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Correlated variation of colour between melanin and carotenoid pigmented plumage areas in great tits

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ABSTRACT

Questions: Are carotenoid and melanin pigmented plumage colour signals independent traits? Is the correlated variation between the two traits condition-dependent and sexually selected?

Background: Previous studies suggested that carotenoid- and melanin-based traits provided different information, but did not assess their correlation or shared information content.

Data description: We recorded reflectance spectra of the black crown and yellow breast plumage of male and female great tits (*Parus major*) after the summer moult (2 years, 192 birds) and during the breeding season (3 years, 126 birds). We used the growth rate of tail feathers as an index of body condition during moult.

Search method: We ran common principal components analyses of crown and breast spectra to identify correlated variation in spectral elevation and spectral shape. We examined the consistency of the shared colour axes between autumn and spring. We used the autumn data to estimate the condition-dependence of the seasonally consistent shared colour axes. Finally, as a measure of sexual selection, we used the spring data to quantify assortative mating patterns in relation to correlated colour variation. We controlled for year- and age-related differences in all analyses.

Conclusions: More than 70% of spectral shape variation in the two plumage regions was correlated. The main directions of correlated variation were similar in autumn and spring. A main axis of spectral covariation reflected body condition during moult. The birds showed significant assortative mating for the same axis.

Keywords: carotenoid, condition dependence, good genes, melanin, multiple signals, *Parus major*, plumage colour, sexual selection.

INTRODUCTION

Systems of multiple sexual signals exist in many species, and represent an enigma for evolutionary biologists (Møller and Pomiankowski, 1993). Different signals in individuals of the

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same species may target different receivers (Andersson *et al.*, 2002), or may provide different information to the same receiver, because of different plasticity and sensitive periods (Møller *et al.*, 1998; Hegyi *et al.*, 2007a). Another potential function of multiple signals is to ensure proper information transmission if display or assessment is context-dependent (Godin and Dugatkin, 1996; Coleman *et al.*, 2004). However, theory predicts that systems where multiple signals provide a similar message to the same receiver may be evolutionarily stable under certain conditions (Johnstone, 1996). Similar information content may arise, for example, if two signals have the same hormonal regulation (Owens and Short, 1995; Blas *et al.*, 2006). Alternatively, multiple condition-dependent traits may correlate because they share the genetic background of context-independent, general body condition [good genes (Rowe and Houle, 1996; Kotiaho *et al.*, 2001)]. Sexual selection on a signal represents indirect selection on its genetic and phenotypic background. If a determinant of genetic quality jointly influences two sexual signals, selection on one signal will influence the information content of the other signal as a correlated response. Therefore, sexual selection on the correlated component of variation in two signals has profound implications for the evolution of the multiple signal system. However, studies that consider the interrelations of multiple display traits (Badyaev *et al.*, 2001; Garamszegi *et al.*, 2006) or evaluate these traits as parts of an integrated signal transmission system (Cooperman *et al.*, 2007; Hegyi *et al.*, 2007a) are rare.

Conspicuous ornamental plumage colour in birds is extremely diverse, and it has long been a popular target of research (Hill and McGraw, 2006). Because of its complexity even within species (e.g. Omland and Lanyon, 2000 vs. Hofmann *et al.*, 2006), plumage colour is a promising system to examine the interdependence of multiple sexual signals. Plumage colour has classically been categorized as pigment-based versus structural, with the former group further subdivided based on pigment type, such as melanin or carotenoid colours (Gray, 1996). However, the expression of pigment-based colour may strongly depend on keratin matrix structure (Shawkey and Hill, 2005) and pigments play important roles in the production of structurally based colours (Shawkey and Hill, 2006). Moreover, a visually based distinction between carotenoid and melanin colour is less straightforward than previously thought (McGraw *et al.*, 2004). In spite of these problems, the use of these three classes of colour and discussions of their relative information content remain common in the literature.

The honesty of a carotenoid colour signal may originate from the fact that these pigments must be ingested with food, and serve many other functions such as antioxidants or immune stimulants (Møller *et al.*, 2000). Several studies have supported the view that carotenoid colour is a more plastic and more informative signal of individual quality than melanin colour (e.g. McGraw and Hill, 2000; Badyaev and Young, 2004; but see Griffith *et al.*, 2006). However, there are several honesty-enforcing mechanisms for melanin-based plumage as well, such as the limitation of specific amino acids (Poston *et al.*, 2005) or the costs of testosterone titres that promote colour exaggeration (Evans *et al.*, 2000). Moreover, melanins are potent antioxidants, similar to carotenoids (Griffith *et al.*, 2006). Therefore, it is not surprising that empirical evidence on the relative information content of melanin versus carotenoid colour is ambiguous (Fitze and Richner, 2002; Parker *et al.*, 2003).

Given the controversy regarding the honesty and evolutionary significance of the two ornamentation types (see Badyaev and Hill, 2003; Bókony *et al.*, 2003), it is important to determine whether colour variation in carotenoid and melanin pigmented plumage areas of the same species is correlated, and whether any common variation reflects individual quality and plays a role in sexual selection. Here we address these topics using a multi-year spectrometric data set from a population of great tits (*Parus major*). The great tit is an ideal model system

with which to address these questions, since not only are both types of colour present, but the whole plumage is replaced at the single autumn moult (Svensson, 1992), so environmentally mediated correlation between the two signal types is plausible.

The carotenoid-based yellow colour of the great tit breast is based on the unmodified deposition of lutein and zeaxanthin from the diet (Partali *et al.*, 1987). Breast colour has been found to reflect environmental pollution (Eeva *et al.*, 1998), other aspects of habitat quality (Hõrak *et al.*, 2001), and body condition during moult (Senar *et al.*, 2003). In an Estonian population, breast colour also differed between the sexes and predicted survival (Hõrak *et al.*, 2001). Although it is well known that the black plumage of the crown differs in luminosity between males and females (Svensson, 1992), this plumage area has rarely been examined as an ornament. A recent study revealed very large sexual dichromatism in the spectral attributes of the crown, with males having much higher relative reflectance in the ultraviolet (UV) range than females. Moreover, the relative UV reflectance of the crown was strongly positively related to body condition during moult, and it was positively correlated between pair members, suggesting mutual sexual selection on this trait (Hegyi *et al.*, 2007b). The reflectance of the yellow breast, on the other hand, was sexually monomorphic and did not appear to be condition-dependent in this population. Assortative mating patterns were also non-significant for this trait (Hegyi *et al.*, 2007b). Finally, it was found that more than 99% of variation in the spectral reflectance of the black breast stripe was explained by monochromatic brightness (Hegyi *et al.*, 2007b, unpublished data).

In this study, we focus on the patterns of common colour variation between the yellow breast and the black crown, because these traits show variability in both spectral elevation and spectral shape. We examine great tits both after the autumn moult (2 years of data) and in the breeding season (3 years of data). First, we look for common axes of variation in the reflectance of yellow and black feathers, and compare these axes between the autumn and spring plumages. Second, we use ptilochronology (measurements of feather growth rate) to assess the relationship between body condition during moult and variation along the common colour axes of the carotenoid and melanin pigmented feathers grown. Third, we examine whether mating patterns in spring suggest mutual sexual selection in relation to correlated differences in colour, when correcting for any year- and age-related variation.

MATERIALS AND METHODS

Field and laboratory procedures

Fieldwork was undertaken in the Pilis Mountains, near Szentendre, Hungary (47°43′N, 19°01′E), at the nestbox plots used for long-term population studies of hole-nesting passerines over the last two decades (for more details, see Török and Tóth, 1988, 1999). Great tits were caught in October and early November 2005 and 2006 with mist-nets at two feeders baited with sunflower seeds. In the springs of 2004, 2005, and 2006, breeding pairs at the plots were captured in the nestbox, during the endothermic phase of nestlings. Birds were ringed with standard numbered metal rings, and their sex and binary age (yearling or older) were determined as described in Svensson (1992). Tarsus length and body mass were measured to the nearest 0.1 mm and 0.1 g, respectively. Moreover, a minimum of eight feathers were collected from standard positions on the yellow breast and the black crown, and put into envelopes for later spectrometric analyses. Individuals moulting their breast or crown feathers were excluded from the analyses. In autumn, we also collected the left and

right second outermost rectrices. The number of birds with complete spectral data was 192 in autumn and 126 ($n = 63$ breeding pairs) in spring, but feather growth rate was not measured on a few autumn birds due to damaged, missing or moulting tail feathers.

Reflectance curves of feathers were measured with an Ocean Optics USB2000 spectrometer, using a bifurcated fibre-optic probe and a Mini-DT deuterium-tungsten-halogen light source [Ocean Optics Europe (see also Hegyi *et al.*, 2007b)]. A black plastic tube was fixed on the probe to standardize measurement distance and exclude ambient light. The probe was held perpendicular to the sample. Eight feathers were placed on top of one another on a piece of black velvet. The shape of the spectral curves made it possible to ensure that the background reflectance of the velvet did not confound the measurements. Two or three scans were taken for each set of feathers in 0.37-nm steps, with a measurement from a WS-2 white reflection standard (Ocean Optics Europe) between each. Reflectance curves were stored using OOIBase software (Ocean Optics Europe). All spectra were taken by the same person, using the same instrument.

Body condition during moult was estimated from the growth rate of tail feathers – that is, ptilochronology (Grubb, 1995; for applications to plumage ornamentation, see Hill and Montgomerie, 1994; Keyser and Hill, 1999; Senar *et al.*, 2003). The method adapted to the great tit by Senar *et al.* (2003) was used (see also Hegyi *et al.*, 2007b). The total width of the first ten visible growth bars (light and dark) from the distal end of the feather was measured using a calliper (nearest 0.1 mm), under intense direct illumination. All feathers were measured by the same person. The left and right feathers of the same individual were measured separately in time, and measurer experience bias was avoided by alternating between the sides after every eight samples. The repeatability of growth rate between the two temporally separate measurements of an individual was high (Pearson $r_{173} = 0.704$, $P < 0.001$). The mean of the two sides was used as growth rate in the analyses.

Statistical analyses

Principal components analysis (PCA) is a method of statistical dimension reduction (Stevens, 1986). It attempts to condense most of the information contained in the original multivariate space into a few principal component axes. Principal component axes are orthogonal to one another, and they are oriented in a way that maximizes the explained variance. There are multiple ways of combining spectral information from several plumage areas using PCA. First, one may calculate derived indices (e.g. brightness, chroma) from spectra of individual plumage regions, and then combine these in a PCA. However, the derived measures may not accurately reflect the main directions of spectral variation in the individual regions, so it is preferable to enter the raw spectral data into a PCA (Cuthill *et al.*, 1999; Peters *et al.*, 2004; Siitari *et al.*, 2007). One may first conduct separate PCAs on spectral data from each area, and then combine the resulting axes in a second PCA. However, this is incorrect for two reasons. First, some of the axes entered into the second analysis are inherently orthogonal, coming from the same PCA. This makes the second PCA mathematically invalid. Second, as the separate PCAs produce orthogonal axes within the given plumage areas, the combination of these will not reflect the main axes of variation in the whole multivariate space defined by the two spectra together. It will reflect only the best way in which the program could combine the orthogonalized axis systems of the two areas. Thus, to reliably determine the extent and the directions of correlated spectral variation in two or more plumage areas, it is

necessary to enter all raw spectral data into the same PCA. This is the method we used here, for the first time to our knowledge. The present analyses were based on the correlation matrix of the original variables, and we report unrotated principal component axes.

We first averaged the reflectance readings from the black crown and the yellow breast into 10-nm spectral bands for each patch on each individual, within the visual range of the closely related blue tit (*Cyanistes caeruleus*) – that is, from 320 to 700 nm (Hart *et al.*, 2000). The resulting $2 \times 38 = 76$ standardized spectral bands were used as input variables in the PCA. That is, both spectra were represented by 38 bands in the analysis. Overall reflectance or brightness strongly changes due to abrasion, and brightness variation constitutes most of the spectral variation of any plumage area (Cuthill *et al.*, 1999; Mennill *et al.*, 2003). Indeed, the first two common principal components axes of raw spectral data in our case described exclusively brightness differences, and explained more than 90% of the variance (see Results). Therefore, to make the analyses sensitive to the shape differences of the spectra, we also standardized each spectrum for its brightness by dividing reflectance at each spectral band with the average reflectance of the whole curve, and ran the PCA again on these data (see, for example, Endler and Théry, 1996; Stein and Uy, 2006). Because of the standardization, the latter analysis detected variation only in spectral shape, not in the elevation of the curve. Analyses using 20-nm averaging and 38 instead of 76 spectral bands produced almost quantitatively identical results, so we can conclude that the large number of input variables does not bias our conclusions.

Separate PCAs were run on autumn and spring birds, because the two groups were analysed separately in the following. This also allowed us to quantify any seasonal difference in the directions of overall colour variation due to the large abrasion of feathers from autumn to spring (Örnborg *et al.*, 2002; Delhey *et al.*, 2006) or other reasons (see Discussion). From the very few repeated measurements of individuals between two autumns or two springs, the first captures were used in the analyses. To check whether the axes detected by the common PCA are only statistical artefacts or indeed correspond to the main axes of variation in the individual ornaments, we also calculated separate PCAs for the two plumage areas, and correlated the scores of the separate principal components with those produced by the common PCA for the respective area.

The relationship of spectral variation along the common axes with sex, binary age, and body condition during moult was examined by general linear models using the autumn spectral data. The dependent variable was the respective principal component (PC), while year, sex, and age were included as fixed factors, and measurement date, tarsus length, and feather growth bar width as covariates. All two- or three-way interactions were tested, except those involving two or more covariates. The spring PCs, on the other hand, were used to analyse mating patterns. First, PC scores of males and females were compared between years and ages in two-way general linear models, also including the interaction between year and age. The PC scores were standardized for these factors where necessary. Thereafter, Pearson correlations between the standardized PC values of social mates were calculated. All general linear models were subjected to stepwise backward model selection with reintroduction of non-significant terms to the final model.

RESULTS

Main axes of colour variation

The PCAs of raw spectral data from the breast and the crown yielded axes that quantified monochromatic brightness variation. However, these axes were grossly inconsistent between autumn and spring (Fig. 1). The first autumn PC (50.5% of variation) correlated positively with both breast and crown brightness, while the second autumn PC (41.3% of variation) correlated negatively with breast and positively with crown brightness. The spring PCs (47.7% and 45.7% of variation), on the other hand, correlated with the brightness of one area but not the other (Fig. 1; correlations of common PCs with the separate PC1 scores of the respective areas, abs(r) > 0.502, $P < 0.001$ in both autumn and spring). This implies that the patterns of parallel brightness variation between the two plumage areas after moult disappear by spring. Therefore, we did not analyse condition-dependence and mating patterns for brightness-related PCs. Instead, we conducted new PCAs using brightness-standardized spectral data to determine whether the shape of the spectra shows seasonally consistent parallel variation between the breast and the crown.

The first two PCs of the brightness-standardized spectra explained most of the variance in colour, irrespective of season (autumn: PC1 42.1%, PC2 30.3%; spring: PC1 42.0%, PC2 30.4%). The correlations between individual 10-nm spectral bands and PC values are shown in Fig. 2. The main axes of individual differences in overall plumage colour were consistent

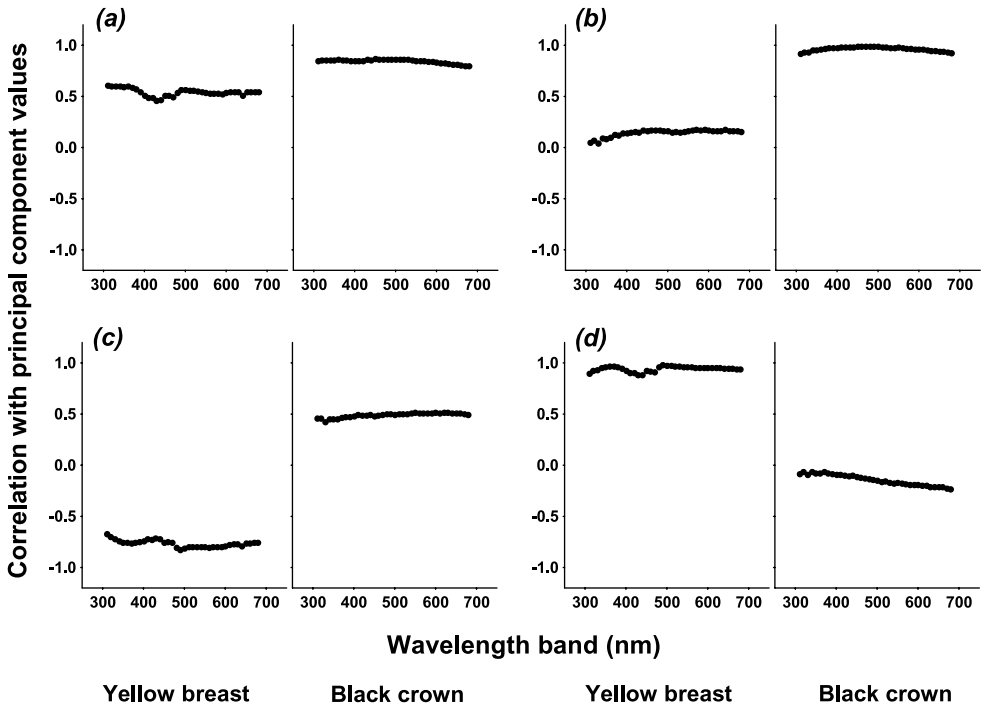

Fig. 1. Common principal components analysis of the raw reflectance spectra of two plumage regions in great tits: autumn and spring relationships between reflectance of the respective area in 10-nm wavelength bands and PC values. (a) PC1, autumn; (b) PC1, spring; (c) PC2, autumn; (d) PC2, spring.

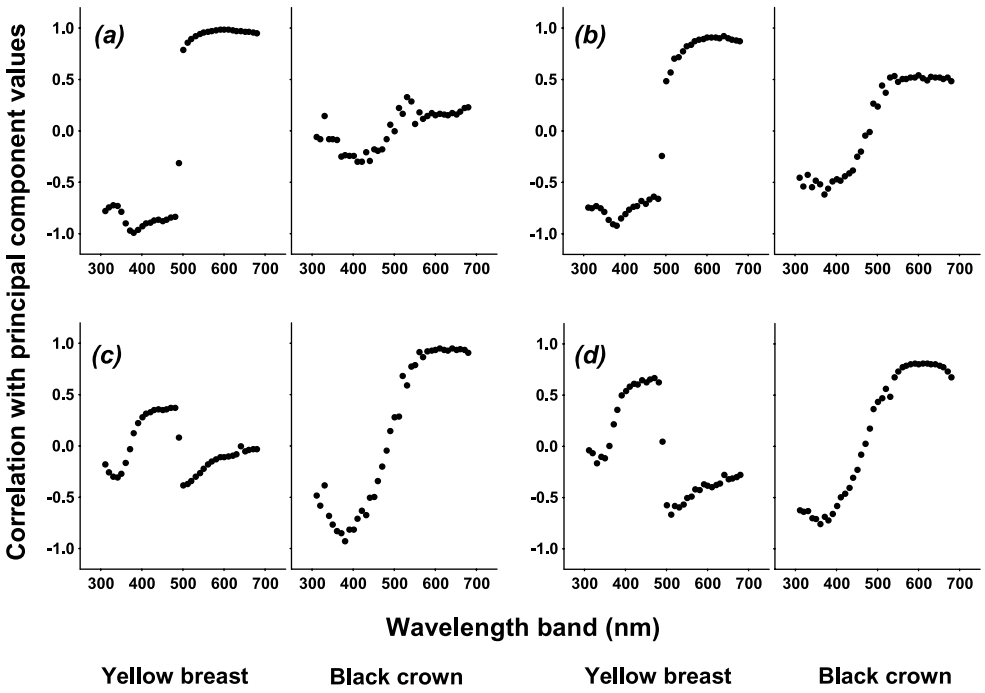

Fig. 2. Common principal components analysis of the brightness-standardized reflectance spectra of two plumage regions in great tits: autumn and spring relationships between reflectance of the respective area in 10-nm wavelength bands and PC values. (a) PC1, autumn; (b) PC1, spring; (c) PC2, autumn; (d) PC2, spring.

between the two seasons (correlations of autumn and spring spectral loadings: PC1, $r_{76} = 0.931$, $P < 0.001$; PC2, $r_{76} = 0.922$, $P < 0.001$). In both autumn and spring birds, the first PC quantified the ratio of high- versus low-wavelength reflectance for both the black crown and the yellow breast. However, the contribution of crown reflectance to this PC increased greatly from autumn to spring. The second PC was very similar between autumn and spring birds. For the black crown, this PC was highly negatively correlated with relative ultraviolet reflectance [correlation with UV chroma ($R_{320–400}/R_{320–700}$): autumn, $r_{192} = -0.731$, $P < 0.001$; spring, $r_{126} = -0.747$, $P < 0.001$]. For the yellow breast, on the other hand, the shape of the correlations with individual spectral bands resembled the inverse of a carotenoid reflectance curve (Bleiweiss, 2004). In other words, this PC quantified the inverse of the saturation of yellow colour on the breast [correlation with yellow chroma ($(R_{700}–R_{450})/R_{700}$): autumn, $r_{192} = -0.285$, $P < 0.001$; spring, $r_{126} = -0.561$, $P < 0.001$). Separate PCAs of brightness-standardized data from the two plumage areas validated the common analysis by producing factor scores that were highly consistent with those of the common axes. Common spectral PC1 correlated with the first PCs of both areas (autumn yellow: $r_{192} = 0.986$, $P < 0.001$; autumn black: $r_{192} = 0.218$, $P = 0.002$; spring yellow: $r_{126} = 0.891$, $P < 0.001$; spring black: $r_{126} = 0.583$, $P < 0.001$). Common spectral PC2 correlated with yellow PC2 (autumn: $r_{192} = 0.557$, $P < 0.001$; spring: $r_{126} = 0.556$, $P < 0.001$) and black PC1 (autumn: $r_{192} = 0.971$, $P < 0.001$; spring: $r_{126} = 0.805$, $P < 0.001$).

The third and fourth common PCs were also similar between autumn and spring (correlations of factor loadings: $r_{76} = 0.835$ and 0.828, respectively). These PCs, however, received strong loadings from only one of the two plumage areas. PC3 apparently quantified a green overtone on the black crown plumage, while PC4 seemed to capture a component of yellow chroma on the breast that was independent of black crown reflectance (details not shown here). In the following, we examine the proximate determination and potential mating consequences of the first two PCs, which are the shared axes of the spectral shape of black and yellow plumage.

Information content

In autumn birds after moult, the first PC of colour was related to the weak main effects of year, age, capture date, and a variety of interactions between year, sex, age, tarsus length, and feather growth rate (Table 1). However, the only significant relationship between PC1 and feather growth rate was a weak positive one in adult males ($P = 0.048$; results of *post-hoc* analyses not shown). The second PC, on the other hand, showed strong effects of year, sex, and feather growth rate (Table 1). The effects of date, body size, and age were non-significant. Males had smaller PC2 values than females (Fig. 3a), and PC2 was highly

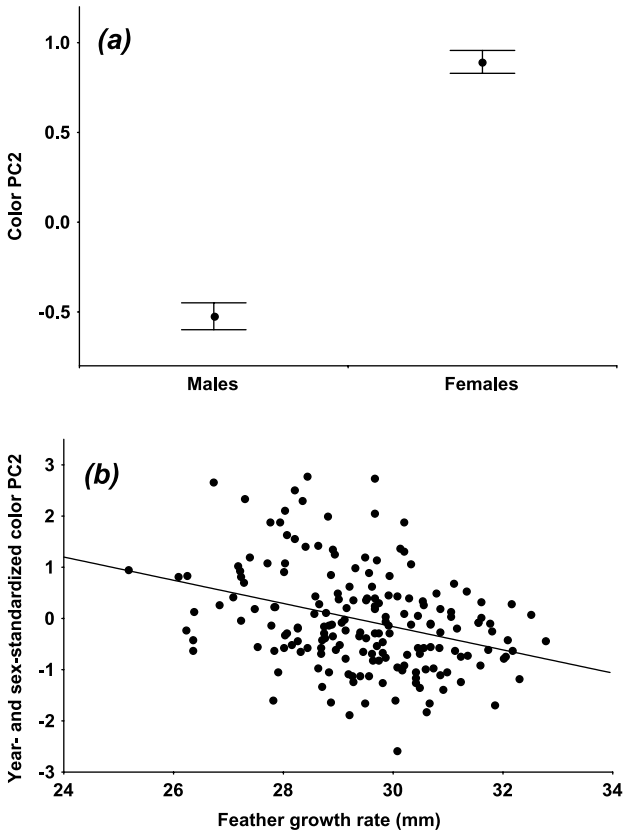

Fig. 3. The second common PC axis of plumage colour in autumn great tits, in relation to: (a) sex and (b) the rate of tail feather growth during the last moult.

Table 1. Common spectral principal components of two plumage areas in autumn great tits, in relation to year, sex, binary age, measurement date, tarsus length, and the rate of tail feather growth

	PC1	PC2
Year	4.34* (1, 169)	46.08*** (1, 177)
Sex	1.60 (1, 169)	216.58*** (1, 177)
Age	4.53* (1, 169)	0.10 (1, 176)
Date	4.90* (1, 169) (–)	0.17 (1, 176)
Tarsus	0.00 (1, 169)	0.95 (1, 176)
FGR	2.44 (1, 169)	18.95*** (1, 177) (–)
Year × sex	6.81** (1, 169)	0.11 (1, 176)
Year × age	0.00 (1, 168)	0.99 (1, 175)
Sex × age	8.96** (1, 169)	0.00 (1, 175)
Year × date	0.12 (1, 168)	0.09 (1, 175)
Sex × date	1.14 (1, 168)	0.22 (1, 175)
Age × date	0.76 (1, 168)	0.30 (1, 174)
Year × tarsus	0.17 (1, 168)	1.75 (1, 175)
Sex × tarsus	1.44 (1, 168)	2.79 (1, 175)
Age × tarsus	0.94 (1, 168)	0.64 (1, 174)
Year × FGR	4.68* (1, 169)	0.28 (1, 176)
Sex × FGR	0.06 (1, 168)	1.58 (1, 176)
Age × FGR	4.45* (1, 169)	0.74 (1, 175)
Year × sex × age	0.00 (1, 168)	0.17 (1, 175)
Year × sex × date	0.73 (1, 168)	0.14 (1, 175)
Year × age × date	0.61 (1, 168)	1.82 (1, 174)
Sex × age × date	0.17 (1, 168)	0.45 (1, 174)
Year × sex × tarsus	6.73* (1, 169)	0.13 (1, 175)
Year × age × tarsus	0.00 (1, 168)	1.05 (1, 174)
Sex × age × tarsus	0.57 (1, 168)	0.01 (1, 174)
Year × sex × FGR	0.13 (1, 168)	3.91* (1, 177)
Year × age × FGR	0.00 (1, 168)	0.95 (1, 175)
Sex × age × FGR	8.57** (1, 169)	0.01 (1, 175)

Note: Signs of relationships are indicated for significant continuous predictors. General linear models using backward stepwise model selection with reintroduction. *F*-values are shown with effect and error degrees of freedom in parentheses. FGR = feather growth rate.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

negatively related to feather growth rate (Fig. 3b). There was a weak three-way interaction between year, sex, and feather growth rate ($P = 0.049$), but no interaction between year and feather growth rate could be detected in either sex ($P > 0.528$). In summary, the only consistent predictor of PC1 was capture date, while PC2 showed strong overall effects of year, sex, and feather growth rate.

Mating patterns

Among the breeding birds, PC1 scores differed significantly among years (females: $F_{2,58} = 6.117$, $P = 0.004$; males: $F_{2,60} = 7.667$, $P = 0.001$). There was no year effect on PC2 in

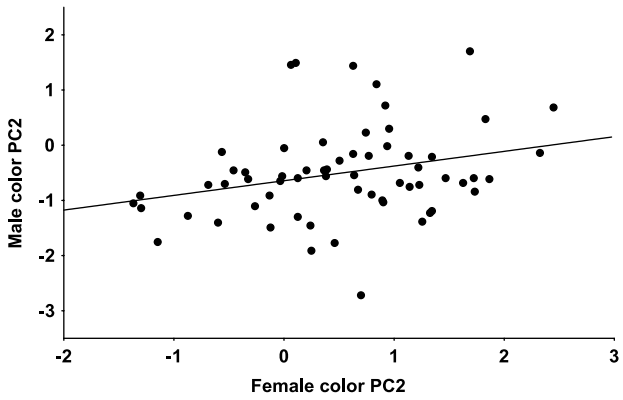

Fig. 4. The correlation of the second common PC axis of plumage colour between the members of great tit social pairs.

either sex (females: $F_{2,58} = 2.001$, $P = 0.144$; males: $F_{2,60} = 1.888$, $P = 0.160$). Age and the year × age interaction, on the other hand, were non-significant for both principal components in both sexes ($P > 0.248$). Assortative mating was not significant for year-standardized spectral PC1 ($r_{63} = 0.098$, $P = 0.444$, lower 95% CI = −0.154, upper 95% CI = 0.338), but significantly positive for PC2 ($r_{63} = 0.279$, $P = 0.027$, lower 95% CI = 0.336, upper 95% CI = 0.493) (Fig. 4). The two correlation coefficients did not differ significantly from each other (see confidence intervals).

DISCUSSION

Melanin and carotenoid plumage pigmentation have been classically regarded as distinct signal types with very different information content (Badyaev and Hill, 2003), although there are at least four different pathways that may cause correlated expression of colour between the two ornament classes. First, feather microstructure is an important determinant of both colour types, and the developmental stability of microstructure should be broadly repeatable across the plumage of an individual (Shawkey and Hill, 2005; Shawkey *et al.*, 2006). Second, the two types of colour may correlate through the limitation and antioxidant function of both carotenoids and melanins (Griffith *et al.*, 2006; Moreno and Møller, 2006). Third, it has recently been realized that testosterone may modulate not only melanin but also carotenoid ornaments, by regulating the levels of lipoproteins that transport carotenoid pigments to peripheral tissues (Blas *et al.*, 2006; McGraw *et al.*, 2006). Finally, carotenoid and melanin colour may both be condition-dependent (Veiga and Puerta, 1996; Hill and Brawner, 1998), and they may correlate if both reflect the common phenotypic or genetic basis of body condition (Parker and Garant, 2004). In any of these cases, however, understanding the possible importance of melanin and carotenoid colour as components of the same complex signal requires the quantification of common variation in the two. In other words, shared information content is unlikely if the main axes of variation in the two traits are independent.

Here we addressed this topic in great tits by performing common principal components analyses of spectra from two plumage regions. The yellow breast is pigmented by carotenoids, while the black crown combines melanin and structural colour. The individual spectral bands of the two regions were weighted equally in our analysis. When looking at the raw

spectra, we found that correlated variation of the brightness of crown and breast was present after moult but disappeared by spring. These patterns are in line with a previous study that showed rapid temporal change in the information content of crown brightness after moult, probably due to a relationship between brightness, melanin content, and feather abrasion (Hegyi *et al.*, 2007b). On the other hand, when correcting the spectra for brightness, we detected strong, seasonally consistent shared variation in the spectral shape of the breast and the crown. Strikingly, it was the first two PCs that described correlated variation between the yellow breast and the black crown, and these correlated changes constituted more than 70% of variation in the spectral shape of the two areas. Independent variation on the breast or the crown loaded into the minor principal components (PC3 and PC4). The first PC quantified the ratio of short to long wavelength reflectance in both plumage areas, while the second PC linked yellow chroma on the breast with relative UV reflectance on the crown. There was a strong correspondence between the common PC axes and the main axes of within-ornament variability as revealed by separate PCAs of the two areas.

Studies in the closely related blue tit have shown that measures of plumage reflectance differed greatly between seasons (Örnborg *et al.*, 2002). Later analyses indicated that these seasonal differences were at least partly due to within-individual changes in reflectance, most likely due to feather abrasion or soiling (Delhey *et al.*, 2006). In the present study, we did not follow the same birds across seasons, so the seasonal change may also reflect selection. Finally, seasonal differences in uropygial gland activity may also contribute to the apparent spectral changes. Therefore, it is a very interesting aspect of our results that the main axes of common variation in the spectral shape of crown and breast plumage were similar between autumn and spring birds. The only major difference was an increase from autumn to spring in the contribution of melanin-based colour to the first PC. This may be due to the fact that melanin content is important to the structural strength of feathers (Bonser, 1995), so the abrasion, ectoparasitic, bacterial or fungal degradation of melanin pigmented regions after moult may contribute to their spectral variability and information content (Kose and Møller, 1999; Goldstein *et al.*, 2004; Hegyi *et al.*, 2007b). In summary, our present analyses indicate that most of the variation in spectral shape is correlated between the yellow and black plumage of great tits, although the degree of correlation varies between autumn and spring. Moreover, the directions of shared variation show seasonal consistency. It is possible, therefore, to proceed to explore the possible information content and role of this variation.

The relative importance of pigments versus feather structure in shaping plumage colour is not well known. In a comparative study of cowbirds, for example, mathematical models incorporating both melanin content and the arrangement of reflective layers were successful in explaining most of the characteristic species differences (Shawkey *et al.*, 2005). Similarly, experiments with carotenoid pigmented yellow feathers of American goldfinches (*Carduelis tristis*) revealed that pigment content and keratin matrix structure were both important determinants of reflectance curves (Shawkey and Hill, 2005). In our case, the first PC of colour quantified the relative amount of short to long wavelength reflectance in both plumage regions. The amount of relative ultraviolet reflectance in eastern bluebird (*Sialia sialis*) feathers was explained by the density of circular keratin rods in the spongy layer of feather barbs (Shawkey *et al.*, 2003). Therefore, our first PC may reflect the regularity of feather microstructure, which is consistent across different plumage regions of the same individual. Colour PC1 was not consistently related to body condition during moult, which suggests that this variable may indicate an individual-specific attribute such as developmental stability (Møller and Swaddle, 1997). In summary, the first axis of common spectral variation

between melanin and carotenoid pigmented regions may be caused by a common structural mechanism.

The second PC, however, linked attributes of crown and breast colour that probably do not have a common mechanistic background. The saturation of yellow on the breast is related to the carotenoid content of feathers (Bleiweiss, 2004; Shawkey *et al.*, 2006). The relative ultraviolet reflectance of the black crown, on the other hand, may be due to structural characteristics (Shawkey *et al.*, 2003). However, both measures of colour have been demonstrated to depend on body condition. For example, brood size manipulations affected both the carotenoid chroma of breast feathers and the relative UV reflectance of tail feathers in blue tit nestlings, although the latter effect appeared only in males (Jacot and Kempenaers, 2007). Our results show that colour PC2 was significantly negatively related to body condition during moult, irrespective of the sex or the age of the bird. In other words, great tits in good body condition simultaneously developed higher UV chroma on the crown and more intense yellow colour on the breast than those in poor condition.

Thus, our second PC of overall colour variation may reflect body condition as a common background. Interestingly, this second, apparently condition-dependent axis of common spectral variation was significantly positively correlated between males and females in social pairs. The mating pattern was not confounded by year, age (see Results), breeding date, body size or body condition (Spearman rank correlations and multiple regressions, results not shown). This relationship may therefore reflect mutual sexual selection and the resulting assortative mating for overall colour (Fitzpatrick and Price, 1997; Siefferman and Hill, 2005; Hidalgo-Garcia, 2006), although experiments are needed to establish the exact causal mechanism [mate choice or sexual competition (Liu *et al.*, 2007)]. In other words, we cannot exclude the possibility that sexual selection based on visual cues does not focus exclusively on individual traits, but instead builds on a combination of multiple, correlated signals. This has important implications for the preferred design of future studies of sexual selection (see also Calkins and Burley, 2003; Badyaev and Young, 2004).

To conclude, our results suggest that great tits looking for a mate may benefit from an integrated signal system if they assess the colour of different plumage regions together. Shared axes of information content in this system may make it possible to convey multiple different types of information more reliably or more efficiently than via independent individual signals. In particular, the position of an individual along the condition-dependent common axis of breast yellowness and crown UV reflectance has the potential to reflect overall viability through the good genes process (Qvarnström, 1999; David *et al.*, 2000; Kotiaho *et al.*, 2001; Parker and Garant, 2004). Further studies with a broad taxonomic coverage are needed to clarify the detailed mechanistic background of intraspecific plumage colour variation (Prum, 2006), and to look for systems of different signals with integrated information content. In the great tit, experiments should be conducted to test the condition-dependence of overall plumage colour as a complex signal system, as well as its role in sexual selection.

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