Sexual dichromatism, size dimorphism and microscale anatomy of white wing stripe in blue tit (*Cyanistes caeruleus*)

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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 is a well-established model species in the studies on colouration, 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 analysed its correlations with current body condition and crown colouration - a trait with established role in sexual selection. Further, we qualitatively analysed microstructural barb morphology underlying covert's colouration. 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. Importantly, UV chroma of covert's vane was positively correlated with crown UV chroma, which suggests that coverts colouration might be also an ornament assessed by females during courtship display. Internal structure of covert barbs within the white dot was similar to the one 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. However, it lacked melanosomes which was the main observed difference. Together with marked sexual dimorphism, it suggests that white dots may have emerged under sexual selection as an apomorphic trait on previously uniformly coloured feathers.

Keywords: achromatic colouration, structural colouration, wing stripe, dimorphism, dichromatism, blue tit

INTRODUCTION

Achromatic plumage patches are widespread in birds. Usually, by developing against much darker or brightly coloured patches, they create contrasting, conspicuous patterns. Although being quite common, white plumage, in comparison to structural or pigment-based types of feather colouration, received so far considerably less attention. One reason may be a relatively simpler mechanism of colour production, based on incoherent scattering of incident light on unspecialised 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 mechanic 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 exposes 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 ecto-parasites (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 collard flycatcher (*Ficedula hypoleuca* and *Ficedula albicollis*), where the size of a white forehead patch constitutes a secondary sexual character in males (Gustafsson et al. 1995, Potti and Montalvo 1991, 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 ducks 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 (*Poecile 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 to 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 (*Ficedula 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 (Kokko 1999; Siitari and Huhta 2002).

The blue tit (*Cyanistes caeruleus*), thanks to its conspicuous, vivid colouration, is an important model species in the studies on bird colouration. However, majority of research so far was devoted to carotenoid-based and structural colouration, usually of yellow breast and blue crown feathers respectively, while 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 colouration was recently investigated in the context of carry-over effects (in the study of Badas et al. 2018), and extra-pair paternity (Badas et al. 2020). Beside 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 the courtship behaviour a male blue tit spreads and shakes its wings to attract a female, making the white stripe clearly visible (Stenning 2018). Based on such observations, we presume that this patch may potentially have a signalling 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 1 A. and 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 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 colouration 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 colour production, there are known cases of white ornaments evolved from feathers with plesiomorphic non-iridesent UV-blue colouration. 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 colouration (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).

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 moult limit present in yearlings between the primary and greater coverts, respectively (Svensson 1994), and body mass, tarsus length and wing length measurements were also taken. A bunch of crown feathers and the second right wing's greater covert were plucked from each individual. Covert feathers were preserved in parchment envelopes, while crown feathers were placed on black paper with transparent double-sided adhesive tape, preserving feather arrangement suitable for further reflectance measurements. 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. Subsequently, feathers were placed on black cardboard and scanned to 300 dpi JPG files together with pieces of graphing paper for scale. As the coverts overlap even when the wing is outstretched, height and area of dots affect the size of the wing stripe more than the width; nevertheless all three dot size parameters were 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 × 400μm illuminating fibres and 1 read fibre held perpendicularly to the sample (Ocean Optics, Dunedin, FL, USA). We took five measurements of the white covert's dot and five measurement of the blue part of outer vane. 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 in R (R Core Team), using the *pavo* package (Maia et al. 2019). To quantify coverts' and crown feathers' colouration, "brightness" and "UV chroma" were calculated as sum of reflectance values over all wavelengths, and total reflectance in the region between 320-400 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 colours we applied the avian tetrahedral colour 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 (*visual* = "bt"). This model allows for representing each reflectance measurement as a point in tetrahedral space, whose vertices correspond to four 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 analysed patches).

Scanning electron microscopy

To characterise the covert's barbs microscale morphology, we applied scanning electron microscopy (SEM). For this analysis we used feather samples from 10 individuals (five males and five females), randomly chosen from among samples from both analysed seasons, by drawing envelopes by a person not involved in the research. Feather cross-sections were made under binocular; a cut within the white dot made perpendicular to the rachis approx. 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 approx. 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 2500x. 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).

Statistical analysis

All covert's parameters, as well as body mass, tarsus length, wing length and crown colour 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 dot size parameters and colour metrics, general linear models were applied. Before the analysis, all models were inspected for normality of residuals and homoscedasticity. The models analysing dimorphism of dot size parameters (height, width and area) 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 models also tested for interaction between covert's length and sex, but it was not significant and therefore removed from all three models. To aid in interpretation of effect sizes between different response variables colour 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 colour metrics (with the following metrics as dependent variables: dot brightness, dot UV chroma, blue vane brightness, blue vane UV chroma) included sex, age and year of study as categorical predictors and dot area to account for relation between patch size and its reflectance properties, with initial models also testing for an interaction between dot are and sex.

To explore the relationship between colour metrics of covert dots and the blue part of covert vane and crown feathers, two linear models were applied, separately for brightness and UV chroma metrics. The models included dot colour metric as a dependent variable, vane colour

metrics and crown colour metrics as a continuous predictors and sex, age and year as categorical predictors. Initial models tested for interactions between sex and both continuous predictors. In each of those models, we controlled for potential multicolinearity of predictors by verifying the VIF values (variance inflation factors). Relation between coverts colouration and current condition (residual body mass) was analysed in linear models including condition as a continuous predictor and sex as a categorical factor. Initial models tested for interaction between condition and sex. In all analyses, non-significant interactions ($p > 0.05$) were sequentially removed from the models (which was the case in all but one model).

To understand the patterns of covariation between dot size parameters and reflectance properties of coverts and crown feathers we performed a principal component analysis (PCA). In the analysed set of variables, we included also covert length and tarsus length as measures of structural body size, and current body condition (residual body mass). The first two components, explaining 24.4% and 18.4 %, respectively, were used in further analyses (Figure 5). To test for sex differences, a simple linear model with PC1 and PC2 as response variables and sex, age and year of study as categorical predictors was applied. All analyses and graphs were done in R using packages: '*lme4*', '*MASS*', '*factoextra*' and '*ggplot2*' (version 3.6.0, R Core Team).

RESULTS

Covert's dot size dimorphism and dichromatism

Height and area of covert's white dot were significantly larger in males (Table 2.A, Figure 2.A and B), while dot width did not differ between sexes (Table 2.A). Neither of dot size parameters differed between age classes (Table 2.A). Measurements of the covert morphological parameters, averaged within sex, are summarized in the Table 1. Dot height and dot width, but not dot area, were significantly lower in the second year of study. All dot size parameters were independent of the covert's length (Table 2.A). Thus, we found significant sexual dimorphism in the size of covert dots, which (taken the arrangement of dots on the wing) should translate into dimorphism in the width of the wing stripe.

Brightness and UV chroma were significantly higher in males both within the white dot and blue part of the outer vane (Table 2.B, Figure 3.A and B). Covert's dot brightness and UV chroma were related with the dot area, but for the latter the estimated effect of this relation was

negative and relatively weak (Table 2.B) Differences between age classes were absent, apart from the UV chroma of the blue part of the covert's vane, which was higher in older birds (Table 2.B). Achromatic contrast between dot and vane was significantly higher in males and was positively correlated with the size of the dot (Table 2.B). In case of UV-blue outer vane colouration, the convex hulls overlap was 41.06% of the volume occupied by the males only (Figure 3.A). For white dots the volume overlap was higher and amounted to 65.48% in relation to volume occupied by males (Figure 3.B). It should be noted that the colour parametrisation used to construct convex hulls does not take into account the differences in brightness. Therefore, despite the partial volume overlap in the tetrahedral colour space, we can conclude that both white dot and blue part of the outer vane are significantly sexually dichromatic patches.

Coverts parameters in relation to crown colouration, current condition

UV chroma of covert's blue vane was highly correlated with UV chroma of crown feathers, but similar relation was absent for brightness (Table 3.A). Covert white dot's brightness was highly, positively related to the blue vane brightness, but not to the crown brightness (Table 3.B). There was a significant interaction between vane UV chroma and sex, with a steeper slope of relationship between dot UV chroma and vane UV chroma among females than among males (Table 3.B, Figure 4.B). Relationship between dot UV chroma and crown UV chroma appeared non-significant in the model, but simple correlation between raw variables showed high positive association (r = 0.66, $p<0.001$, Figure 4.C). The only covert colour metric significantly, positively related with current condition (residual body mass) was blue vane UV chroma (Table 4).

PC1 exhibited strong negative loadings for all analysed parameters (Table S2.) except for the brightness of covert's outer vane (0.148). Within PC2, variables clustered into two groups: the first one with positive loadings included variables associated with achromatic characteristics – dot dimensions (height, width and area with respective loadings: 0.562, 0.555, 0.621), brightness (0.434) and achromatic contrast (0.382) between dot and vane brightness. The second group exhibited negative loadings and clustered variables related to chromatic characteristics and condition, e.g. dot UV chroma (-0.614), crown UV chroma (-0.469) and current condition (-0.138) (loadings for all variables for both PCs can be found in Table S1.). In both models with PC components as response variables, we found significant sex differences (Table S1). In the model with PC1, there was also significant difference between age classed,

with older individuals exhibiting higher values of all analysed parameters (Table S2, Figure 5).

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 between 4 to 6 centrally located air vacuoles (Figure 1.C). Mean diameter and area of barb cross-section in the blue part of covert was 40.45 μm and 719.55 μm^2, respectively, while 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 the Table 1.B. Barb cross-sections from the white dots lacked completely melanin granules and had smaller amount of keratinocytes in the medullary part, with between 2 to 4 air vacuoles surrounded by thin, but well developed ß-keratin spongy nanostructure (Figure 1.D).

DISCUSSION

Here, we provide the first evidence for sexual dimorphism in the patch size of achromatic dots on greater coverts creating wing stripe in the blue tit, as well as sex-related dichromatism in reflectance properties of achromatic dot and adjacent blue region of the coverts. Height and area of white dots were larger in males, independently of covert's length, but dot width did not differ between sexes. It might be explained by the fact that the width of the dot is largely determined by the width of the feather itself, which in turn is a much more conservative feature than the area of the achromatic patches. Moreover, covert feathers overlap, even when the wing is unfolded, therefore the height and dot area are more important for determining total area of the wing stripe, as it can be seen by other birds. Further, despite appearing monochromatic to a human eye, spectrophotometry revealed that white dots of males are both brighter and more UV chromatic than those of females. The same hold true for the adjacent blue outer vane, which also occurred to have higher brightness and UV chroma in males. Thus, by describing new dichromatic region, our results expanded those of Hunt et al. (1998) that reported five dichromatic plumage regions of the blue tit (blue and white part of crown, nape, tail, and back feathers), and later study of Griggio et al. (2009) showing sexual differences in reflectance of white 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*) the size of this patch was shown to be a sexually selected trait in a mate choice experiment (Senar et al. 2005). 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 colouration generated by deposition of carotenoids) reflects male's foraging abilities. However, regardless of the colour 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 behaviour 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 male spreads 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 colouration, together with the characteristic courtship behaviour, give the ground to investigate whether white wing stripe in the blue tit might have a signalling function in a mate selection.

Although our data are correlative, they allow us to draw wary conclusions as for the signalling function of the white stripe. Among blue tit plumage regions, the crown is most often considered as a sexually selected trait, as its brightness was shown to be related to male biased sex ratio (Sheldon et al. 1999; Griffith et al. 2003, although see objections in the meta-analysis of Parker 2013). We found that UV chroma of structural blue outer vane was highly correlated with crown UV chroma, but analogous relation was not found for the brightness. In contrast to higher crown brightness, which is considered to be positively related with bird quality, higher brightness of covert's vane does not necessarily have to be beneficial as due to the lower contrast with the white dot it will make it less conspicuous. Moreover, neither dot brightness nor it's UV chroma was significantly associated with crown metrics (Table 3). However, this pattern might stem from a different mechanisms of colour production between the blue and white parts of feathers, and therefore, as suggested by multiple message hypothesis (Møller, and Pomiankowski 1993), the achromatic white stripe may bear information on different aspects of a bird's condition compared to patches with blue structural colouration. Additionally, there was a sex specific pattern of relationship between dot's UV chroma and vane's UV chroma, with steeper slope, but overall lower of both variables values observed in females (Figure 4.B), yet the reason why this relationship would be stronger in females remains unclear. Further, we found no relation between dot size and current condition (residual body mass), which is in line with results of Hegyi et al. 2019. Perhaps, to comprehensively verify the link between body condition and plumage quality, future studies should take into account other measures of condition, that could reflect more long-term trends, like lipid reserve accumulation or lipid reserve depletion (as suggested in Hegyi et al. 2019).

The Principal Component Analysis revealed further interesting relationships between structural and colour metrics. In the first PC, the only variable with loading value opposite to the rest, was blue vane brightness (Figure 5). Together with a positive correlation between the achromatic contrast (of bordering white dot and blue parts of the outer vane) and the area of white dot this suggests that the larger dots also tend to be more contrasting. The relationship between dot area and vane brightness, in spite of having a (expected) negative slope, was not statistically significant (estimate = -5.835, p = 0.734; Table 2.B), therefore this trend ought to be treated with caution. Within the PC2, variables clustered into two well separated groups: the first one associated with achromatic characteristics of the dots, and the second one grouping chromatic variables together with body condition and structural size. This separation likely results from the different factors that determine the expression of achromatic colouration and structural colouration. This possible divergence of plumage patches' signalling contents was also visible in the negative relationship between dot area and dot UV chroma (Tab. 4, Fig. 4.D), which may further suggest that those two traits signal different aspects of bird quality. As indicated by Kose et al. (1999) and Moreno-Rueda and Hoi (2012), the signalling value of the area of white patches might be associated with ability to maintain it in good condition and/or with resistance to ectoparasites. On the other hand, UV chroma of white patches was shown to be positively related to arrival date at the breeding sites and reproductive success in pied flycatchers (Siitari and Huhta 2002).What is also particularly interesting, is that chromatic variables of crown and blue part of vane clustered close to each other (Figure 5, but also see Figure 4.A) and to the metrics of the current body condition and structural size. It may suggest that coverts colouration, similarly to the crown feathers, may play a signalling function, although this assumption will need further experimental verification.

An obvious question arises: what is the function of white wing stripes in blue tit females? The primary explanation might be that it emerged as a by-product of sexual (via mate choice) or viability (e.g. via social interactions) selection acting on males and is expressed in females as a result of strong genetic correlations in plumage characteristics between sexes (Price 1996). Alternatively, it may play a role in male mate choice or social competition between females (Doutreland 2020). Future research should therefore assess, preferably using mate-choice experiments, whether there is clear preference expressed in males and/or in females towards particular white stripe ornaments.

In terms of possible origin of the white stripe, our microscopic analysis revealed that the microstructure of barbs within the white dot is homologous to the one 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. It indicates that the white colour of covert dots results from withheld deposition of melanosomes in barbs within the white area. This suggests that, similarly to the snowy-capped manakin *Lepidothrix nattereri* and the white-fronted manakin *Lepidothrix serena* (Prum 2006), the plesiomorphic state might have been a homogenously coloured feather, while the achromatic dot evolved under sexual selection as an apomorphic trait. Similar mechanism of white colour 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 colour, however lacking the melanin layer. Therefore, in this case, there are no premises to assume that the production of white ornament is less costly. Moreover, although analysed on the restricted sample, there was a tendency for males to have thicker barbs, with higher number of vacuoles, within both white dot and blue part of the vane.

Noteworthy, within the complex of subspecies of the closely related African blue tit (*Cyanistes teneriffae*), there can be seen 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 (between 12-13 mm in azure tit and 3-5 mm in Pleske's tit) (Ławicki 2012). Furthermore, this trait is present in some representatives of other genera of the Paridae family (e.g. great tit *Parus major* and coal tit *Periparus ater*), and absent in others (e.g. crested tit *Lophophanes cristatus*, willow/marsh tit *Poecile montanus/plustris*). The question arises whether this appeared independently in several lines, or was it present in the common ancestor of tits and was lost in some descendant lines. A simple ancestral state reconstruction, using average white stripe figures for different species (Figure 6) suggests, that white stripes are an ancestral trait in Paridae, apomorphically lost in some specific lineages. We believe the *Cyanistes* genus, 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

signalling content of multiple ornaments (e.g. the multiple message hypothesis, the redundant signal hypothesis; Møller and Pomiankowski 1993).

Conclusions

To summarize, with this initial study we want to draw attention to the previously neglected white wing stripe of adult blue tits and raise questions of its signalling function and evolutionary pressure that led to its emergence. We demonstrate that white wing stripe is both 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 the signs of assortative mating for the wing stripe size and reflectance properties. Furthermore, since 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 colour traits of the blue tit, and between sexes within the white stripe traits.

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TABLES

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**	
A.	**Males**	**Females**
Covert length (mm)	19.81 ± 1.00	18.89 ± 0.98
Dot height (mm)	1.98 ± 0.43	1.8 ± 0.44
Dot width (mm)	3.96 ± 0.78	3.89 ± 0.78
Dot area (mm^2)	6.84 ± 1.93	6.22 ± 1.91
B.		
White dot barb cross-section		
diameter (µm)	24.07 ± 46.28	23.80 ± 2.39
area (μm^2)	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 (μm^2)	727.06 ± 70.96	712.32 ± 71.92
number of vacuoles	4.56 ± 0.86	4.39 ± 0.92

Table 2. Results of linear models analysing dimorphism in dot size parameters (**A**) and dichromatism of dot and blue part of covert vane (**B**). **A.** The models included dot size parameter (height, width and area) 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 predictors. **B.** The models included colour metric: brightness or UV chroma of dot or blue part of the vane as a dependent variable. Dot area 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 predictors. Colour metrics were scaled to zero mean and unit standard deviation (SD).

		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	***
Dot width	Intercept	3.765	0.736	5.113	<0.001	***
	Covert length	0.024	0.039	0.621	0.535	
	Sex	0.042	0.074	0.565	0.572	
	Age	-0.096	0.068	-1.414	0.158	
	year	-0.678	0.068	-9.959	<0.001	***
Dot area	Intercept	5.535	2.016	2.746	0.006	**
	Covert length	0.039	0.107	0.363	0.717	
	Sex	0.565	0.202	2.803	0.005	**
	Age	0.003	0.186	0.018	0.985	

	Year	-0.063	0.186	-0.336	0.737	
B.						
Dot brightness	Intercept	-0.951	0.169	-5.624	<0.001	***
	Dot area	0.113	0.024	4.777	<0.001	***
	Sex	0.238	0.091	2.618	0.009	**
	Age	0.118	0.092	1.281	0.201	
	Year	0.146	0.095	1.534	0.126	
Dot UV chroma	Intercept	-0.162	0.148	-1.094	0.275	
	Dot area	-0.066	0.021	-3.196	0.001	**
	Sex	1.037	0.080	13.032	<0.001	***
	Age	-0.030	0.081	-0.375	0.708	
	Year	0.313	0.083	3.756	<0.001	***
Blue vane brightness	Intercept	0.179	0.174	1.032	0.303	
	Dot area	-0.008	0.024	-0.340	0.734	
	Sex	-0.284	0.093	-3.040	0.003	**
	Age	-0.013	0.095	-0.132	0.895	
	Year	0.047	0.098	0.476	0.895	
Blue vane UV chroma	Intercept	-0.909	0.100	-9.112	<0.001	***
	Dot area	-0.001	0.014	-0.080	0.936	
	Sex	1.622	0.054	30.213	<0.001	***
	Age	0.179	0.055	3.279	0.001	**
	Year	0.193	0.056	3.432	<0.001	***
Achromatic contrast	Intercept	-1.130	0.190	-5.949	<0.001	***
	dot area	0.121	0.027	4.563	<0.001	***
	sex	0.522	0.102	5.113	<0.001	***
	age	0.131	0.104	1.260	0.208	
	year	0.099	0.107	0.930	0.353	

Table 3. Results of linear models analysing relation between colouration of covert's blue vane (A.) and white dots (B.) and crown feathers. The models accounted for colour metrics of covert vane and crown (in respective models: brightness and UV chroma) as a continuous predictors and sex, age and year as a categorical predictors. Colour metrics were scaled to zero mean and unit standard deviation (SD).

	Estimate	**SE**	**t**	**p**	
A. Blue vane					
Brightness					
Intercept	0.260	0.179	1.448	0.148	
Crown Brightness	0.038	0.053	0.709	0.479	
Sex	-0.335	0.105	-3.192	0.002	**
Age	-0.037	0.097	-0.378	0.706	
Dot area	-0.016	0.025	-0.626	0.532	
year	0.051	0.098	0.520	0.603	
UV chroma					

Intercept	-0.666	0.109	-6.121	<0.001	***
Crown UV chroma	0.202	0.038	5.377	<0.001	***
Sex	1.346	0.073	18.462	<0.001	***
Age	0.103	0.057	1.802	0.072	
Dot area	-0.009	0.014	-0.622	0.534	
Year	0.123	0.057	2.147	0.032	*
B. White dot					
Brightness					
Intercept	-1.099	0.162	-6.776	<0.001	***
Vane brightness	0.389	0.044	8.934	<0.001	***
Crown brightness	-0.013	0.048	-0.283	0.778	
Sex	0.427	0.096	4.465	<0.001	***
Age	0.079	0.087	0.902	0.368	
Dot area	0.119	0.023	5.242	<0.001	***
Year	0.163	0.089	1.834	0.067	
UV chroma					
Intercept	0.064	0.019	3.383	<0.001	***
Vane UV chroma	0.599	0.069	8.717	<0.001	***
Sex	0.069	0.028	2.515	0.012	*
Crown UV chroma	0.028	0.019	1.479	0.140	
Dot area	-0.001	0.000	-3.493	<0.001	***
Age	-0.002	0.001	-2.072	0.039	*
Year	0.002	0.001	2.050	0.041	*
Vane UV chroma: Sex	-0.243	0.095	-2.561	0.011	*

Table 4. Results of linear models analysing relation between coverts colouration and current condition. Each model included condition index as a continuous variable and sex as a categorical factor. The models included also predictors that appeared to be significantly related with coverts colour metrics: dot area in models with dot brightness and UV chroma and age and year in blue vane UV chroma. Colour metrics were scaled to zero mean and unit standard deviation (SD).

		Estimate	**SE**	**T**	**p**	
Dot brightness	Intercept	-0.883	0.161	-5.482	<0.001	***
	Dot area	0.118	0.024	4.997	<0.001	***
	Condition	0.108	0.082	1.309	0.191	
	Sex	0.230	0.091	2.527	0.012	*
Dot UV chroma	Intercept	-0.043	0.144	-0.299	0.765	
	Dot area	-0.069	0.021	-3.258	0.001	**
	Condition	0.076	0.074	1.028	0.305	
	Sex	1.028	0.081	12.625	<0.001	***
Blue vane brightness	Intercept	0.147	0.065	2.254	0.025	*
	Condition	0.050	0.086	0.584	0.560	
	Sex	-0.300	0.094	-3.211	0.001	**

Blue vane UV chroma	Intercept	-0.911	0.049	-18.719	<0.001	***
	Condition	0.102	0.049	2.061	0.040	*
	Sex	1.615	0.054	30.106	<0.001	***
	Age	0.167	0.055	3.008	0.003	**
	Year	0.192	0.057	3.389	<0.001	***

FIGURES

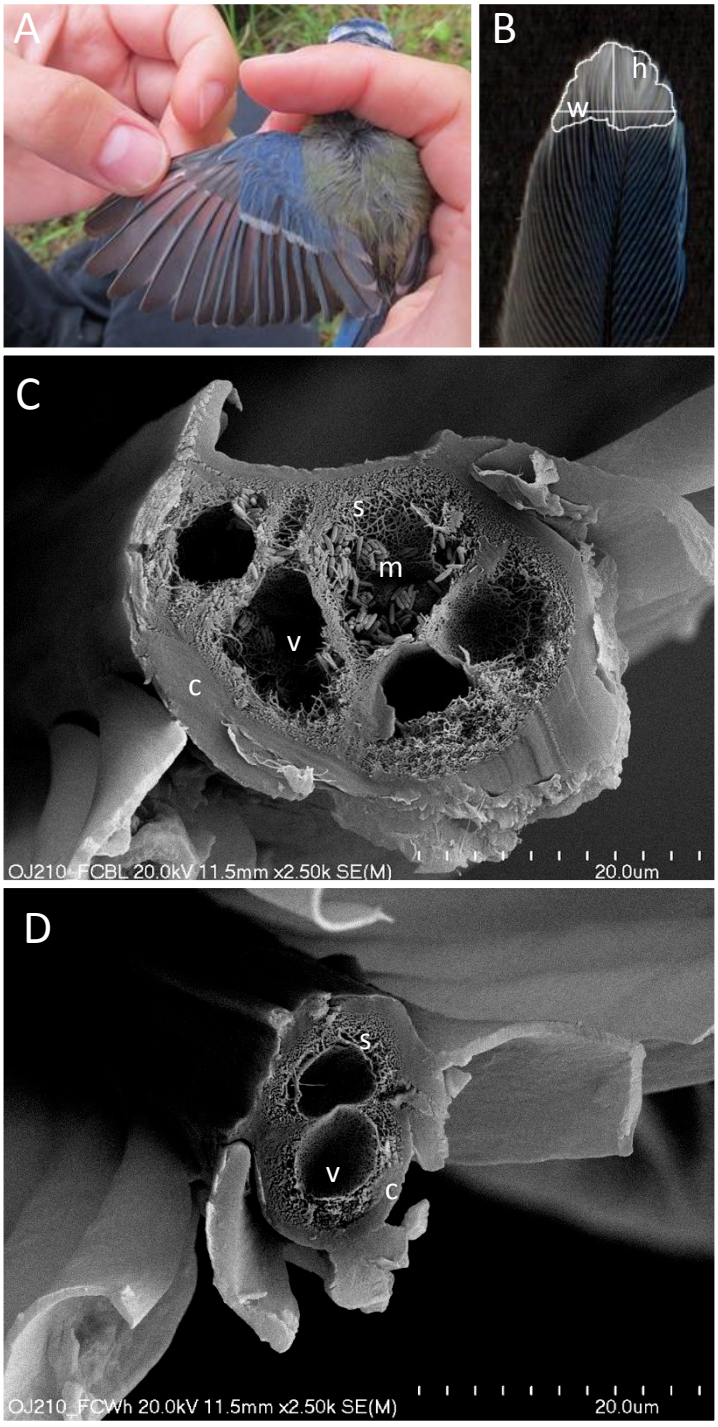

Figure 1. A. Adult blue tit wing patch (fot. D. Lutyk). **B.** Close-up of the upper part of the covert, with width, height and area of the white dot marked. **C.** and **D.** Scanning electron

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).

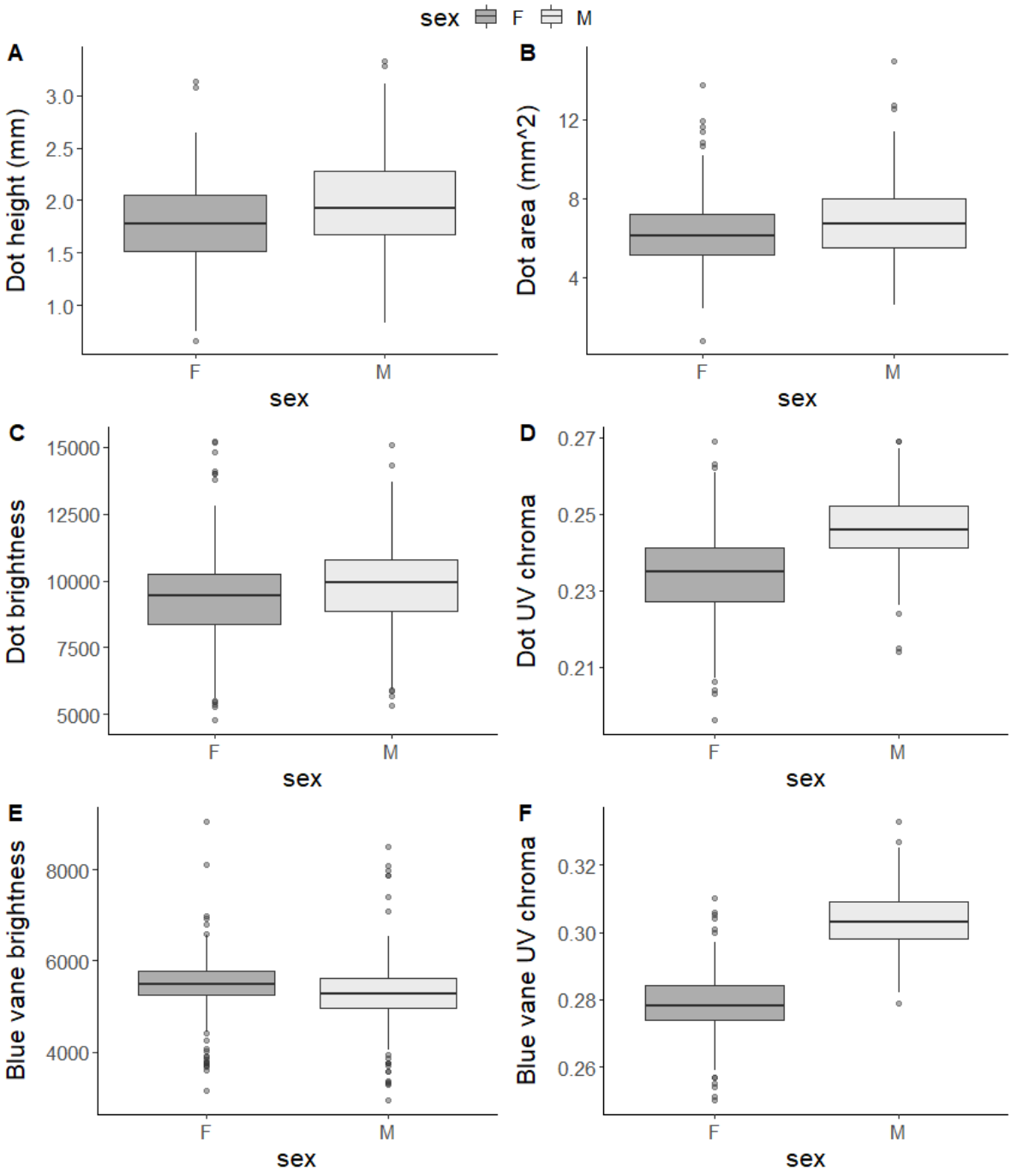

Figure 2. Box-plot showings sexual dimorphism in covert's dot height (A) and dot area (B) and sexual dichromatism in dot brightness (C), dot UV chroma (D), brightness of blue outer vane (E) and UV chroma of blue vane (F). Horizontal bars indicate median, lower and upper bounds indicate respectively 1st and 3rd quartile and whiskers indicate minimum and maximum values. Males and females are marked with light and dark grey colour, respectively.

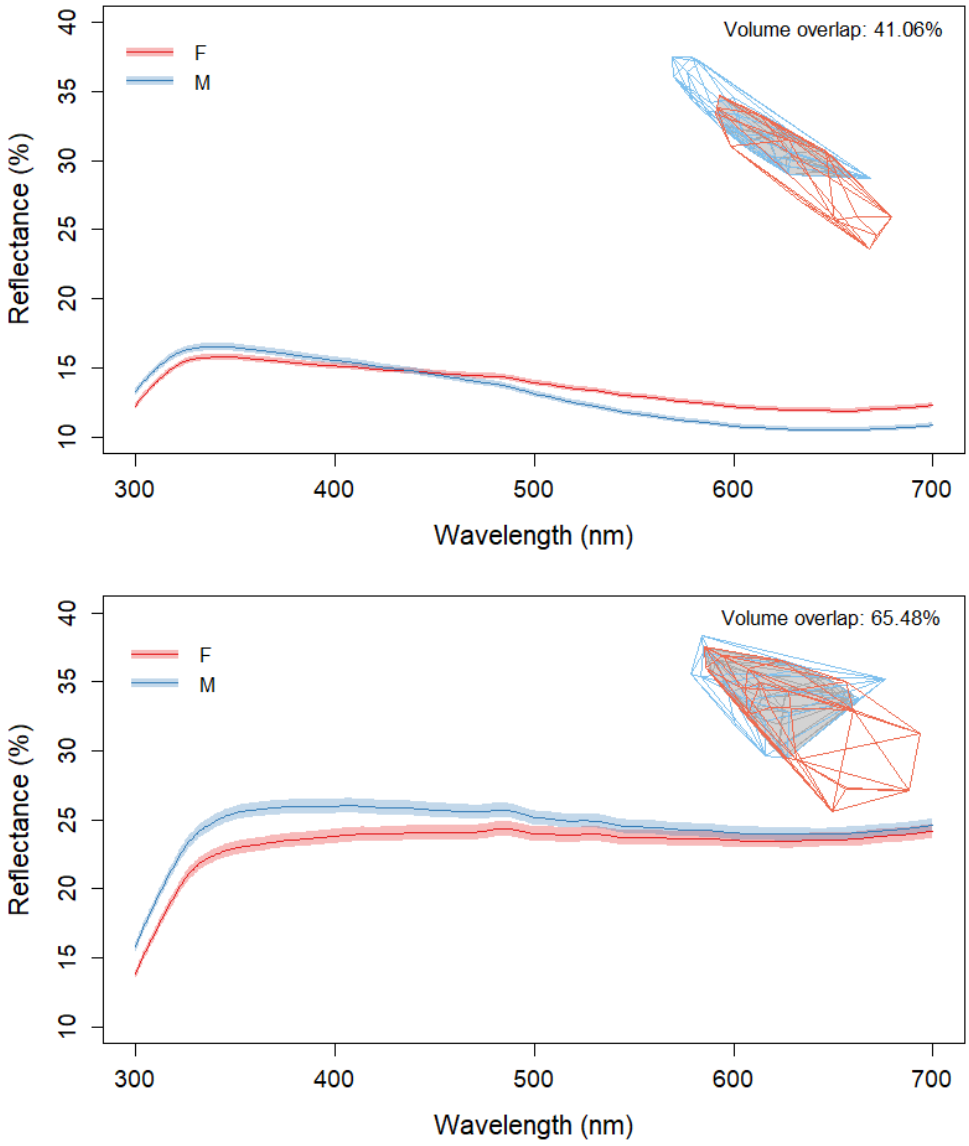

Figure 3. Reflectance spectra of blue tit covert's outer vane (A) and white dot (B). Blue and red lines denote, respectively male and female mean reflectance, while shading indicates 95% confidence intervals. The pictures in the top right corner represent convex hulls volume overlap from the tetrahedral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

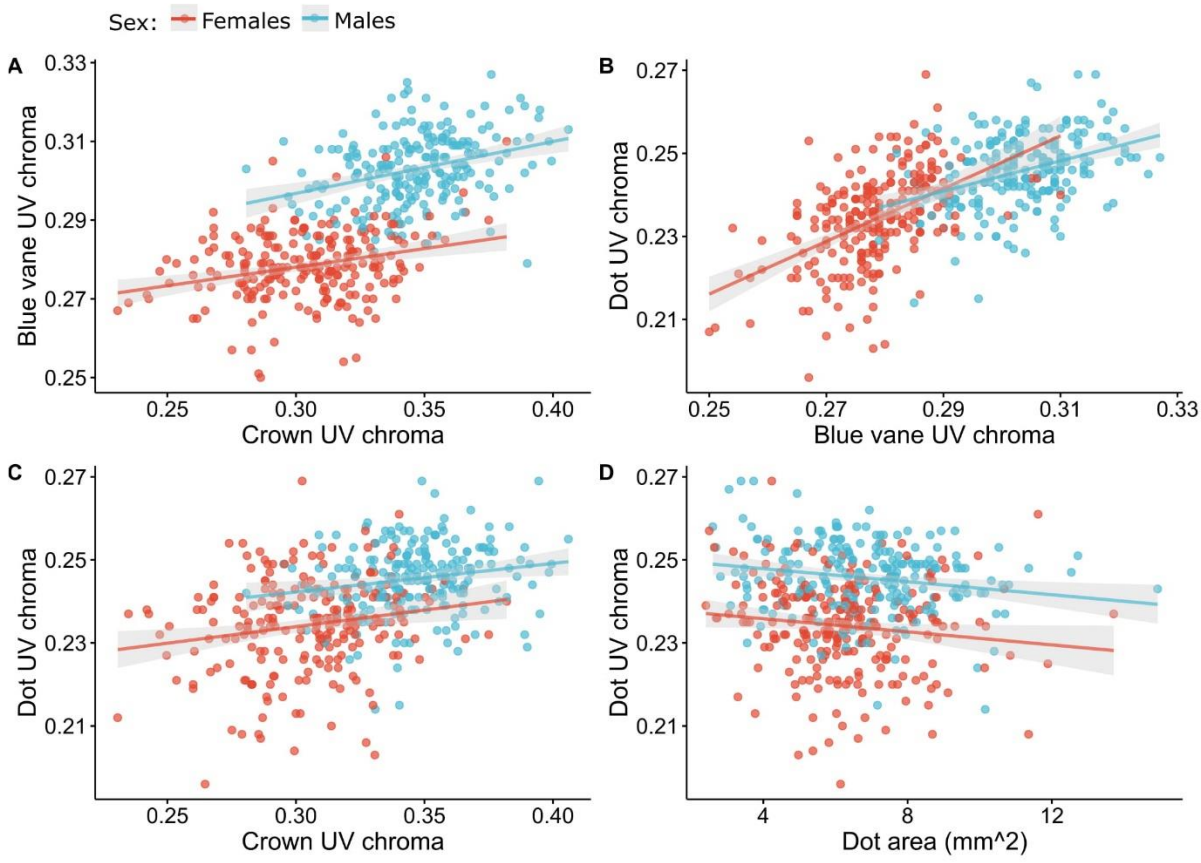

Figure 4. Relationship between blue vane UV chroma and crown UV chroma (A), dot UV chroma and blue vane UV chroma, dot UV chroma and crown UV chroma and dot UV chroma with dot area (mm^2). Red and blue points indicate respectively females and males, while grey shaded areas represents confidence intervals.

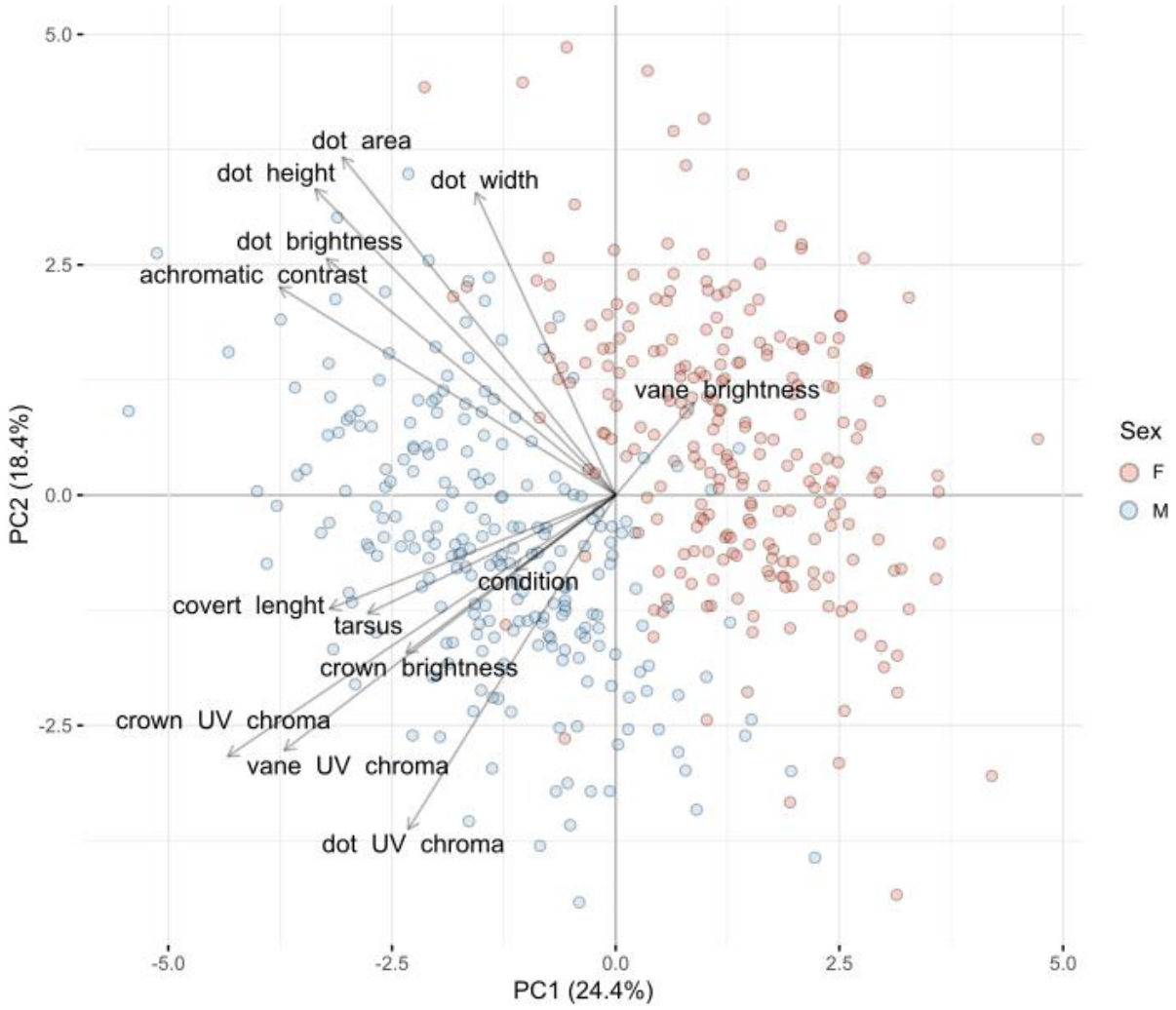

Figure 5. PCA biplot showing first two principle components. Females scores are marked with red brick colour and males scores are marked with blue colour.

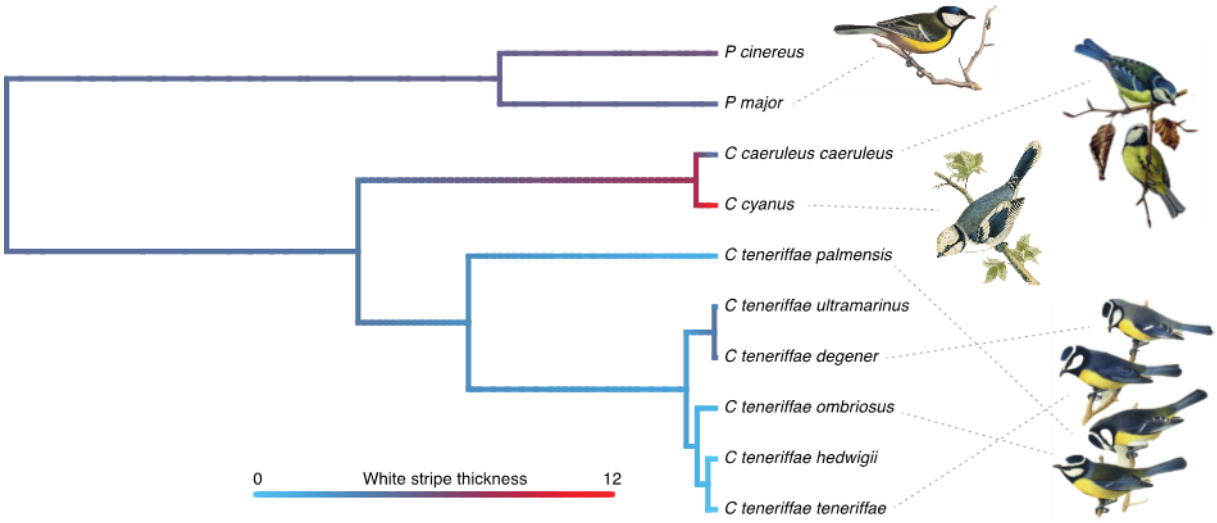

Figure 6. Simplified reconstruction of ancestral state of the white stripe in selected representatives of the Paridae family. The thickness of the stripe is ranked from 0 to 12, where 0 indicates no wing stripe and 12 denotes maximum width of the stripe (12 mm in Cyanistes cyanus). Ancestral values along the branches reconstructed using the re-rooting method in phytools (Revell 2012). Phylogeny based on the genetic topology from Gohli et al. (2015). Bird pictures by Henrik Gronvold (1920, CC BY-SA 3.0).

Supplementary material

Table S1. Values of first two principal component loadings for each of analysed variables.

Variable	PC1	PC2
covert length (mm)	-0.541	-0.209
dot height (mm)	-0.568	0.562
dot width (mm)	-0.264	0.555
dot area (mm^2)	-0.517	0.621
crown brightness	-0.396	-0.290
crown UV chroma	-0.627	-0.469
tarsus (mm)	-0.469	-0.217
dot brightness	-0.547	0.434
dot UV chroma	-0.392	-0.614
vane brightness	0.148	0.170
vane UV chroma	-0.734	-0.480
achromatic contrast	-0.636	0.382
condition	-0.189	-0.138

Table S2. Results of linear models analysing sex differences in first two PC components. The models included sex, age (second calendar year or older), and year of study as a categorical predictors.

	Estimate	SE	t	p	
PC1					
Intercept	1.494	0.100	14.884	<0.001	***
Sex	-2.648	0.108	-24.625	<0.001	***
Age	-0.510	0.108	-4.713	<0.001	***
Year	0.084	0.109	0.772	0.441	
PC2					
Intercept	0.954	0.121	7.91	<0.001	***
Sex	-1.158	0.129	-8.967	<0.001	***
Age	-0.195	0.130	-1.502	0.134	
Year	-0.195	0.130	-1.502	<0.001	***