Melanin, carotenoid and structural plumage ornaments: information content and role in great tits *Parus major*

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The importance of plumage colour as an indicator of individual quality and the basis of sexual selection has long been recognized. Of the three generally distinguished classes of plumage colours, melanin-based ornaments are traditionally considered to provide less reliable information than carotenoid-based traits. However, the role of structural ornaments in multiple signalling systems has rarely been examined, and no study has compared the information content and role of the three ornament types simultaneously. Here we investigated three plumage ornaments in great tits *Parus major*: the size of the melanin-based breast stripe, the carotenoid-based colour of the yellow breast and the structurally based reflectance properties of the black crown. We worked on both the mechanistic and the functional levels. First, we assessed the dependence of ornaments on body condition during moult using ptilochronology. Second, we estimated assortative mating for these traits, as a measure of mutual sexual selection. Only the spectral attributes of crown feathers correlated with body condition during moult. However, breast stripe size was related to age, while the brightness of the yellow breast indicated body size. Relative crown ultraviolet reflectance was much higher in males than in females. Assortative mating was strongest for crown ultraviolet reflectance, but composite measures suggest that a system of multiple sexually selected traits with different information content may work in this population. These data support the accumulating evidence that the condition-dependence of melanin and carotenoid coloration is not qualitatively different. They also suggest that more research should target the reflectance properties of dark plumage areas in general, and ultraviolet crown ornamentation in tits in particular.

Mate choice in animals often makes use of multiple signal traits (Candolin 2003). According to theoretical models, different signals in the same system may provide similar information or multiple messages (Møller and Pomiankowski 1993, Johnstone 1996), but some ornaments may also be unreliable or even threshold cues (Holland and Rice 1998). Theory (Pomiankowski and Iwasa 1998) and empirical data (Wiens 2001) suggest that the evolution of multiple signal systems is rapid. Recent studies have detected systems of multiple ornaments within the same general morphological traits (Møller and Petrie 2002, Badyaev and Young 2004), but also among very different and clearly distinct traits (Møller et al. 1998, Andersson et al. 2002). Among the most promising subjects for multiple ornamentation studies are avian plumages.

Some plumage ornaments vary in the spatial extent of a distinct patch or pattern (Møller 1988, Yezerinac and Weatherhead 1997), while in other cases the variability involves the light reflectance, that is, the colour of a certain plumage area (Siitari and Huhta 2002, Safran and McGraw 2004). It has also been recognized that the size and the colour of a patch may be correlated, and even more subtle interrelations among different attributes may make a single plumage ornament a multiple signal (Badyaev et al. 2001).

It is possible to classify plumage colours according to their metabolic origin. The two traditionally assessed types are based on the deposition of melanins and carotenoids. As access to carotenoids is limited to ingestion with food (Goodwin 1984), while melanins are synthesized by the birds themselves (Griffith et al.

2006), it has been proposed that melanin-based ornaments are less sensitive indicators of body condition or individual quality than carotenoids (Gray 1996, Badyaev and Hill 2000). Support for this claim is, however, very mixed (Griffith et al. 2006). Carotenoid based ornamentation was related to body condition in some studies (McGraw and Hill 2000, Senar et al. 2003), but not in others (Fitze and Richner 2002, Parker et al. 2003). Similarly, melanin-based ornaments seemed to reflect body condition in a few studies (Slagsvold and Lifjeld 1992, Veiga and Puerta 1996), but not in others (Hill and Brawner 1998, McGraw et al. 2002). Most of the above studies have measured or even experimentally manipulated body condition during moult (e.g. Veiga and Puerta 1996, McGraw and Hill 2000, Senar et al. 2003), which makes these conclusions robust. Finally, the contradictory results obtained when comparing the information content of melanin and carotenoid traits in the same population (McGraw and Hill 2000, Fitze and Richner 2002, Parker et al. 2003, Senar et al. 2003) also suggest that more research is needed before drawing conclusions on the relative importance of these two ornament types as sexually selected indicators of quality.

Structural plumage coloration is based on the structure rather than the biochemical constitution of the feather (Shawkey et al. 2003). Recent surveys have revealed widespread variation in structurally based ultraviolet reflectance both among and within bird species (Eaton and Lanyon 2003, Bleiweiss 2004, Eaton 2005). Ultraviolet reflectance of plumage has been shown to be subject to sexual selection via male competition (Alonso-Alvarez et al. 2004, Siefferman and Hill 2005a), mate choice (Andersson et al. 1998, Siitari et al. 2002) and extrapair paternity (Johnsen et al. 2001, Doucet et al. 2005). Studies have also found links between male ultraviolet reflectance and postmating reproductive investment by females, such as sex ratio adjustment (Griffith et al. 2003) and nestling feeding rates (Johnsen et al. 2005). The importance of ultraviolet ornamentation as part of a multiple signalling system is less clear (Siefferman and Hill 2003). The information conveyed by a sexual signal may also depend on the sex (Price and Burley 1994, Siefferman and Hill 2005b) and the age of the signaller (Badyaev and Duckworth 2003, Hegyi et al. 2006). Until now, few studies have evaluated the information conveyed by multiple traits while considering sex- and age related differences, and to the best of our knowledge, no study has simultaneously examined the condition-dependence and relative role of carotenoid, melanin and structural plumage ornaments.

The plumage colour of great tits *Parus major* is to a large extent a combination of black and yellow surfaces. The most striking yellow areas are those on the sides of the breast. The colour of these is known to result from an environmentally limited deposition of carotenoids (Hõrak et al. 2001, Fitze et al. 2003), but its significance in sexual selection is completely unknown. The black patches of the plumage are due to melanin deposition, and include the most widely examined ornament of the species, the breast stripe. The breast stripe size of males has been found to confer a mating advantage (Norris 1990) and indicate parental quality (Norris 1990) as well as offspring survivorship (Norris 1993, but see Kölliker et al. 1999). Finally, a large black area on the great tit plumage is the crown. The size of this trait does not vary, but its appearance is often strikingly luminous on males while duller on females (Svensson 1992). It seems therefore fruitful to investigate whether crown reflectance represents a sexually dimorphic, structural plumage ornament such as in the closely related blue tit *Cyanistes caeruleus* (Hunt et al. 1998 and many consecutive studies).

Our first goal in this study is to assess the information content of plumage coloration in great tits, including carotenoid-based yellow breast colour, the size of the melanin-based breast stripe, and the structurally based ultraviolet ornamentation of the black crown. We examine: (i) if body condition during the single postnuptial moult predicts the expression of ornaments (Grubb 1995, Senar et al. 2003), (ii) if any relationship with condition persists after moult (Cotton et al. 2004), (iii) whether ornamentation indicates body size, a probable determinant of social dominance (Koivula et al. 1993), and (iv) whether ornament expression and dependence on body condition during moult differ between the sexes, and between recently fledged young and older birds. As a second goal, we examine breeding data to test for assortative mating in relation to components of ornamentation, as a measure of mutual sexual selection for these plumage traits (Jawor et al. 2003, MacDougall and Montgomerie 2003, Safran and McGraw 2004).

Materials and methods

Quantifying ornamentation

Here we aimed to measure ornamentation in three areas of the great tit plumage. A comprehensive study of colour on a plumage consisting of distinct patches would involve measurements of patch size and reflectance for each area. In our case, the yellow on the breast is poorly outlined, while the crown is always totally black, so the only attributes we could study for these surfaces were the reflectance curves. For the distinct breast stripe, we only measured patch size, but not reflectance. This could potentially lead to loss of information. However, data collected in another study showed that the brightness of the breast stripe was

significantly positively related to that of the crown, while variation in the relative ultraviolet reflectance of the breast stripe was negligible (G. Hegyi et al. unpubl. data). Thus, we can conclude that independent information provided by the melanin-based ornamentation of the breast is appropriately quantified by the size of the breast stripe (also see Results).

Field procedures

Non-breeding great tits (n = 89 individuals) were caught by mist-nets at two feeders baited with sunflower seed on 8 October and 5 November 2005 in Pilis Mountains, Hungary, on the plots of nestboxes used for a long-term study of hole-nesting species (e.g. Török and Tóth 1999). Great tits undergo a single annual moult from summer to early autumn (Svensson 1992). We excluded the few birds still moulting their contour or flight feathers in October from the analyses of the respective plumage area. Only two individuals were caught twice, and we omitted their second observation from the analyses. We ringed birds with standard numbered metal rings, and determined age (first-year or older) and sex as described in Svensson (1992). We measured tarsus length with a calliper (nearest 0.1 mm) and body mass with a spring balance (nearest 0.1 g). To quantify the size of the black breast stripe, we took a digital picture of the breast plumage outdoors, from a fixed distance. We collected approximately ten feathers from standard positions on the yellow of the breast and on the black crown, and stored them in envelopes for later spectral measurements in the laboratory. Finally, we also collected the left and right second outermost tail feathers from each bird and stored these individually in envelopes until measurements of growth rate. We caught breeding individuals in the springs of 2004 and 2005 at the same area as the non-breeders, when they were feeding approximately eight day old nestlings. The ringing and measurement of the birds was the same as that described above, except that we did not collect tail feathers from these individuals. We caught a total of 39 and 18 pairs in 2004 and 2005 respectively, but sample sizes vary among analyses due to missing data. For example, only a subset of the 2004 birds was sampled for black crown feathers. We caught two females in both years, but only their 2004 measurements are used here.

Feather growth rate

To measure body condition during moult, we used ptilochronology (Grubb 1995). This method measures the growth rate of tail feathers based on the breadth of the visible daily growth bars on their surface. Since the replacement of flight feathers impairs the manoeuvring capacity of birds, there is directional selection for high rate of feather growth, which is, however, an energetically expensive process (Murphy 1996). As a consequence, the rate of tail feather growth is strongly positively related to nutritional condition (Grubb 1989, 1992, also see Mennill et al. 2003). Several studies have successfully used ptilochronology to assess the condition-dependence of plumage ornaments (e.g. Hill and Montgomerie 1994, Keyser and Hill 1999). We used the method adapted to our study species by Senar et al. (2003). Under intense direct illumination, we measured with a calliper the total width of the first ten visible pairs of growth bars (light and dark) from the distal end of the feather to the nearest 0.1 mm. To avoid bias due to measurer experience, we ordered samples of individuals according to their arbitrary number. We measured the left feathers of eight birds, the right feathers of another eight birds, and so on, until having measured one side on all individuals. The whole process was then repeated for the remaining sides of the birds. Feather growth rate was highly repeatable between the two temporally separated measurements of the same individual (Lessells and Boag 1987, $r = 0.587 \pm 0.059$ SE, $n = 78$, $P < 0.001$).

Breast stripe sizes and spectral measurements

To take a picture of breast stripe size, we first smoothed the plumage and then placed the fully extended bird in a box, upholstered with foam material to prevent it from moving. A reference ruler was fixed on the box for calibration of patch areas. We analysed the pictures with the public domain program NIH Image (available on the Internet at http://rsb.info.nih.gov/nih-image/). To calculate breast stripe surface, we followed the method described by Figuerola and Senar (2000) which they showed to be the most repeatable measure of the breast stripe size. We measured the stripe surface as the area of the black band between the point of inflexion, where the ventral stripe widens to a throat patch, and the posterior end of the stripe, to the nearest square millimetre. The repeatability (Lessells and Boag 1987) of the breast stripe area measurement calculated from individuals which were photographed twice in succession was highly significant ($r > 0.96$, SE < 0.04, $P < 0.001$ for every season).

We measured the spectral reflectance of crown and yellow breast feathers using an USB2000 spectroradiometer with a Mini-DT deuterium-halogen light source (Ocean Optics Europe). We used a bifurcated fibre optic probe on which a black plastic tube was fixed to standardize measuring distance and exclude ambient light. The diameter of the measurement spot was 3 mm. We held the sampling optic perpendicular to the sample. We placed eight feathers on black velvet on

the top of one another and measured colour in an area of their visible surface. The velvet itself has a characteristic reflectance curve with high reflectance above 650 nm, which made it possible to detect and avoid the influence of background reflectance on our measurements. We took two or three scans for each set of feathers in 0.37 nm steps, with a measurement from a WS-2 white reflection standard (Ocean Optics Europe) before each scan.

Statistical analyses

We used principal component analyses (PCA-s) to condense the information contained in the reflectance spectra of black and yellow feathers into a few axes (Cuthill et al. 1999). Principal components solve the problem of correlation among classical spectral measures (e.g. hue, chroma, saturation), make it easier to interpret multimodal spectra (including those of yellow feathers, Bleiweiss 2004), and capture the directions of difference in spectral shape which are most characteristic to the sampled population, which may not be true for classical spectral measures (Cuthill et al. 1999). First, we averaged the two or three spectra for a plumage area of an individual, and then averaged the readings within the resulting spectra for every 10 nm from 320 to 700 nm (the range visible to tits, Hart et al. 2000). We then used the resulting 38 bands as input variables for the PCA. Separate PCA-s were conducted for yellow and black feathers, but males and females were analysed together, because we were also interested in the sex differences of colour. We performed separate PCA-s for breeding and autumn birds, because of the potentially substantial seasonal changes in colour caused by wear (Örnborg et al. 2002), and because we analysed the two datasets separately.

The component loadings of individual spectral bands for the first two PCs of autumn yellow and black feathers are shown in Fig. 1. Both yellow and black PC1 reflected monochromatic brightness ("whiteness" and "greyness", respectively). Yellow PC2 was very highly correlated with the classical spectral measure yellow chroma ($r = 0.949$, $P < 0.001$), so we interpreted it as yellow chroma or "yellowness". Yellow chroma ($(R_{700} - R_{450})/R_{700}$) should directly indicate the influence of carotenoids on colour since lutein, the dominant carotenoid in great tit feathers, absorbs most strongly at 450 nm (Partali et al. 1987, Johnsen et al. 2003). Finally, black PC2 decreased with increasing reflectance at low wavelengths relative to high wavelengths. It was highly negatively correlated with the classical spectral measure UV chroma ($R_{320 - 400}/R_{320 - 700}$, $r = -0.776$, $P < 0.001$). We therefore refer to an increase of this PC as a decrease of relative UV reflectance. Loadings calculated for spring PC-s were
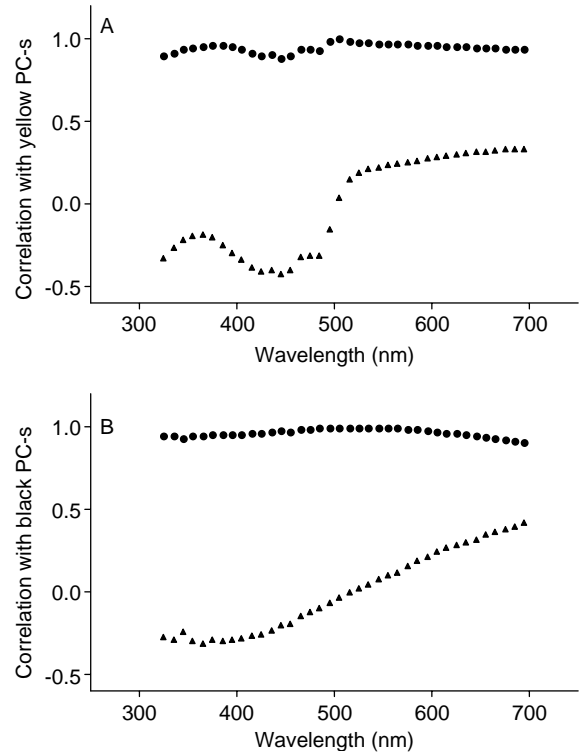

Fig. 1. Correlations of the first and second principal components of: (A) yellow breast, and (B) black crown feathers with individual 10 nm wavelength bands. Circles, PC1, and triangles, PC2.

very highly correlated to their autumn counterparts (from $r = 0.885$ to $r = 0.981$, all $P < 0.001$), which indicates that they contained very similar information, and feather wear did not confound our results.

To assess the dependence of ornamentation on body condition during moult, we ran general linear models. The dependent variable was breast stripe size or a single PC. We entered sampling date (first or second occasion), sex and binary age as fixed factors, and tarsus length (standardized for sex), body mass (standardized for sex and date) and feather growth rate as covariates. We standardized mass and tarsus length (mean $= 0$, SD $= 1$) to eliminate confounding covariation among discrete and continuous independent variables (Norman and Streiner 2000). We also considered two- and three-way interactions, with the exception of interactions involving two or three continuous variables, which are difficult to interpret. We backward removed nonsignificant terms, and reintegrated them to the final model one by one, to avoid nonsignificance due to overparameterization of the model (e.g. Peters 2000, Török et al. 2004). The selection procedure converged in all cases.

To assess assortative mating on the spring data, we first looked for differences among years in the mean or

the variance of the respective spectral variable (Student t tests and F tests, respectively). We conducted these tests separately for male and female data. In case of any year effect, we standardized the respective variable to a mean of 0 and a standard deviation of 1 among the two years. Thereafter, we pooled the two years and conducted separate Pearson correlations for each of the five colour measures of pair members. We did not evaluate differences in slopes between years because of the relatively few data points from 2005. We report means with their standard errors. Probability values are two-tailed throughout, and we use $P = 0.05$ as significance threshold. Statistical analyses were conducted in Statistica 5.5 (StatSoft 2000).

Results

Interdependence of ornament measures

First, we assessed pairwise correlations between the five plumage attributes analyzed here, to reveal whether they can be regarded as independent traits. Black PC1 and PC2, as well as yellow PC1 and PC2 are by definition orthogonal. All other pairwise correlations between attributes were nonsignificant ($r < 0.118$, $P > 0.271$), except that between breast stripe size and black PC2 ($r = -0.647$, $n = 86$, $P < 0.001$). This correlation was caused by sex-differences in both traits, as there was no significant relationship in sex-standardized data ($r = 0.030$, $n = 86$, $P = 0.783$). The five variables can therefore be treated as independent in the following analyses.

Breast stripe size

Results for the information content of ornaments are summarized in Table 1. Mean breast stripe size increased from October to November. The breast stripe was also larger for males than females, and for old birds compared to first-years. However, no relationship with feather growth rate could be detected. Assortative mating for this trait was non-significantly positive ($r = 0.218$, $n = 51$, $P = 0.125$).

Yellow breast reflectance

Yellow PC1 was positively related to tarsus length, and exhibited a date- and sex-dependent relationship with body mass at capture, but it was unrelated to feather growth rate. Yellow PC2 was also unrelated to feather growth rate, but it was negatively correlated with body mass at capture, and its relationships with age and tarsus length were date-dependent. Assortative mating was non-significantly positive for yellow PC1 ($r = 0.221$,

$n = 52$, $P = 0.115$), but very far from significance for yellow PC2 ($r = 0.043$, $n = 52$, $P = 0.760$).

Black crown reflectance

Black PC1 was significantly negatively related to feather growth rate, but the interaction of the covariate with capture date indicated that the relationship was significant for November ($F_{1,24} = 5.796$, $P = 0.024$), but not for October ($F_{1,52} = 0.349$, $P = 0.557$). Black PC2 was much smaller in males than in females (Fig. 2a), with a slightly smaller sex-dependence in November than in October (data not shown). Black PC2 was strongly negatively related to body condition at moult as reflected by feather growth rate (Fig. 2b). There was also a weak three-way interaction between capture date, sex and feather growth rate, but separate analyses revealed no significant interaction between date and feather growth rate in either sex (not shown). Assortative mating was non-significantly positive for black PC1 ($r = 0.253$, $n = 40$, $P = 0.116$), and significantly positive for black PC2 ($r = 0.320$, $n = 40$, $P = 0.044$, Fig. 3).

Overall patterns of assortative mating

Obtaining nearly significant relationships in the same direction for multiple independent variables by chance is unlikely, so we analyzed the assortative mating statistics of the four plumage ornaments which were found to indicate at least one aspect of individual quality (breast stripe size: age, yellow PC1: body size, black PC1 and 2: body condition during moult) in a Fisher combined probabilities test (Sokal and Rohlf 1981). This test was significant ($\chi^2 = 19.040$, $df = 8$, $P = 0.015$), which implies that the overall pattern of mating correlations suggests assortative mating for these four ornaments.

Discussion

Here we examined the dependence of three potential plumage signals on body condition during moult in male and female great tits. We found significant condition-dependence in the spectral attributes of the black crown. This ornament also exhibited strong sexual dichromatism in the ultraviolet range. The size of the black breast stripe and the colour of the bordering yellow areas were unrelated to body condition at moult, but indicated age and body size, respectively. Finally, we detected the strongest signs of assortative mating for ultraviolet crown colour, but found similar tendencies for the other three quality-indicator ornaments. These

Table 1. Descriptors of the plumage colouration of great tits after moult, in relation to capture date (October or November 2005), sex, binary age, tarsus length (standardized for sex), body mass (standardized for date and sex), and feather growth rate (FGR). General linear models with backward stepwise model selection, see Methods for details. Only the terms contained in the final models are shown here. Signs in parentheses indicate the direction of a significant relationship. —, not in the final model; *, P <0.05; **, P <0.01; ***, P <0.001.

|	Breast stripe size			Yellow breast PC1			Yellow breast PC2			Black crown PC1			Black crown PC2		
	F	df1	df2	F	df1	df2	F	df1	df2	F	df1	df2	F	df1	df2
Date	22.658***	1	73	6.699*	1	74	2.430	1	73	3.749	1	76	0.179	1	74
Sex	111.206***	1	73	1.293	1	74	—			—			73.761***	1	74
Age	3.974*	1	73	—			0.393	1	73	—			—		
Tarsus	—			7.389** (+)	1	74	0.003	1	73	—			—		
Mass	—			5.538* (—)	1	74	6.175* (—)	1	73	—			—		
FGR	—			—			—			6.441* (—)	1	76	11.729** (—)	1	74
Date ×sex	—			—			—			—			4.447*	1	74
Date ×age	—			—			7.153**	1	73	—			—		
Date * tarsus	—			—			10.365**	1	73	—			—		
Date ×FGR	—			—			—			3.970*	1	76	—		
Date ×sex ×mass	—			4.018*	1	74	—			—			—		
Date ×sex ×FGR	—			—			—			—			4.526*	1	74
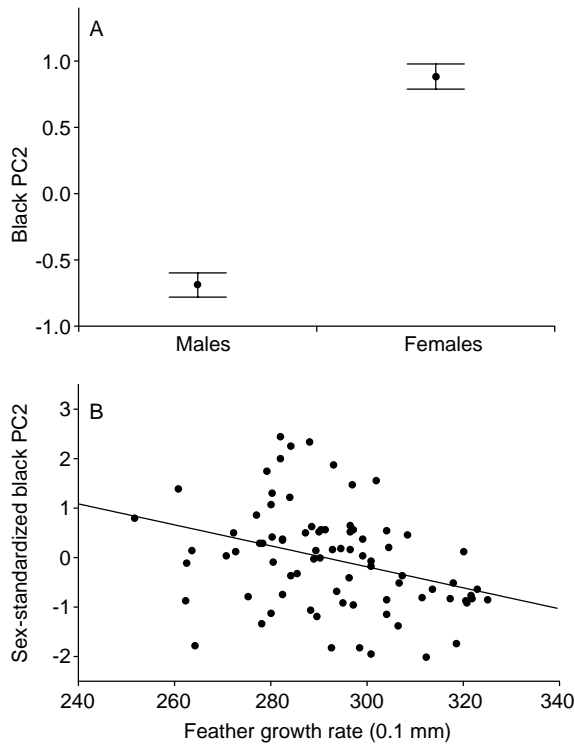
Fig. 2. The second principal component of the reflectance of black crown feathers of great tits after moult, in relation to: (A) sex (means ± SE), and (B) feather growth rate.

Fig. 3. Assortative mating in great tits in relation to black crown PC2.

findings have implications for the link between metabolic origin and the information content of ornaments, and the evolution of multiple ornament systems in birds.

The condition-dependence of melanin-based breast stripe size has been experimentally demonstrated by manipulating ectoparasite loads of breeding birds and measuring subsequent changes in ornament size (Fitze and Richner 2002). We failed to confirm this conclusion by estimating body condition during moult. The recent findings of Senar et al. (2003) by a similar method are in line with our results, suggesting no condition-dependence. Collectively, these studies suggest variation in the information content of the same plumage ornament among populations (Hegyi et al. 2006). Alternatively, specific metabolic costs rather than general condition may limit the expression of badge size (Poston et al. 2005). Previous studies suggested a role for breast stripe size in numerous communication contexts, including social dominance (Järvi and Bakken 1984), territoriality (Wilson 1992), social mate choice (Norris 1990), sex recognition (Slagsvold 1993) and sex allocation (Kölliker et al. 1999). We cannot exclude the possibility that the trait is used in mutual sexual selection in our population, but
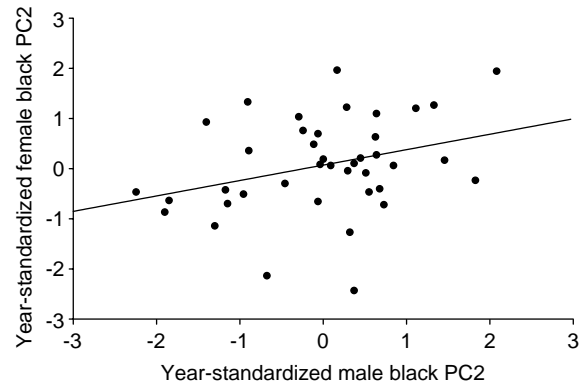
more, preferably experimental data are needed to clarify this point.

Birds obtain carotenoids exclusively from their diet, and utilize them for sexual signalling, immunomodulation and immunostimulation, as well as antioxidant defence (Olson and Owens 1998). This suggests that carotenoid-based colours should be reliable indicators of individual quality, which has been confirmed by several studies (Hill and Montgomerie 1994, Badyaev and Young 2004). In great and blue tits, most of the studies on the proximate determination of breast yellowness were conducted on nestlings (e.g. Slagsvold and Lifjeld 1985, Johnsen et al. 2003, Tschirren et al. 2003), although nestling and adult breast colour are different traits (Fitze et al. 2003). In sexually mature birds, yellowness differed between sexes and habitats (Hõrak et al. 2001), positively correlated with survival (Hõrak et al. 2001), and it was suggested to indicate the ability of parents to find carotenoid-rich food (Fitze et al. 2003), or to provide good rearing conditions for their young (Senar et al. 2002). Our results did not support a direct dependence of yellowness on body condition during moult, which is in line with some studies (Fitze and Richner 2002) but contradicts others (Senar et al. 2003). We therefore conclude that the dependence of breast yellowness on moult conditions varies among contexts or populations.

Yellow PC2 in our population largely reflects yellow chroma, which is a measure of the carotenoid content of breast plumage (Johnsen et al. 2003). We found that this PC was negatively related to body mass after moult, which may indicate the costs of acquiring carotenoid pigments from the environment (e.g. Slagsvold and Lifjeld 1985, Tschirren et al. 2003). Yellow PC1 (breast whiteness), on the other hand, was positively correlated with body size. Surprisingly, to our knowledge, no study to date has estimated sexual selection on yellow colour in tits. In our data no sexual dichromatism could be detected in measures considering the whole reflectance

spectrum visible to birds, and there was no assortative mating with respect to PC2, although there was a tendency for PC1. More studies are therefore needed to elucidate the information content and role of yellow breast colour in this species.

The ultraviolet reflectance of the blue crowns of blue tits has recently received much attention as a sexually selected signal of individual quality (Andersson et al. 1998, Hunt et al. 1998, Delhey et al. 2003, Griffith et al. 2003). Here we evaluated the information content and possible sexual selection role of crown colour in the great tit, where the appearance of this black plumage area is visibly different between males and females (Svensson 1992). The first principal component axis we obtained reflected achromatic brightness, that is, higher values of this variable indicated increasingly grey crowns. Crown greyness was significantly negatively related to body condition at moult, that is, birds in good condition grew darker crowns. This result is in line with two previous studies in other species which suggested that achromatic brightness of black plumage areas declined with increasing general body condition at moult (Mennill et al. 2003) or an increased availability of specific melanin precursors in the diet (Poston et al. 2005). It is interesting that crown greyness in our birds reflected nutrition at moult only after some abrasion, but not immediately after moult. This suggests that food availability affected the structural strength of crown feathers grown by the birds through the quantity of melanin deposited (Bonser 1995). We found no significant assortative mating in relation to crown greyness, but the trait may still have a role in unidirectional (e.g. female-only) mate choice or other mechanisms of sexual selection.

An increase of the second principal component of black crown colour corresponded to a decrease in relative UV reflectance. Relative UV reflectance was much higher in males than in females, and it also increased with body condition during moult. Moreover, we detected positive assortative mating for this colour variable, which may indicate that the trait is used during mate choice in both sexes (see e.g. Andersson et al. 1998, Roulin 1999, Jawor et al. 2003, Kraaijeveld et al. 2004). Alternative explanations for this pattern could include age-assortative mating, and the confounding influences of breeding date, body size and body condition. However, black PC2 was unrelated to age (see above) and first egg dates (results not shown), and there was no assortative mating in the present dataset with respect to body size or body mass (results not shown). The relative UV reflectance (UV chroma) of feathers may reveal nutrition during moult via quantitative effects on keratin deposition in the spongy layer of feather barbs (Shawkey et al. 2003). There is now evidence from several species that ultraviolet plumage reflectance is condition-dependent in nestlings

(UV chroma and hue; Johnsen et al. 2003), adult males (brightness; Siefferman and Hill 2005c) and adult females (brightness, chroma and hue; Siefferman and Hill 2005b). Our study indicates that the small reflectance of black crown feathers in the ultraviolet (generally less than 5% of the white standard, see also Eaton and Lanyon 2003) contains information on sex and individual quality, and may be used in the context of mutual mate choice. These data, combined with previous findings (Siitari et al. 2002, Mennill et al. 2003), suggest that more attention should be paid to the spectral attributes of dark plumage areas.

Our data have important implications in the context of sexual selection for multiple ornaments. At first sight, the results suggest that only structural plumage colour reflected body condition at moult and played a role in mutual mate choice. However, other traits were found to indicate other aspects of quality (age, body size), and we found tendencies for positive assortative mating in all four quality-indicator traits (P <0.15), a correlation pattern significantly deviating from chance expectations. This strongly suggests that mutual mate choice uses multiple signals of different information content, that is, multiple messages (Møller and Pomiankowski 1993) in our population. Studies of the condition-dependence of multiple traits in the same sample provide more reliable inferences of phenotypic plasticity than those analyzing single traits (Cotton et al. 2004). Studies comparing melanin- and carotenoid-based signals in the same species brought equivocal results, with carotenoid ornaments being the more reliable indicators of condition in some studies (Hill and Brawner 1998, McGraw and Hill 2000, Senar et al. 2003), but melanin ornaments in others (Fitze and Richner 2002, Parker et al. 2003). Given that the information content of ultraviolet ornaments has usually been assessed in isolation (but see McGraw et al. 2002, Siefferman and Hill 2003, 2005b), no general conclusion between trait types can be drawn at this point. Our results support the pattern that carotenoid-based colours may not be consistently better signals of body condition than melanin-based colours, and contribute to the evidence that structural colours are particularly reliable indicators of individual quality. Further studies should be conducted to assess the generality of one type of ornamentation indicating body condition better than other types, the uniformity of trait information content among populations of a single species, and the use of multiple ornaments on multiple levels and pathways of sexual selection.

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