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Moult speed affects structural feather ornaments in the blue tit

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Abstract

Growing evidence suggests that structural feather colours honestly reflect individual quality or body condition but, contrary to pigment-based colours, it is not clear what mechanism links condition to reflectance in structural feather colours. We experimentally accelerated the moult speed of a group of blue tits (*Cyanistes caeruleus*) by exposing them to a rapidly decreasing photoperiod and compared the spectral characteristics of their structural feather colours with those of control birds. Blue tits were sexually dimorphic on the UV/blue crown and on the white cheek feathers. Moult speed, however, dramatically reduced brightness and the saturation only on the UV/blue crown feathers, whereas structural white on the cheek feathers was basically unaffected by moult speed. Given that the time available for moulting is usually confined to the period between the end of the breeding season and migration or wintering, UV/blue colours, but not structural white, may convey long-term information about an individual's performance during the previous breeding season. The trade-off between fast moulting and structural colour expression may represent a previously unrecognized selective advantage for early-breeding birds.

Introduction

Coloured feathers are among the commonest sexually selected ornaments in birds (Hausmann *et al.*, 2003; Senar, 2006). Feather colours can derive either from pigments (usually melanins or carotenoids) deposited in the protein matrix of the feathers during growth (McGraw, 2006a,b) or from the regular microstructures of feathers that interact with incident light via a process of coherent scattering (Prum, 2006). Different feather colours are therefore produced through different metabolic pathways, which implies that they are subject to different constraints/costs and may convey different information to prospecting mates (Hill & McGraw, 2006). Plumage colours often result from a combination of light reflected by the structural components of the feathers, and the light absorption by incorporated pigments (Shawkey & Hill, 2005; Prum, 2006). Structural colours are produced by regular nanometer-scale physical structures which can be composed of keratin/air structures, pigment granules forming regular nanolayers/structure embedded in the keratin matrix, or a nanometer-scale keratin structure underlain by a layer of absorbing pigment (usually melanin granules and in some cases carotenoids, Prum, 2006). Thus, both pigment deposition and regularity of nanostructures (layers of keratin, air and pigments) contribute, separately or in combination, to the spectral properties of the feather colour signals.

Numerous studies have found evidence that carotenoid and melanin-based feather ornaments function as honest indicators of the quality of the bearer (Griffith *et al.*, 2006; Hill, 2006; McGraw, 2006a,b; Griggio *et al.*, 2007; Hoi & Griggio, 2008). Growing evidence suggests also that noniridescent structural feather colours correlate with individual quality or conditions (McGraw *et al.*, 2002; Johnsen *et al.*, 2003; Hill *et al.*, 2005; Hegyi *et al.*, 2007; Jacot & Kempenaers, 2007; Peters *et al.*, 2007; Siefferman & Hill, 2007; Doutrelant *et al.*, 2008). Unlike melanin- and carotenoid-based feather ornaments, for which a physiological link between feather colouration

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and condition has been proposed (e.g. Endler, 1980; Lozano, 1994; Owens & Wilson, 1999; Mackintosh, 2001; Jawor & Breitwisch, 2003; McGraw, 2007; Bokony *et al.*, 2008), it is not clear which mechanism links condition to reflectance in the structural feather colours (Prum *et al.*, 1998; Prum, 2006), although nutritional stress has been proved to affect structural feather colours (Keyser & Hill, 1999; Doucet, 2002). It has been suggested that variation in saturation and in hue of plumage structural colours (UV/blue, violet or green) is caused by variation in the regularity of a feather's microstructure (Andersson, 1999; Shawkey *et al.*, 2003, 2005; Doucet *et al.*, 2006; Prum, 2006), as the precision of microstructural elements determines the spectral saturation of colour (Shawkey *et al.*, 2003). The honesty of structural signals may therefore be maintained by the cost of accurately producing these nanoscale structures: because feather synthesis is a long-lasting and physiologically costly process (Siikamaki *et al.*, 1994; Klaassen, 1995; Nilsson & Svensson, 1996), any developmental stress during moult will alter the regularity of feather microstructure. For example, it has been suggested that only high-quality individuals, which may have unrestricted access to nutrients, are able to grow feathers with a regular microstructure that translates into more saturated structural colours (Keyser & Hill, 1999). Contrary to this view, Prum (2006) noted that the precise nanostructure producing the structural feather colouration is the result of the intrinsic chemical properties of the macromolecules involved in the nanostructures, as these affect the chemical bonds among macromolecules and hence their spatial assemblage, making condition dependency less likely for this kind of colouration.

Structural colours have been suggested to degrade more rapidly than long wavelength colours, as worn plumage is proportionally less saturated in the UV range that in the long wavelength spectrum in the blue tit (*Cyanistes caeruleus*, Örnborg *et al.*, 2002). Experimental evidence also suggests that structural, short wavelength colours may be particularly sensitive to the accumulation of dustiness and hence maintenance (Zampiga *et al.*, 2004; but see Montgomerie, 2006a). Substantial variation in the saturation of structural colours is present, however, in freshly produced feathers, and clearly plumage maintenance cannot account for this variation. In a study on the effect of experimental inoculation with coccidian parasites in male wild turkeys (*Meleagris gallopavo*), infected males showed proportionately less UV reflectance in their wing covert and breast feathers, thus suggesting that parasites can reduce the expression of structural plumage colouration (Hill *et al.*, 2005). Similarly, exposure to experimentally elevated levels of testosterone resulted in impaired expression of UV colours in black grouse (*Tetrao tetrix*, Siitari *et al.*, 2007). A satisfactory mechanistic explanation of the condition dependence of structural colours is still lacking.

Two recent papers (Serra *et al.*, 2007; Ferns & Hinsley, 2008) provide evidence that carotenoid-based feather colour signals are influenced by moult speed. Despite the fact that the moult (and more generally feather growth) is clearly a process crucial to the production of colour feather ornaments, its role in the regulation of ornamental colouration has received little attention. In particular, late breeders and juveniles from late clutches have less time to moult before the arrival of the winter or the beginning of migration (Bojarinova *et al.*, 1999). Delay of moult start (and hence less time available for growing the new plumage) has been found to correlate positively with brood size (Bensch *et al.*, 1985; Siikamaki *et al.*, 1994; Nilsson & Svensson, 1996), suggesting a trade-off between current reproduction and subsequent moult. Birds can cope with a delayed moult onset by increasing their moulting speed (Dawson, 2004), or by overlapping moult and migration (Norris *et al.*, 2004). A faster moult occurs, however, at the expense of the quality of flight feathers (Dawson *et al.*, 2000; Serra, 2001; Hinsley *et al.*, 2003). Whether or not a faster moult also affects feather colour signals has recently been investigated in two species with carotenoid feather ornaments, the rock sparrow (*Petronia petronia*, Serra *et al.*, 2007) and the great tit (*Parus major*, Ferns & Hinsley, 2008). Results were similar in the two species, demonstrating that moult speed negatively affects colour saturation of carotenoid-based feather ornaments. A trade-off between moult speed and quality of carotenoid feather ornaments would mediate a selective advantage for early breeders and juveniles from early clutches, as these birds have more time to renew their plumage (Svensson & Nilsson, 1997; Hemborg *et al.*, 2001). As early breeders are often the individuals in the best condition, moult speed contributes to explain the observed condition dependence of carotenoid colours reported in several species (Griffith *et al.*, 2006). Whether or not moult speed also affects structural colours, however, is currently not known.

To investigate the effect of moult speed on structural feather colours, we experimentally increased the speed of the post-breeding moult of a group of blue tits by rapidly decreasing the photoperiod and compared the spectral characteristics of three colour feather patches with those of a group of individuals whose photoperiod decreased at a slower rate, using a standard procedure (Dawson *et al.*, 2000; Serra *et al.*, 2007; Ferns & Hinsley, 2008). We considered three body areas with different mechanisms of feather colour production: the blue and ultraviolet crown, the yellow belly and the white cheeks.

In the blue tit, the UV colour of the crown is sexually dimorphic and subject to directional intra- and intersexual selection (Hunt *et al.*, 1999; Delhey *et al.*, 2003; Alonso-Alvarez *et al.*, 2004). Structural, noniridescent UV/blue ornaments such as the crown feathers in the blue tit are generated by coherent scattering of light within the medullary 'spongy layer' of feather barbs in

which regular nanolayers of keratin, air and melanin granules are alternated (Prum, 2006; but see Doucet *et al.*, 2006). The yellow colour of the belly is carotenoid-based (McGraw, 2006a) and, although direct experimental evidence of its ornamental function is lacking, it is assumed to be subject to sexual selection because it correlates with individual quality/condition (Senar *et al.*, 2002; Doutrelant *et al.*, 2008). The white colour of the cheek feathers is structural, and it is created by incoherent scattering of all wavelengths at the interface between keratin and the large air-filled cavity that lies at the centre of the keratinocytes (Prum, 2006). Air-filled cavities are variable in size, resulting in the reflectance of all spectrum wavelengths, from which the white colour derives. As far as we know, the signalling role of the white cheek patches has not been investigated in the blue tit. In other tit species, however, the brightness of this area is sexually dimorphic and related to individual quality (Mennill *et al.*, 2003; Ferns & Hinsley, 2004; Galván & Sanz, 2008), suggesting that it may play a role as a sexual signal in the blue tit also. Analysis of the effect of moult speed on the spectral characteristics of these three plumage ornaments offers the possibility to analyse if and how the reflectance properties of structural colours that do (crown) or do not involve (cheeks) the deposition of pigments (melanins) in feather matrix are influenced by moult speed, possibly shedding light on the mechanisms responsible for condition dependence in feather structural colours.

Material and methods

We used 26 blue tits (14 males and 12 females, all nestlings). They were caught in nest-boxes near Vienna, Austria (48°13′N; 16°17′E, $n = 12$) and Agrigento, Italy (37°37′N; 13°36′E, $n = 14$), between 14 May and 4 June 2006. Experiments were carried out at the Istituto Nazionale Fauna Selvatica, Ozzano Emilia Bologna (44°28′N; 11°30′E), where birds were housed in individual indoor cages ($58 \times 33 \times 31$ cm) and fed *ad libitum* with a commercial insect food (Orlux Insect Pate, see http://www.orlux.be for details); fresh apples were given twice a week.

Birds were kept in natural light conditions until 21 June 2006. On this date, they were divided in two groups, housed in two neighbouring indoor rooms and exposed to artificial daylight of 18 h light and 6 h dark (18L: 6D) until 31 July 2006, when all individuals were observed to have commenced body moult. From that date, each group was exposed to two decreasing photoperiods. The 'slow-decreasing photoperiod' group (five females and eight males) was exposed to a 2 min day^{-1} decrease, the 'fast-decreasing photoperiod' group (seven females and five males) to an 8 min day^{-1} decrease (Serra *et al.*, 2007). Each room was illuminated by eight white fluorescent tubes (36 W each). An incandescent lamp (400 W) with a starting rheostat was switched on for 30 min twice a day to simulate dawn and dusk from/to total darkness.

Morphometrics and sex determination

At the beginning of the experiment, we took standard measurements of flattened wing chord length and tail length to the nearest 1 mm, and tarsus length to the nearest 0.1 mm. Body mass was recorded to the nearest 0.1 g every week from 31 July to the end of the moult (dates ranging from 4 October to 22 December). Birds were sexed with molecular markers (Griffiths *et al.*, 1998). Briefly, PCR amplifications were carried out in a total volume of 20 μL. Conditions were as follows: 50 mM KCl; 10 mM Tris–HCl pH 9 (25 °C); 1.5 mM MgCl 2; 0.1% Triton X-100; 200 μM of each dNTP; 200 ng of each primer and 0.9 units of *Taq* polymerase (Promego, Italia S.R.L., Milan, Italy). PCR was performed in a PTC-100 programmable thermal controller (MJ Research Inc., Waltham, MA, USA). An initial denaturing step at 94 °C (for 1 min 30 s) was followed by 30 cycles of 48 °C for 45 s, 72 °C for 45 s and 94 °C for 30 s. A final run of 48 °C for 1 min and 72 °C for 5 min completed the programme. PCR products were separated by electrophoresis for 45–60 min at 7–10 V cm^{-1} in a 3% agarose gel stained ethidium bromide.

Moult recording and weighing

From 31 July to the end of the moult, moult progress was checked every 7 days. Moult was scored for each plumage area (crown, cheeks and breast) as follows: 0 = all old feathers, 1 = moult start (1–30% growing or new feathers), 2 = about half feathers in moult (31–60%), 3 = approaching the end of moult (61–99%), 4 = moult finished (all feathers renewed). Moult duration was estimated for each plumage area, and a mean moult duration estimate was obtained by averaging the three values. One individual died just before completing the moult, when very few new feathers were still growing. This individual was therefore omitted from the analysis of moult duration, but was included in the spectrometric analyses. Exclusion of this individual from the spectrometric analyses, however, did not change the results substantially. Birds were weighed every other week during the length of the experiment.

Spectrometry

At the end of the moult, we measured spectral reflectance of the breast, crown and cheek with an Ocean Optics S2000 Spectrometer (Dunedin, FL, USA). A fibre-optic measuring probe (Ocean Optics) was used to transfer the light from a deuterium tungsten halogen light source (Ocean Optics DH-2000-BAL) to the feathers and to pass the light reflected back to the spectrometer. The probe was held at a 90° angle to the feather surface.

Five consecutive measurements were taken from the patch, the probe being lifted and replaced each time, and averaged for each bird. Reflectance spectra were recorded with OOIBase32 software (Rev 2.21).

Reflectance spectra were restricted to wavelengths between 320 and 700 nm, corresponding to the typical visual range of blue tits (Hart *et al.*, 2000), for the calculation of colour indices. Each spectrum comprised 1104 data points (the reflectance from 320 to 700 nm at approx. 0.34 nm intervals). To summarize objectively the overall variation in reflectance in orthogonal variables, we performed principal components analysis (PCA) on standardized reflectance spectra (Endler, 1990; Hunt *et al.*, 1998; Cuthill *et al.*, 1999). We first summarized mean reflectance values in 10-nm-wide bins, resulting in 38 mean reflectance values between 320 and 700 nm for each spectrum. The largest source of variation between spectra is represented by brightness (mean reflectance), whereas the major objective of this study was to characterize the effect of moult speed on the shape of the reflectance spectrum (Endler, 1990; Cuthill *et al.*, 1999). To separate the analysis of mean reflectance and spectral shape at an early stage, we therefore standardized each spectrum (the reflectance from 320 to 700 nm at 10-nm binned intervals) by subtracting its mean reflectance across all wavelengths. PCA (using nonrotated solution) was then performed on these standardized measurements, separately for each body region (crown, cheeks and breast).

To facilitate the interpretation of our principal component scores, we calculated six commonly used colorimetric variables relevant to the three dimensions of animal colour vision (brightness, hue and four saturation indexes, Hailman, 1977) and compared these with our PC scores. We calculated total brightness as the average percentage of reflectance from 320 to 700 nm, and hue as the wavelength at maximum reflectance. We calculated four measures of chroma (UV, blue, green, red) to approximate variation in saturation across the entire bird-visible spectrum, according to peak sensitivity for the ultraviolet-sensitive (UV chroma), short-wavelength-sensitive (blue chroma), medium-wavelength-sensitive (green chroma), and long-wavelength-sensitive (red chroma) cone types in passerines (Endler, 1990; Cuthill *et al.*, 1999). Thus, we calculated UV chroma as the proportion of total reflectance occurring between 320 and 415 nm, blue chroma as the proportion of total reflectance occurring between 416 and 510 nm, green chroma as the proportion of total reflectance occurring between 511 and 605 nm, and red chroma as the proportion of total reflectance occurring between 606 and 700 nm (Montgomerie, 2006b). In the subsequent analysis, however, we concentrated on the UV chroma for crown and cheeks, and on green chroma for breast feathers, as these two indexes should capture the variation in colour saturation in structural and carotenoid feather colours, respectively.

Statistical analyses

Univariate ANOVAS were carried out to model the effect of sex and treatment (factors) on biometric and morphological variables pre- and post-experiment. All variables were tested for homogeneity of variance and normality of distribution. Statistical analyses were carried out with SPSS version 15 (SPSS Inc., Chicago, IL, USA).

Results

Effect of photoperiod manipulation on moult speed and body mass

Birds from the experimental group exposed to a rapidly decreasing photoperiod increased moult speed and completed their body moult in 58.4 days ± 5.18 SD (range = 52.7–70.0), in contrast with the 82.8 days ± 9.95 SD (range = 69.3–108.3) taken by the birds subject to the slowly decreasing photoperiod. We found no effect of sex or population origin on moult speed (sex, $F_{1,21} = 0.60$, $P = 0.45$; population, $F_{1,21} = 0.06$, $P = 0.81$; photoperiod, $F_{1,21} = 52.62$, $P < 0.0001$). Similar results were obtained for the moult duration of the three plumage areas separately (sex, all $P > 0.10$, photoperiod, all $P < 0.002$).

Birds from the two populations did not differ in any of the morphometric (body mass, wing length and tarsus length) and spectrometric measures (all $P > 0.17$) and the data from the two populations were therefore pooled in the subsequent analyses. Body mass did not differ between the two experimental groups at the beginning of the experiment (moult speed, $F_{1,26} = 1.23$, $P = 0.28$; sex, $F_{1,26} = 0.23$, $P = 0.64$), when birds were half-way through their moult (moult speed, $F_{1,25} = 2.21$, $P = 0.15$; sex, $F_{1,25} = 0.01$, $P = 0.99$) and at the end of their moult (moult speed, $F_{1,25} = 0.16$, $P = 0.69$; sex, $F_{1,25} = 0.85$, $P = 0.37$).

Colour and interpretation of PC scores

The reflectance spectra of crown feathers showed a peak in the UV region, whereas reflectance of cheek feathers generally increased across the bird-visible spectrum, and the reflectance spectra of the breast feathers were typically bimodal, with a plateaux between 510–700 nm and a secondary peak around 375 nm, typical of carotenoid feather colours (e.g. Johnsen *et al.*, 2003; Shawkey *et al.*, 2006; Fig. 1). For the PCA on crown feathers, PC1 explained 85.5% of the variation in reflectance, and PC2 explained 14.4% of the variation. For the PCA on cheek feathers, PC1 explained 87.1% of the variation in reflectance, and PC2 explained 10.4% of the variation. For the PCA on breast feathers, PC1 explained 85.3% of the variation in reflectance, and PC2 explained 14.0% of the variation. For all body regions, the factor loadings for PC1 were strongly

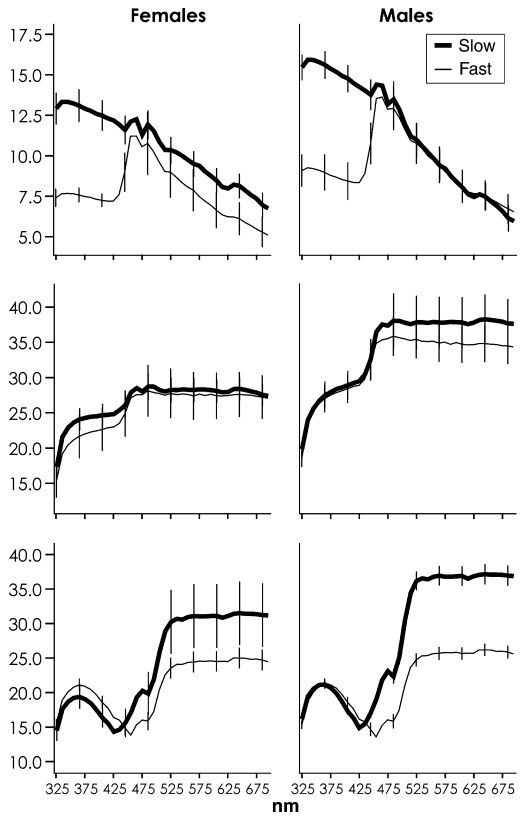

Fig. 1 Reflectance (%, mean ± SE) spectra of the blue tit feathers in three body parts, from the top: UV/blue crown, white cheeks, and yellow breast (thin line, fast moult; thick line, slow moult).

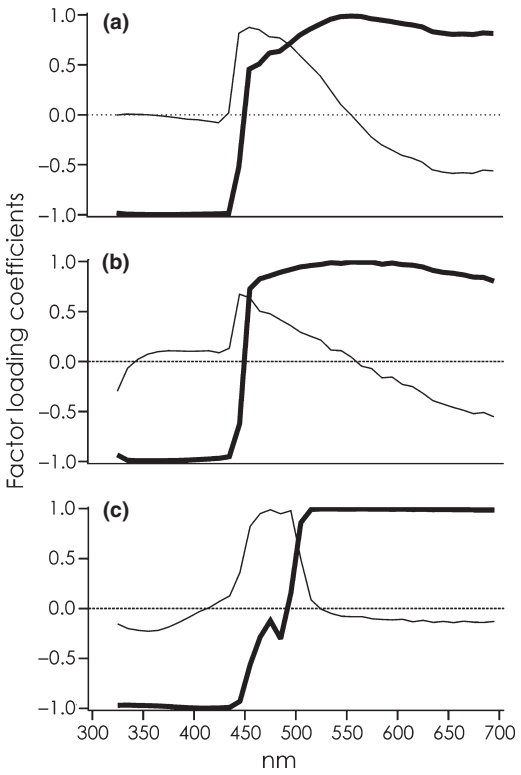

Fig. 2 Association between principal component factor-loading coefficients PC1 (thick lines) and PC2 (thin lines) and wavelength for UV/blue crown (a), white cheek (b) and yellow breast feathers (c).

negative in the short wavelength spectrum (< 450 nm for crown and cheeks and < 485 for breast feathers) and strongly positive above (Fig. 2). Indeed, PC1 was highly negatively correlated with UV chroma and positively correlated with green chroma and red chroma for all body regions (Table 1). In contrast, factor loadings for PC2 varied in direction and magnitude at different wavelengths in the three different body parts (Fig. 2). In particular, it was positively correlated with blue chroma and brightness on the crown and the cheeks and with blue chroma only in the breast (Table 1). PC2 were negatively correlated with hue and red chroma in cheeks only. Altogether, these results suggest that PC1 captured the variance in saturation, i.e. the relative reflectance in short (UV) vs. long (green and red) wavelengths, whereas PC2 captured the variance in intermediate (blue) wavelength reflectance.

Effect of moult speed on feather colour

Fast moulting was associated with a remarkable reduction of the short wavelength reflectance on crown feathers. In particular, fast-moulting birds showed a strongly reduced reflectance below 450 nm as compared with their slow-moulting counterparts. In contrast, reflectance spectrum above 450 nm was comparable between treatments (Figs 1 and 4a). The reduction of UV reflection on the blue crown feathers resulted in an overall reduction in brightness and an increase in hue (i.e. a significant shift of peak reflectance towards long wavelengths, Table 2, Fig. 3a). As already found in previous studies, crown blue feathers were sexually dimorphic in the short wavelength part of the reflectance spectrum, as suggested by the significant difference between sexes along the PC2 axis (Table 2, Fig. 3a). Reflectance spectrum of the white cheek feathers was also dimorphic, with males showing higher brightness, UV chroma and PC1 scores than females (Table 2, Fig. 3b). In contrast with the UV/blue crown feathers, white feathers of the cheeks were not significantly affected by moult speed (Table 2, Figs 1, 3b and 4b).

On the yellow breast feathers, fast-moulting birds had on average a reflectance spectrum characterized by lower reflectance above 450 nm, as compared with slow-moulting birds (Fig. 1). As a consequence, fast-moulting birds showed, on their breast feathers, lower brightness, PC1 scores and green chroma (Table 2, Fig. 3c) than their slow-moulting counterparts. In contrast, we did not find any sexual dimorphism in breast colouration in our

Table 1 Correlation coefficients between PC scores and reflectance indexes (see methods for details). Significant correlations are in bold.

	Crown		Cheeks		Breast	
	PC1	PC2	PC1	PC2	PC1	PC2
UV chroma (320–415 nm)	−0.96	−0.11	−0.83	0.36	−0.95	−0.18
Blue chroma (415–510 nm)	0.21	**0.71**	0.18	**0.92**	−0.29	**0.84**
Green chroma (510–605 nm)	**0.96**	0.07	**0.86**	−0.28	**0.93**	−0.13
Red chroma (605–700 nm)	**0.78**	−0.29	**0.58**	−0.76	**0.89**	−0.23
Hue (λ_{Rmax})	**0.81**	0.20	−0.14	−0.84	−0.01	−0.03
Brightness (mean *R*)	−0.26	**0.55**	**0.81**	**0.41**	**0.89**	0.05

Table 2 Effect of moult duration and sex on colour indexes of blue tit plumage reflectance of three body parts (for details on colour indexes see methods). Significant effects are in bold (*b* = standardized beta values).

		PC1			PC2		
Body part	Factor	*b**	*F*	*P*	*b**	*F*	*P*
Crown	Sex	0.200	2.045	0.166	−0.423	**5.649**	0.026
	Moult speed	**0.831**	**52.735**	< 0.0001	0.101	0.117	0.736
Cheeks	Sex	−0.570	**10.896**	0.003	0.051	0.107	0.746
	Moult speed	−0.080	0.128	0.724	0.282	1.715	0.203
Breast	Sex	−0.212	1.922	0.179	0.215	1.129	0.299
	Moult speed	−0.695	**22.860**	< 0.0001	−0.233	1.299	0.266
		Hue			Brightness		
Crown	Sex	−0.067	0.263	0.613	−0.236	1.611	0.217
	Moult speed	**0.773**	**35.142**	< 0.001	−0.452	**5.930**	0.023
Cheeks	Sex	0.022	0.012	0.914	−0.520	**8.262**	0.009
	Moult speed	−0.282	1.948	0.176	−0.116	0.414	0.526
Breast	Sex	0.099	0.222	0.642	−0.211	1.344	0.258
	Moult speed	0.034	0.027	0.872	−0.490	**7.219**	0.013
		UV chroma			Green chroma		
Crown	Sex	−0.069	0.233	0.634	0.130	0.781	0.386
	Moult speed	−0.747	**27.649**	< 0.0001	**0.728**	**24.657**	< 0.0001
Cheeks	Sex	**0.418**	**4.885**	0.037	−0.400	**4.352**	0.048
	Moult speed	−0.101	0.287	0.598	0.074	0.150	0.702
Breast	Sex	0.199	2.020	0.169	−0.275	3.366	0.080†
	Moult speed	**0.751**	**28.696**	< 0.0001	−0.689	**20.758**	< 0.0001†

*Male and slow moult as reference levels.
†After arcsine transformation.

sample for any of the spectrometric indexes considered here (Table 2, Fig. 4c). The large variation observed in the group of slow-moulting females (Fig. 3, left bottom panel) was due to a single individual who showed very little yellow on the feathers. Removing this outlier did not change significantly the results.

Discussion

Day-length change resulted in an increased moult speed in the individuals exposed to a more rapidly decreasing photoperiod, in agreement with previous experiments (e.g. Dawson *et al.*, 2000; Serra *et al.*, 2007). The duration of the body feather moult was within the range reported for the blue tit and other passerine species (Jenni & Winkler, 1994), suggesting that our experimental manipulation was within the natural variation. We found a significant effect of moult speed on the reflectance properties of two of three feather sexual signals that were examined in our blue tits, that is, UV/blue crown feathers and yellow breast feathers. Despite the significant differences between slow- and fast-moulting birds in brightness and saturation, the shape of the spectra we obtained was qualitatively similar to that reported in other studies (e.g. Andersson *et al.*, 1998; Hunt *et al.*, 1998; Örnborg *et al.*, 2002; Alonso-Alvarez *et al.*, 2004; Doutrelant *et al.*, 2008), suggesting that our experimental manipulation did not cause unnatural effects on plumage colours.

In contrast, white cheek feathers were substantially unaffected by moult speed, although they showed significant sexual dimorphism, with males exhibiting

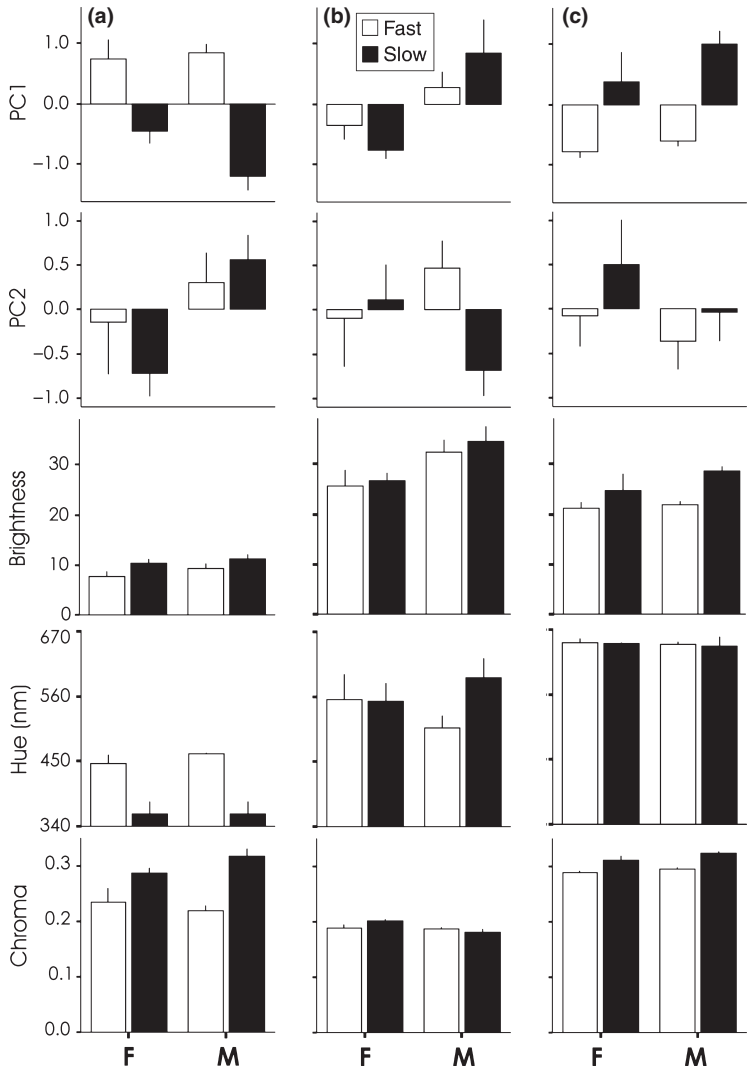

Fig. 3 Mean ± SE of principal component scores, brightness (mean reflectance), hue (λ_{Rmax}) and chroma of blue tit feathers in three body areas: (a) UV/blue crown feathers, (b) white cheeks feathers, and (c) yellow breast feathers (open bars, fast moult; black bars, slow moult; M, males, F, females). UV chroma (320–415 nm) and green chroma (510–605 nm) are reported for crown and cheek feathers, and for breast feathers respectively.

higher brightness than females. A previous study on the blue tit did not find evidence of sexual dichromatism on the white feathers on the cheeks (Hunt *et al.*, 1998), whereas sexual dichromatism in white cheek feathers has been reported for another parid, the black-capped chickadee (*Poecile atricapilla*, Mennill *et al.*, 2003). Interestingly, whereas in this species other sexually dimorphic sexual traits (black crown) have been found to covary with male quality/condition (rank), brightness of white cheek feathers was not associated with an individual's rank. A lack of association between structural white feather colour and condition has also been reported in other species (Shawkey *et al.*, 2006). It would be interesting to see if females show a preference for males with brighter cheeks or not.

The effect of moult speed differed substantially between blue crown feathers and yellow breast feathers. Birds whose moult speed was experimentally increased grew crown feathers with strongly reduced UV reflectance compared with their slow-moulting counterparts. In contrast, crown feather reflectance at long wavelengths (i.e. > 450 nm), which is negatively correlated with the concentration of melanins in the feather keratin matrix (McGraw, 2006b), was unaffected by moult speed. This result suggests that, as far as feather melanization can be estimated from the reflectance in the long-wave length spectrum, fast moulting was not associated with a measurably reduced melanin deposition. Similarly, an experiment on American goldfinches (*Carduelis tristis*), which were subjected to a coccidial infection, did not evidence any significant effect on melanin pigmentation (McGraw & Hill, 2000).

Yellow breast feathers of fast-moulting birds showed, in contrast, a significant reduction of total reflectance and yellow saturation, as indicated by the significant decrease in brightness and green chroma. A similar reduction of

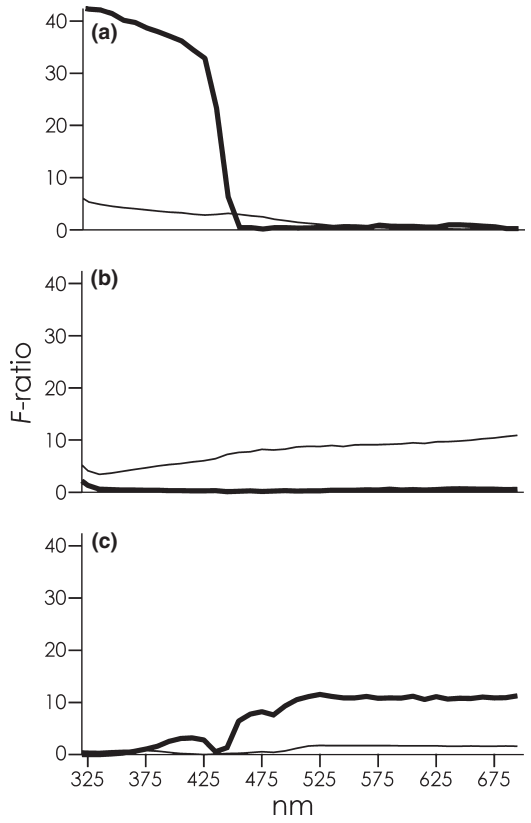

Fig. 4 *F*-ratio values of binned (10 nm) reflectance values of the three feather ornament types (a, crown; b, cheeks; c, breast). *F*-ratios were obtained from a GLM in which sex (thin line) and moult speed (thick line) were entered as factors.

yellow saturation in carotenoid feather signals, associated with fast moulting rate, has been found in two other passerine species, the rock sparrow *Petronia petronia* (Serra *et al.*, 2007) and the great tit *Parus major* (Ferns & Hinsley, 2008). Presumably a faster moult resulted in the base feathers, to which the carotenoids are added during growth, being greyer and having a lower overall brightness, as suggested by the reduction of the reflectance along the whole curve. However, the reduction of reflectance was more pronounced above 525 nm than between 425 and 575 nm, resulting in a lower yellow saturation. Whether this effect is also due to a reduction of the carotenoid deposition rate during the moult, to a different carotenoid composition (as the shift of the curves towards longer wavelength observed between 425 and 525 nm may suggest) or to different reflectance properties of the keratin/carotenoid microstructure would require direct measurement of feather carotenoid content and microstructural analyses.

If moult speed does not affect melanin content of the crown feathers, the observed decrease of UV reflectance associated with fast-moulting must be because of changes in the nanoscale structures responsible for coherent light scattering. The comparison of the effects of moult speed on the reflectance properties of white (cheeks) and UV/blue (crown) feathers may, however, allow some speculation as to the mechanism responsible for the reduced UV reflectance of the crown feathers in fast-moulting birds. It has been shown that the colour of the ultraviolet UV-blue feather of the eastern bluebird (*Sialia sialis*), which has a reflectance spectrum very similar to blue tits, is caused by coherent scattering of light within the medullary 'spongy layer' of feather barbs and that the size of spongy-layer elements, the depth of the spongy layer and hence by the distance from the underlying melanin layer are critical to UV-blue colour production (Shawkey *et al.*, 2003). Structural white, which is instead generated by air/keratin nanostructures of variable size scattering light of all wavelengths (Prum, 2006), was not affected by moult speed in our blue tits. It is therefore unlikely that fast moulting hindered the formation of the smallest nanostructures or caused a reduction of the depth of the spongy layer as this would have affected both UV/blue and white feathers. Therefore, the effect of moult speed on structural crown colours may rather be due to a decrease in the regularity of the size of the air/keratin or melanin nanostructures involved in light coherent scattering. For example, a fast feather growth may not allow sufficient time for the melanin molecules to be arranged in regular crystal-like structures (Prum, 2006). A microstructural analysis of feather structure is necessary to distinguish between these and other possible explanations.

In temperate regions moult is usually temporally constrained between the end of reproduction and the onset of migration or wintering (Holmgren & Hedenström, 1995). Our results revealed that moult duration has a strong negative effect on the expression of structural UV/blue feather ornaments, suggesting that structural colours may therefore signal not only condition during (or immediately preceding) the moult but also an individual's performance during the whole previous breeding season (or the time of birth in the case of first-year birds). Indeed, low-quality birds, which usually reproduce later in the season, and consequently their offspring, may have less time to complete their moult after breeding. Thus, the moult-constrained expression of UV feather signals may therefore represent an additional, previously not recognized, long-term cost of late breeding (O'Donald, 1972; Norris *et al.*, 2004), i.e. poorer quality signals in the following breeding season in both parents and offspring. The difference in the duration of body feather moult between the two experimental groups of blue tits was on average 22.5 days, a variation of the same magnitude as observed in natural conditions (Jenni & Winkler, 1994). It is therefore likely that variation in moult speed contributes to the phenotypic variation in the expression of structural as well as pigment feather signals (Serra *et al.*, 2007; Ferns &

Hinsley, 2008). In addition to other nonmutually exclusive mechanisms, like intra-sexual competition (Alonso-Alvarez *et al.*, 2004), the observed link between moult duration and UV chroma and brightness of structural UV/blue crown feathers may explain how the honesty of this signal is maintained, creating a link between, for example, reproductive effort and future reproductive success or survival (Dawson *et al.*, 2000).

It may be noted, however, that feather structural colours are widespread in tropical species, where the constraint between the end of the breeding season and the time available for moulting may be less strong than in temperate regions. However, moult is energy-demanding process and it may interfere, or traded-off against other nonexclusive functions. Indeed, it has been estimated that moulting passerine birds show an increase in metabolic rate of around 30% (Campbell & Lack, 1985; Cyr *et al.*, 2008). Feather production costs include more than the costs for keratin synthesis and they mainly consist of the costs of maintaining the tissues necessary for feather production (Lindstrom *et al.*, 1993). Slow moulting birds are therefore likely to incur in higher metabolic costs as compared to fast moulting birds simply because they have to maintain these tissues for a longer time. Not surprisingly, experimental studies indicate that strong immune reactions have a negative effect on the moult process, suggesting a trade-off between immune reactions and moult (Ilmonen *et al.*, 2000; Sanz *et al.*, 2004). Also, it is true that the immune system seems to be more active in tropical species because of a stronger parasite pressure (e.g. Møller, 1998). So, it has been suggested that in tropical species may exist a moult-immune system activation trade-off (Moreno, 2004). Following this reasoning, structural colours, mediated by moult speed and moult costs, may reveal immune quality. Indeed, a direct evidence of the link between parasite infection and structural feather colours has been found, although in a temperate species (Hill et al., 2005).

In conclusion, our results suggest that the time available for moult, and hence the speed at which new feathers grow, may be a previously undervalued explanation of the observed condition-dependence of structural feather colours in natural populations.

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