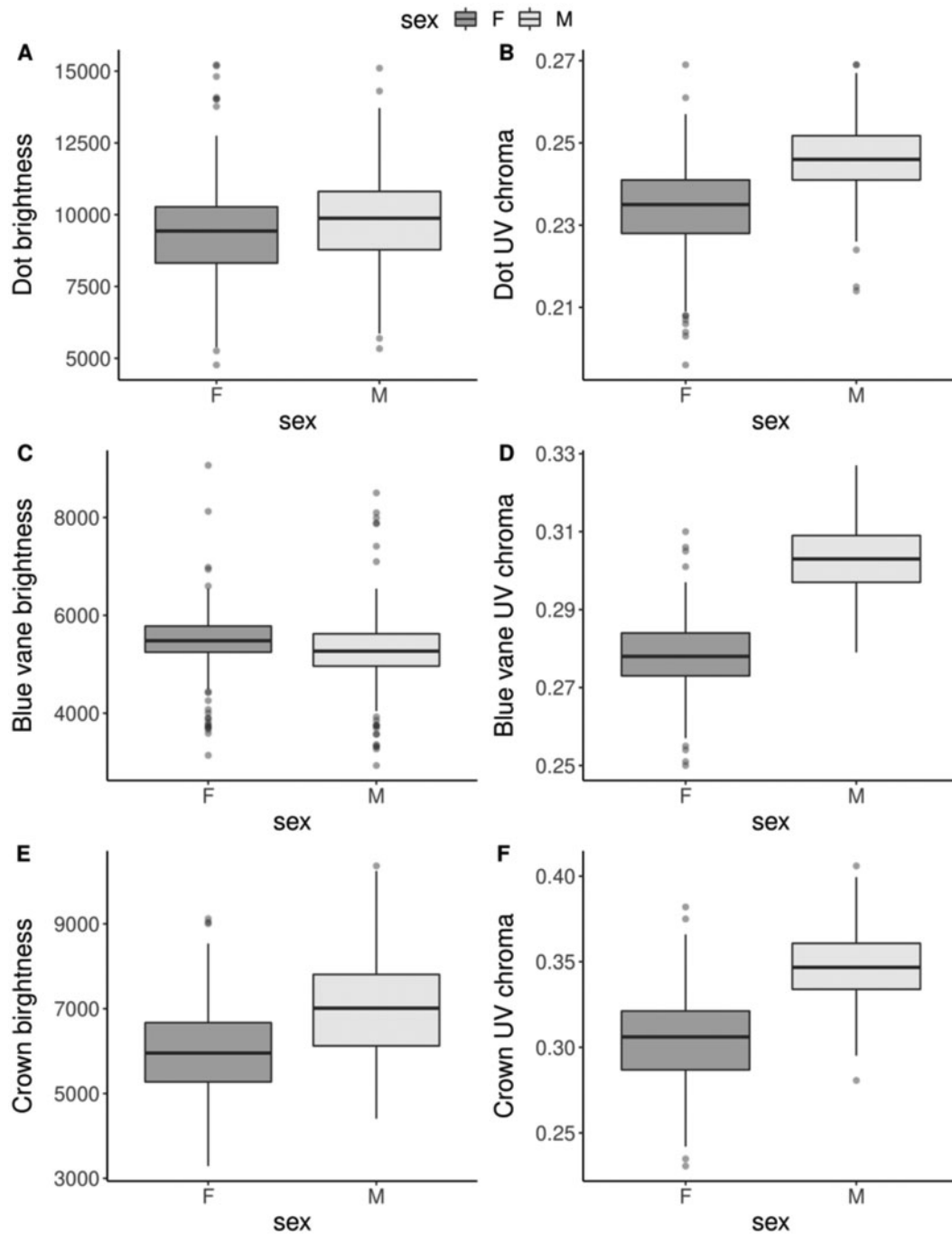

Figure 2. Box-plot showings sexual dichromatism in dot brightness (A), dot UV chroma (B), brightness of blue outer vane (C) and UV chroma of blue vane (D), crown brightness (E), and crown UV chroma (F). Horizontal bars indicate medians, lower and upper bounds indicate, respectively, first and third quartiles and whiskers indicate minimum and maximum values. Males and females are marked with light and dark grey cooler, respectively.

on the wing) should translate into sexual dimorphism in the width of the white wing stripe. Dot height was independent of the covert's length (Table 2A) and not differed between age classes (Table 2A). Brightness and UV chroma were both significantly higher in males within the white dot. In the blue part of the outer vane, brightness was higher in females whereas UV chroma in males (Table 2B and Figure 2C,D). Achromatic contrast between the dot and the vane was significantly higher in males and was positively correlated with the size of the dot (Table 2B). Covert's dot brightness was positively related to the dot height, whereas relationship between the UV chroma and the dot height was negative (Table 2B). Differences between age classes were present in the UV chroma of the blue part of the covert's vane and the crown's UV chroma, with older individuals expressing higher values of both metrics (Table 2B). However, the interaction between sex and age in those patches was not significant.

The extent to which colors of males and females overlap in the avian tetrahedral color space model (Stoddard and Stevens 2011) might be an indicator of the degree of sexual dichromatism—the smaller is the overlapping fraction of volume of both solids, the greater is the dichromatism between analyzed groups. In the case of UV-blue outer vane coloration, the convex hulls overlap was 41.06% of the volume occupied by the males only (Figure 3A). For white dots, the volume overlap was higher and amounted to 65.48% in relation to volume occupied by males (Figure 3B). However, it should be noted that the color parametrization used to construct convex hulls does not take into account the differences in brightness. Therefore, despite the partial volume overlap in the
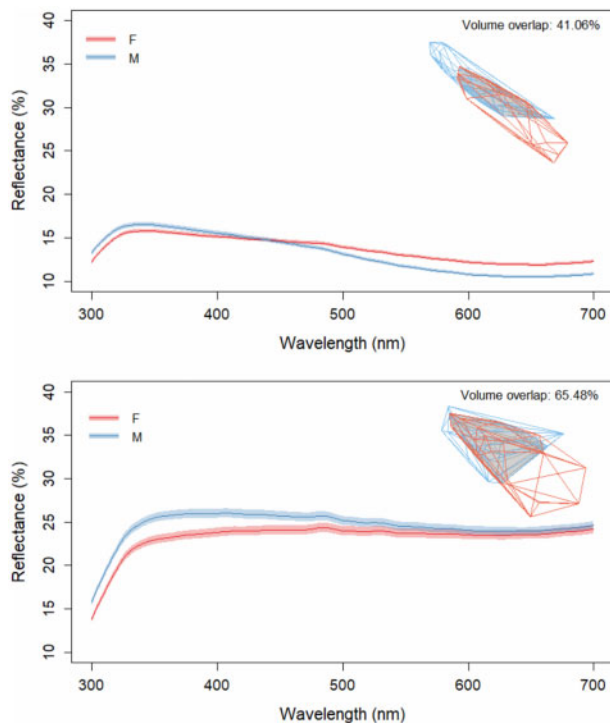

Figure 3. Reflectance spectra of blue tit covert's outer vane (A) and white dot (B). Blue and red lines denote, respectively male and female mean reflectance, whereas shading indicates 95% confidence intervals. The pictures in the top right corner represent convex hulls volume overlap from the tetrahedral color space model, with overlap percentage calculated in relation to lower volume convex. The hulls marked in blue and red represent respectively males' and females' measurements. Grey area represents the volume common for both convex hulls.

tetrahedral color space, we can conclude that both white dot and blue part of the outer vane are significantly sexually dichromatic patches.

Path analysis

In the set of models accounting for UV chroma color metrics, Models 2 and 5 had the fit, with indistinguishable fit indices (i.e., with AIC = 3117.38, CFI = 0.986, and RMSEA = 0.092). We preferred Model 2 as being more parsimonious (Supplementary Table S2). After running Model 2 separately on the subset of males and females, we found a sex-specific relationship between the current condition index and the blue vane's UV chroma that was significant only in males (Table 3 and Figure 4). The relationships between the current condition and the UV chroma of all 3 analyzed plumage components in females were not significant. In both sexes, we found strong negative associations between the dot height and the dot UV chroma (Figures 4 and 5B). The covariances between the UV chroma of crown feathers, the covert's white dot, and the blue vane were all positive and significant.

Among the models focused on brightness metrics (Supplementary Table S3), the best fit came from Model 4 (with AIC = 3468.411, CFI = 0.971, and RMSEA = 0.05). In both sexes, neither of the paths linking the current condition index and the color metrics were significant (Table 4). However, we found a strong positive association between the dot height and its brightness, present in both males and females (with a slightly larger estimate in the latter). The blue vane's brightness significantly predicted dot brightness in both sexes, whereas the covariance between crown and white dot brightness was not significant.

Microstructure

The barbs in the blue part of the covert consisted of dead keratinocytes in the medullary area, with channel-type ß-keratin spongy nanostructure, dense layer of melanin granules and 4–6 centrally located air vacuoles (Figure 1C). Thus, the mechanism of the structural color production in this patch is analogous to the 1 found in the blue tit crown and tail feathers (Hegyi et al. 2018; Janas et al. 2020). Mean diameter and area of barb cross-section in the blue part of covert was 40.45 μm and 719.55 μm^2, respectively, whereas in the white part those values were markedly lower: 24.07 μm and 267.02 μm^2. Mean and standard deviations of covert barb parameters, averaged within sex, are shown in Table 1B. Barb cross-sections from the white dots completely lacked melanin granules and had smaller number of keratinocytes in the medullary part, with 2–4 air vacuoles surrounded by thin, but well developed ß-keratin spongy nanostructure (Figure 1D).

Discussion

Here, we provide the first evidence for sexual dimorphism in the plumage patch size formed by achromatic dots on greater coverts, creating a white wing stripe in the blue tit. We also show sex-related dichromatism in both achromatic dot and adjacent blue region of the coverts. The height of white dots was significantly larger in males, regardless of the length of the coverts, indicating the detected dimorphism is independent from the sexual body size dimorphism. Further, despite appearing monochromatic to the human eye, we spectrophotometrically revealed that white dots of males are both brighter and more UV chromatic than those of females. The same held true for the UV chroma of the adjacent blue vane, yet the

Table 3. Full results of the best fitted structural equation model for the color metric UV chroma, analyzed separately on the subset of males ($N = 210$) and females ($N = 214$)

		Estimate	SE	z-value	P(>|z|)
Males					
Regressions:					
Crown UV chroma ~	Condition	0.133	0.124	1.073	0.283
Blue vane UV chroma ~	Condition	0.339	0.122	2.781	0.005[**]
Dot UV chroma ~	Condition	0.15	0.117	1.276	0.202
	Dot height	−0.529	0.135	−3.903	< 0.001[***]
Covariances:					
Crown UV chroma ~~	Blue vane UV chroma	0.312	0.072	4.368	< 0.001[***]
	Dot UV chroma	0.133	0.066	2.013	0.044[*]
Blue vane UV chroma ~~	Dot UV chroma	0.363	0.069	5.242	< 0.001[***]
Variances:					
Crown UV chroma		1.005	0.098	10.247	< 0.001[***]
Blue vane UV chroma		0.972	0.095	10.247	< 0.001[***]
Dot UV chroma		0.9	0.088	10.247	< 0.001[***]
Females					
Regressions:					
Crown UV chroma ~	Condition	0.192	0.131	1.473	0.141
Blue vane UV chroma ~	Condition	0.1	0.131	0.764	0.445
Dot UV chroma ~	Condition	0.086	0.126	0.68	0.497
	Dot height	−0.633	0.157	−4.022	< 0.001[***]
Covariances:					
Crown UV chroma ~~	Blue vane UV chroma	0.275	0.071	3.854	< 0.001[***]
	Dot UV chroma	0.207	0.068	3.06	0.002[**]
Blue vane UV chroma ~~	Dot UV chroma	0.473	0.074	6.39	< 0.001[***]
Variances:					
Crown UV chroma		1.001	0.097	10.344	< 0.001[***]
Blue vane UV chroma		1.012	0.098	10.344	< 0.001[***]
Dot UV chroma		0.938	0.091	10.344	< 0.001[***]

The table shows estimates, standard errors, z-values and P-values. Regression is marked with "~" and covariances with "~~". Significant values are marked with the asterisks (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

brightness was higher in females. Thus, by describing this new dichromatic region, our results expanded those of Hunt et al. (1998), who reported 5 dichromatic plumage regions of the blue tit (blue and white part of crown, nape, tail, and back feathers), and the later study of Griggio et al. (2009) demonstrating dichromatism in cheek feathers.

Wing stripes are predominantly thought to play a role in social communication (e.g., Beauchamp and Heeb 2001) and so far only in siskins *Carduelis spinus* has the size of such a patch been shown to be a sexually selected trait in a mate choice experiment (Senar et al. 2004). The benefits for the female seem clear in the siskin, as previous studies have shown that the size of a yellow wing stripe in this species (with coloration generated by deposition of carotenoids) reflects male's foraging abilities. However, regardless of the color production mechanism, the presence of sexual dichromatism might indicate that a given trait is a sexually selected ornament (Delhey and Peters 2017). Moreover, the courtship behavior of the blue tit (described in details in Stokes 1960, see also an example under this link to a video record: https://bit.ly/BTCourtship), includes moth flight and dance, during which a male spreads its wings and shakes them with great frequency, thereby making the white stripes clearly visible to the female. Thus, the dichromatism we found in covert's coloration, together with the characteristic courtship behavior, give the ground to investigate whether white wing stripe in the blue tit might have a signaling function in a mate selection.

Among the blue tit plumage patches, the crown is most often considered as a sexually and socially selected trait (e.g., Sheldon et al. 1999; Griffith et al. 2003; Alonso-Alvarez et al. 2004), although Parker (2013) in his critical meta-analysis showed that these assumptions require further research. We found that the UV chromas of greater covert's white dots and blue vanes were highly associated with the UV chroma of crown feathers in both males and females (Table 3 and Figure 5). On the other hand, no significant associations were found between the brightness of the covert's characteristics and crown feathers. We presume that the UV chroma of other dichromatic traits of the blue tit—the tail and cheek feathers –, might also covary with the crown's and greater covert's UV chromas. To our knowledge, no study so far examined these associations, which might give grounds to further exploration of the evolution of multiple color ornaments in this species (Møller and Pomiankowski 1993).

Crown coloration in the blue tit is known to be more elaborate in older individuals (Andersson et al. 1998; Örnborg et al. 2002; Delhey and Kempenaers 2006), yet there are population-specific differences as to which of the color components (UV chroma or brightness) differ between age-classes. In line with Delhey and Kempenaers (2006), in our population older birds expressed higher UV chroma of the crown and blue vane of greater coverts, regardless of their sex. A similar tendency was also observed in the bluethroats (*Luscinia svecica*), where older males express higher UV chroma of structural blue throat patch (Johnsen et al. 2001). Given that in passerines age-related differences in trait expression are often present in secondary sexual traits (Delhey and Kempenaers 2006), the pattern we found in the UV chroma of the blue part of greater
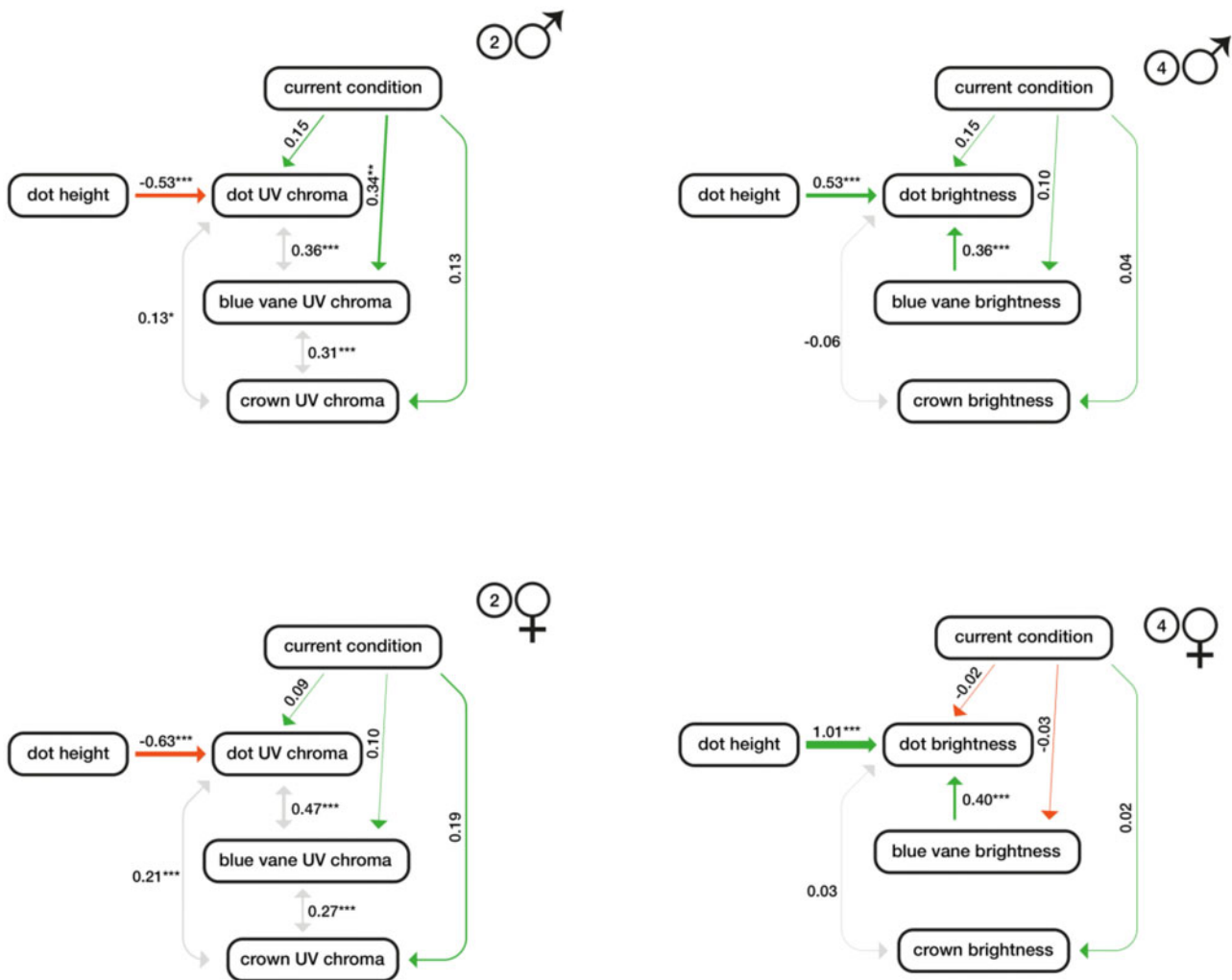
Figure 4. The path diagrams of best-fitted structural equation models exploring relationships between color metrics, dot height, and current condition, separately for males (♂) and females (♀). Green and red arrows denote positive and negative relations, respectively, whereas grey arrows indicate covariances. The width of the arrows represent the absolute size of the estimates. Significant relationships are marked with asterisks ($^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

coverts might potentially suggest a signaling role of this patch in both sexes.

Although the condition dependence of structural coloration is currently still under debate, the recent meta-analysis of White (2020) demonstrated a positive association between the expression of structural colors, body condition, and immune function. In line with this result, we found a male-specific positive relation between the current condition index (residual body mass) and the covert's blue vane UV chroma. This result may be especially interesting in the light of the condition capture models, and one of their main predictions—a heightened condition dependence of color elements in males (Zahavi 1977; Grafen 1990; Cotton et al. 2004). Thus, (contrary to age-differences found in both sexes) the observed pattern may suggest a signaling role of the blue part of the greater covert in males only. However, a similar relationship was not found for the crown and white dot UV chromas, and in none of the brightness metrics. Furthermore, we found no relationship between the dot size and the current condition, which is in line with the results of Hegyi et al. (2019). We also acknowledge that the current condition index (residual body mass), although commonly used in bird studies, is a proxy that should be interpreted with caution. The factors that

could bias its values might include, among others: the current level of hydration, fat reserves, and stomach contents. Condition index measured during breeding may also not reflect body condition experienced by individuals during molt, that is, when the ornamented feathers are produced. Nevertheless, we strongly encourage further studies to explore the condition dependence of the greater coverts coloration, perhaps with the application of other condition indices that could reflect more long-term trends, like lipid reserves accumulation or lipid reserves depletion (as suggested in Hegyi et al. 2019).

Therefore, the question arises whether the white wing stripe itself can act as a signal or is it only a signal amplifier for the blue part of the cover? The size of the white covert's dots, in both sexes, was positively related to its brightness, suggesting that larger dots are also the ones reflecting more light. In contrast, we also found that, irrespectively of sex, there was a strong negative relationship between the dot height and its UV chroma. Such pattern would therefore indicate the role of dots as white "standards" enhancing the perception of the blue vane's color by the signal receivers. Moreover, the coloration of dots was also not related to the condition index, which would be in agreement with the "quality amplifier" hypothesis (Hasson 1991). Alternatively, the achromatic white stripe may bear information on
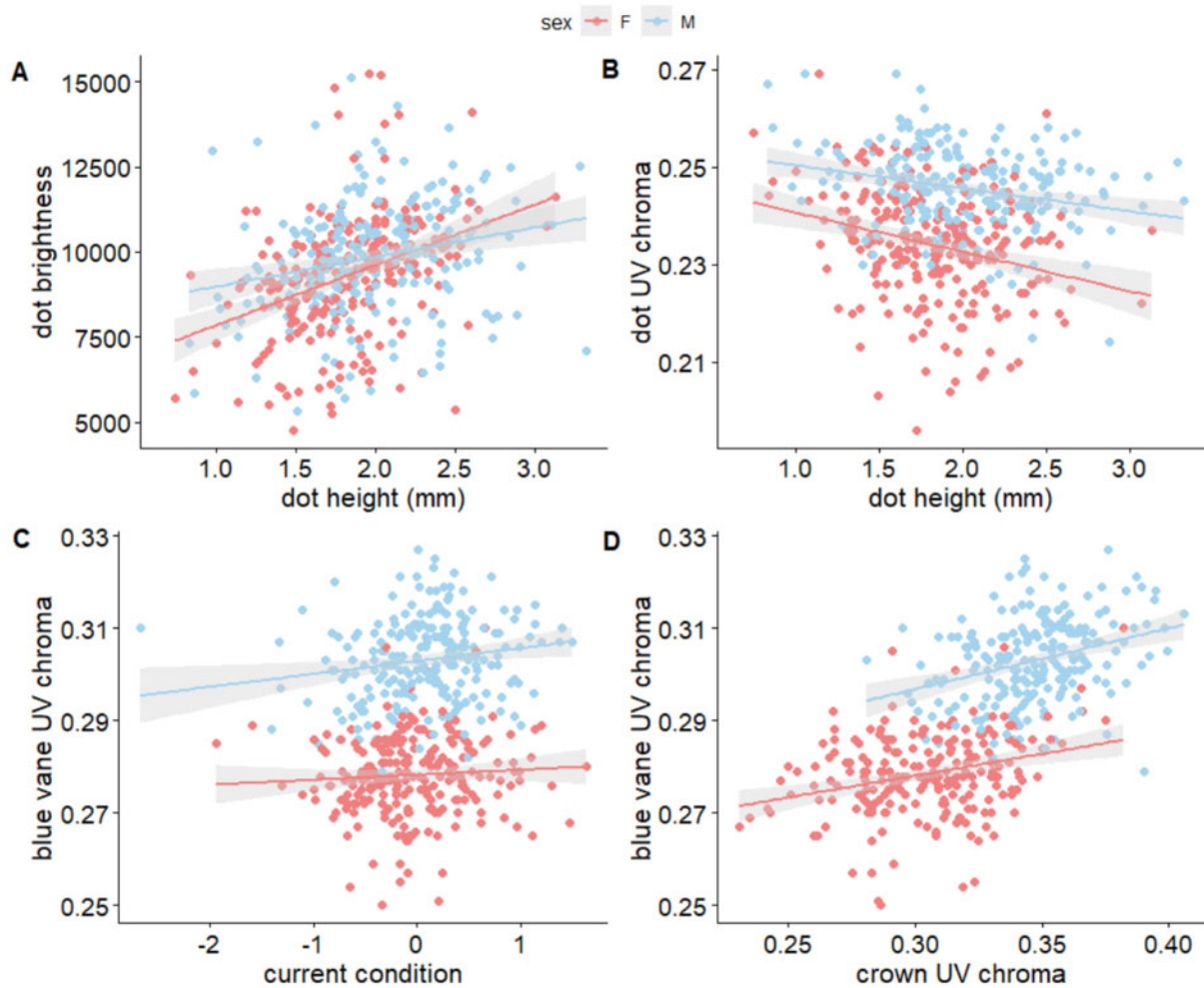
Figure 5. Relationships between dot brightness and dot height (mm) (A), dot UV chroma and dot height (B), blue vane UV chroma and current condition (C), and blue vane UV chroma and crown UV chroma (D). Red and blue points indicate respectively females and males, whereas grey shaded areas represent confidence intervals.

different aspects of a bird's condition compared with patches with blue structural coloration. As indicated by Kose et al. (1999) and Moreno-Rueda and Hoi (2012), the signaling value of the area of white patches might be associated with the ability to maintain it in good condition or with individual resistance to ectoparasites.

Another important question is whether the white wing stripe has a signaling function in the blue tit females. The primary explanation might be that it emerged as a by-product of sexual (via mate choice) or viability selection (e.g., via social interactions) acting on males and is expressed in females as a result of a strong inter-sexual genetic correlations in plumage characteristics (Lande 1980; Price 1996). Conversely, it may play a role in male mate choice or act as a badge of status in the social competition for nesting cavities or food between females (Doutrelant et al. 2020). Although predominantly studied in males, recently there has been more evidence that female plumage can also act as a badge of status (reviewed in Doutrelant et al. 2020). The white color seems to be particularly predisposed to this function as it is considered to be less costly to produce than ornaments with pigment-based coloration (which is important to the sex investing more in reproduction). Moreover, by being more sensitive to mechanical abrasion and ectoparasites, it can honestly signal the condition during molting. Indeed, in both collared and

pied flycatchers (*F. albicollis* and *F. hypoleuca*), the female's white wing stripe was demonstrated to be a badge of status, affecting aggressive behavior, incubation attendance, and vigilance (Hegyi et al. 2008; Plaza et al. 2018). To our knowledge, in the blue tit, none of the white plumage elements has been examined in this context, yet the previous study of Midamegbe et al. (2011) showed that the UV-blue crown coloration acted as a badge of status in females, inducing more aggressive behavior. In our population, we found both the dot height and brightness to be larger in males. Yet, the slope of the relationship between those 2 variables was significantly steeper in females (Table 2B and Figure 5A). Future research should, therefore, assess, preferably by modifying the size of a wing stripe, whether the size of the wing patch in blue tit females can affect intrasexual competition behavior.

In terms of the possible origin of the white stripe, our microscopic analysis revealed that the microstructure of barbs within the white dot is homologous to the 1 found in barbs from the blue part (i.e., with a medullary area consisting of dead keratinocytes containing channel-type ß-keratin spongy nanostructure and centrally located air cavities), with the lack of melanosomes as the most important difference. This indicates that the white color of covert dots results from withheld deposition of melanosomes in barbs within the white

Table 4. Full results of the best fitted structural equation model for the color metric brightness, analyzed separately on the subset of males ($N = 210$) and females ($N = 214$)

		Estimate	SE	z-value	P(>|z|)
Males					
Regressions:					
Crown brightness ∼	Condition	0.041	0.12	0.341	0.733
Blue vane brightness ∼	Condition	0.096	0.119	0.809	0.418
Dot brightness ∼	Blue vane brightness	0.357	0.065	5.485	< 0.001***
	Condition	0.148	0.113	1.311	0.19
	Dot height	0.527	0.141	3.735	< 0.001***
Covariances:					
Crown brightness ∼∼	Dot brightness	−0.059	0.061	−0.956	0.339
Variances:					
Crown brightness		0.949	0.093	10.247	< 0.001***
Blue vane brightness		0.931	0.091	10.247	< 0.001***
Dot brightness		0.832	0.081	10.247	< 0.001***
Females					
Regressions:					
Crown brightness ∼	Condition	0.041	0.12	0.341	0.733
Blue vane brightness ∼	Condition	0.096	0.119	0.809	0.418
Dot brightness ∼	Blue vane brightness	0.357	0.065	5.485	< 0.001***
	Condition	0.148	0.113	1.311	0.19
	Dot height	0.527	0.141	3.735	<0.001***
Covariances:					
Crown brightness ∼∼	Dot brightness	−0.059	0.061	0.061	0.339
Variances:					
Crown brightness		0.949	0.093	10.247	< 0.001***
Blue vane brightness		0.931	0.091	10.247	< 0.001***
Dot brightness		0.832	0.081	10.247	< 0.001***

The table shows estimates, standard errors, z-values, and *P*-values. Regression is marked with "∼" and covariances with "∼∼". Significant values are marked with the asterisks (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

area. This suggests that, similarly to the snowy-capped manakin *L. nattereri* and the white-fronted manakin *L. serena* (Prum 2006), the ancestral state might have been a homogeneously colored feather, whereas the achromatic dot evolved under sexual selection as an apomorphic trait. Similar mechanism of white color production occurs in the amelanotic Steller's jay (*Cyanocitta stelleri*) described by Shawkey and Hill (2006), in which barbs of white tail feathers (normally deep blue with thin darker stripes) possessed well developed spongy structure of sufficient size and regularity to produce blue color but lacked the melanin layer. Moreover, although analyzed on a restricted sample, our data indicated a tendency for males to have thicker barbs, with higher number of vacuoles, within both the white dot and the blue part of the vane.

Noteworthy, within the complex of subspecies of the closely related African blue tit *Cyanistes teneriffae*, we can see a whole phenotypic spectrum of white wing stripe patterns: from completely absent to well-developed (from *C. t. teneriffae* and *C. t. hedwigii* exhibiting homogenously blue coverts, through *C. t. palmensis*, and *C. t. ombriosus* with white stripe on coverts only barely marked, to *C. t. degener* with well visible wing patch (Svensson and Shirihai 2018)). On the other hand, in the azure tit (*Cyanistes cyanus*), as well as in hybrids between blue tits and azure tits, called the "Pleske's tit" (*Cyanistes × pleskei*) the wing patch is markedly wider (Ławicki 2012). Preliminary ancestral state reconstruction on the monophyletic *Cyanistes* clade indicates that a uniformly colored covert without achromatic dots is an unlikely ancestral state. Therefore, in the *Cyanistes* group, white dots seem not to have evolved as a secondary apomorphic trait. Taken the substantial variation in this achromatic character in the *Cyanistes*
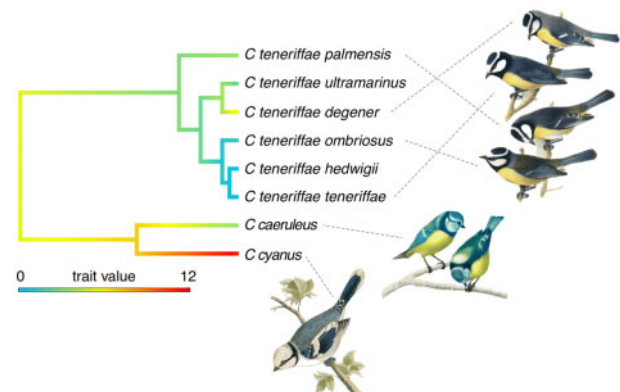

Figure 6. Preliminary reconstruction of ancestral states of the white stripe in the *Cyanistes* genus. The thickness of the stripe is ranked from 0 to 12, where 0 indicates no wing stripe and 12 denotes maximum width of the stripe (12 mm in *C. cyanus*). Bird pictures by Henrik Gronvold (1920, CC BY-SA 3.0). Detailed description of the analytical approach used to produce this figure can be found in the Supplementary Materials.

genus (Figure 6), we believe these species—and especially the complex of African blue tit subspecies—might be a very promising model for studying the genetic background of the emergence of white wing patches—and achromatic ornaments in general, and more broadly to test the hypotheses explaining the presence and signaling content of multiple ornaments (e.g., the multiple message hypothesis, the redundant signal hypothesis; Møller and Pomiankowski 1993).

To summarize, with this preliminary study, we wanted to draw attention to the previously neglected white wing stripe of adult blue tits and raise questions of its signaling function and possible evolutionary pressures that led to its emergence. We demonstrated that the white wing stripe is both sexually dimorphic and dichromatic in the blue tit. To explain whether sexual selection was a driving force that led to the evolution of this trait, further studies with direct mate choice experiments are needed to check for mate preference with respect to the wing stripe size and reflectance properties. Another goal for future research should be the evaluation of social selection (e.g., selection resulting from competition between conspecifics) acting on the blue tit's wing stripe in both sexes. Furthermore, because distribution of melanin in feathers is known to be under genetic control (Lin et al. 2013), quantitative genetics analyses are necessary to estimate heritability of dot size and to explore genetic correlations with other color traits of the blue tit, and between sexes within the white stripe traits.

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Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

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