Stable correlation structure among multiple plumage colour traits: can they work as a single signal?

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The presence of multiple distinct ornamental traits in the same species is frequently explained by context-specificity and different information content. However, the expression of multiple ornaments is often correlated, and such traits may therefore function as a single, integrated signal. Delayed use of an integrated signal relative to production requires temporal stability in integration, which has seldom been examined. We used autumn and spring reflectance data from the breast, breast stripe and crown of great tits (*Parus major*) to assess the stability and mating implications of colour signal integration, as well as the repeatability of any integrated colour trait and its correlation with condition during moult. We found high levels of stability between seasons, years, sexes and ages in the correlation patterns of colour measures across the three plumage areas. The first principal component colour axis described joint variation of UV reflectance on the crown and the breast stripe, thereby representing an among-trait UV chroma axis. However, only breast yellow chroma showed condition-dependence, while temporally consistent and significant assortative mating was restricted to crown UV chroma. Our results therefore do not support the ideas that the overall UV chroma of the breast stripe and the crown is special in condition-dependence and repeatability, or it plays a specific role in mutual sexual selection as an integrated signal. Our results show that stable association between display traits is an existing phenomenon. They also indicate that even in the presence of correlated traits, functional trait integration among these requires further scrutiny.

ADDITIONAL KEYWORDS: body condition – feather abrasion – moult – plumage colour – redundant signal – sexual selection.

INTRODUCTION

Research of sexually selected ornamental traits has always been at the forefront of evolutionary ecology studies (Darwin, 1871; Andersson, 1994; Hill & McGraw, 2006). With the exploration of an increasing number of different sexual traits, recent studies increasingly emphasize the variety of sexual signals and their different information content, even within a single species. This focus on signal variety is well illustrated by the recent introduction of the concept of within-species ornament diversity (Chen *et al.*, 2012). Regarding colour traits, classically used categories include pigment-based (Svensson & Wong, 2011) and structurally based ornaments (Srinivasarao, 1999). Both of these occur in bird feathers (Gray, 1996; Badyaev & Hill, 2003). On the other hand, some recent work indicated that many colours (in birds nearly all plumage colours) in fact combine pigment- and structurally based mechanisms (Rutowski *et al.*, 2005; Doucet *et al.*, 2006; Kuriyama *et al.*, 2006; Shawkey *et al.*, 2006; Wilts *et al.*, 2012), and highlighted similarities in information content among classical categories of colour (Blas *et al.*, 2006; Griffith, Parker & Olson, 2006). This indicates that seemingly distinct ornamental traits of the same species may not behave independently at the developmental and functional levels.

Multiple sexual traits may function in the same sexual selection process, either conveying different information or reinforcing each other (Møller & Pomiankowski, 1993; Johnstone, 1996), but they can also be used in different contexts (Pryke *et al.*, 2001; Dunn *et al.*, 2010). The concept of composite sexual signals emerged relatively early, but classical studies of this phenomenon examined the separate information content and role of multiple aspects of the same conspicuous trait (Badyaev *et al.*, 2001; Møller & Petrie, 2002). By contrast, if the same species apparently displays multiple traits indicating phenotypic quality, these may actually indicate one or few common aspects of quality (Badyaev, 2004; Martín & López, 2009). For example, multiple ornaments may depend on the overall physiological state or body condition of the individual (Rowe & Houle, 1996) and thereby share some of their genetic and environmental background (Tomkins *et al.,* 2004). This raises the possibility that even perceptually distinct traits of different developmental origins, for example, patches of different pigmentary basis in the same plumage, may correlate in their expression and may even constitute a single integrated signal (Hebets & Papaj, 2005). A much more straightforward case is when multiple distinct traits of similar developmental origin convey similar information (Peters *et al.*, 2008). Functional integration among these may occur simply due to a shared sensory processing pathway, i.e. sensory exploitation (Partan & Marler, 2005), although this will not happen if the possibility to assess the individual traits is different or context-specific (Hebets & Papaj, 2005). In sum, multiple processes may cause developmental integration, functional integration, or both, among some components of a system of multiple display traits.

An increasing number of studies investigate multiple conspicuous traits in the same species (Candolin, 2003). Some studies have estimated among-trait correlations and treated multiple unrelated ornaments as separate signals (Reudink *et al.,* 2009), while others pooled correlated ornaments into a single trait (Merilä, Sheldon & Lindström, 1999) or examined them separately despite their interrelation (Guindre-Parker *et al.*, 2013). However, many authors do not even consider the possibility of relationships among multiple traits of different developmental origins. Therefore, there are only a handful of studies that suggested sexual selection on complexes of multiple distinct but nevertheless integrated ornaments. Examples for such integration include pairs of structurally based and melanin-based (Siefferman & Hill 2003), depigmented white and melanin-based (Laczi *et al.,* 2011), and carotenoid- and melanin-based plumage colour traits (Hegyi *et al.*, 2008). Likewise, in general, investigations of possible adaptive mate choice for the parallel variation or occurrence of conspicuous character states are still relatively rare (Møller *et al.*, 1998; McGlothlin *et al.*, 2005; Lancaster, Hipsley & Sinervo, 2009).

A fundamental but seldom examined question concerning signal integration is whether it is sufficiently consistent (among contexts) and stable (in time) to permit receivers to pay special attention to the signal complex rather than the univariate traits *per se*. Recent research puts particularly strong emphasis on fluctuating information content and fluctuating selection in multiple signal systems (Bro-Jørgensen, 2010). To the contrary, there are two cases in which the evolutionary persistence of sexual selection on an integrated signal as opposed to multiple independently processed signals requires stability in trait correlation structure and information content across seasons: when the signal complex is used in multiple seasons, and when the production and the use of the signal complex are temporally separated. Ornaments produced in a limited time window but maintained year-round are subject to seasonal wear and other damages (staining, chemical degradation etc.). For instance, feather abrasion contributes to the expression of various types of plumage signals (Delhey *et al.*, 2010) and it may therefore also affect correlations among them. Feather structures including melanins and carotenoids presumably reduce and increase, respectively, the susceptibility of feathers to abrasion (Bonser, 1995; Bleiweiss, 2004), so abrasion (and other processes) may differentially alter the coloration of such areas across seasons (Figuerola & Senar, 2005; Adamík & Vanáková, 2011). Many species replace their ornaments well before the main period of sexual displays, while others may use them year-round. If seasonal wear increases, reduces or reorganizes developmental integration among multiple ornaments, this will have implications for their functional integration. In the case of delayed ornament use relative to production, integration at production may be completely different or absent at use. In the case of prolonged use, integration may gradually change with time. This may in turn reduce or reverse the adaptive value of paying attention to the trait complex.

In addition, year-specific environmental conditions may exert major influences on sexual signal expression (Saino *et al.,* 2004; Scordato, Bontrager & Price, 2012; Molnár, Bajer & Török, 2012), and there may be different year-specific effects on different ornaments of the same species (Hegyi *et al.,* 2007a). This may cause among-year variation in the magnitude and pattern of integration among multiple ornamental traits. Sexual ornamentation often drastically changes with age and this may involve shifts in information content (Hegyi *et al.*, 2006; Grunst, Rotenberry & Grunst, 2014). With differential delayed maturation of different ornamental components (Hawkins, Hill & Mercadante, 2012) relationships among these components may also undergo an age-related change. Finally, the proximate determination of homologous sexual traits in males versus females is sometimes similar (Doutrelant *et al.*, 2012) but in other cases very different (Murphy & Pham, 2012), which may cause sex differences in signal interrelation. If the ornamentation of the two sexes is qualitatively similar, the degree of similarity in ornament integration between males and females is informative regarding the evolution of ornament integration and it is therefore relevant to the stability of integration. For example, integration may have evolved similarly or differently in the two sexes, and the evolution of integration may have preceded or followed the evolution of sexual dichromatism.

Here we use a long-term spectral dataset from great tits (*Parus major*) to examine the integration of plumage colouration and the above mentioned four aspects of signal consistency. Some components of the plumage ornamentation of great tits have been abundantly studied (see e.g. Fitze & Richner, 2002; Senar, Figuerola & Domènech, 2003; Jacot *et al.,* 2010; Romero-Diaz *et al.*, 2013). However, despite suggestions of similarity in information content among multiple traits (Galván, 2010), their correlation structure and especially the temporal stability of this correlation structure have not yet been explored (but see Hegyi *et al.,* 2008 for a partial attempt; see also Senar & Quesada, 2009). We analyze the autumn and spring reflectance descriptors of birds caught in seven year-specific moult cycles (autumn or the following spring). This species has a single complete moult in summer (Gosler, 1993; see also Methods) and shows strong within-individual changes in plumage colour expression across seasons (Figuerola & Senar, 2005; Adamík & Vanáková, 2011), so it is ideal for testing the relative importance of moult and plumage abrasion for signal expression and integration. We assess reflectance variation and spectral integration among three ornamental plumage areas: the melanised breast stripe (Norris, 1993) and crown (Hegyi *et al.*, 2007b), and the carotenoid containing breast (Partali *et al.*, 1987). The reflectance of melanised traits has very scarcely been studied in great tits (this is the first study of breast stripe reflectance and the second study of crown reflectance), while studies of the yellow breast principally involved nestlings (reviewed in Hegyi *et al.*, 2007b). The role of these traits in signalling is unknown at present. The seasonal consistency of colour integration in this system would be important if some integrated component of reflectance had a role in mate choice (which occurs many months after moult) and would be especially important if the integrated component had a signalling role both within and outside the breeding season, as previously observed for breast stripe area in the same species (Norris, 1990; Lemel & Wallin, 1993).

Here we first compare signal integration patterns between the sexes and between yearling and older birds. Second, we quantify changes in trait interrelation from autumn to spring due to abrasion. Third, we compare the structure of trait correlation matrices among years. Fourth, we define composite colour traits and test the within-individual repeatability of univariate and composite traits in the face of different combinations of moult and abrasion, as well as their relationship with body condition during moult. Finally, we assess assortative mating patterns (the degree of correlation in colour between pair members) to compare one potential indicator of sexual selection between single and composite plumage colour traits. If univariate colour traits are developmentally integrated as an ancestral character state and functionally integrated throughout the year in both sexes, we predict similar colour trait interrelation patterns across sexes, ages, seasons and years. If the costly assessment of composite traits is adaptive, we also predict that the dominant composite colour axis will provide additional or more reliable information compared to the univariate traits, and accordingly show stronger or more consistent assortative mating than the univariate traits.

MATERIAL AND METHODS

FIELD METHODS

Great tit feather samples were collected in our nestbox plots near Szentendre, Hungary (Török & Tóth, 1999). The time span of our present data ranges from autumn 2006 to spring 2013. For comparability, we consider seven pairs of seasons belonging to the same summer moult (e.g. autumn 2006 and spring 2007). Therefore, we have seven ‘moult years’ from 2006 to 2012 (hereafter, ‘year’). In the autumn and early winter (6 October to 28 January in this dataset), great tits were caught using mist nets at two feeders baited with sunflower seed. In spring (5 May to 26 June), parents feeding 8- to 10-day old nestlings were caught in the nest box. All birds received numbered aluminium rings and their age (yearling or older) and sex were determined based on wing covert contrast and breast stripe size respectively (Svensson, 1992). The category “yearling” refers to first-years (1Y) in autumn and second-years (2Y) in spring. The plumage of both “yearling” categories was grown at the post-juvenile summer moult. The category “older” refers to second-years (2Y) and after-second-years (2Y+) in autumn and 2Y+ in spring, both of which have plumage grown at a post-breeding summer moult. Body mass was measured using a Pesola spring balance (nearest 0.1g) and tarsus length using callipers (nearest 0.1mm). Feather samples were collected from the black crown, yellow breast and black breast stripe. We collected approximately ten feathers from each area, which is at the lower threshold of sample size to ensure reliable spectrometric results (Quesada & Senar, 2006). Ten feathers are a small proportion of the plumage in each area. The location of feather collection is hardly visible after sampling and the feathers are regrown in a few weeks (our pers. obs.). The total number of birds sampled was *N* = 425 in autumn and *N* = 433 in spring (sample sizes for autumn and spring respectively; 2006: 91+25; 2007: 48+36; 2008: 49+94; 2009: 90+65, 2010: 59+41; 2011: 29+121; 2012: 59+51), and they were also *N* = 90 recaptures (*N* = 21 in autumn and *N* = 69 in spring). In autumn, we also collected the two second outermost rectrices to estimate body condition during moult from the width of daily feather growth bars (see below). It is important to state here that, in line with some southern populations of the species (Svensson 1992), all or nearly all juvenile birds in our area replace their rectrices at the post-juvenile moult (see Supplementary Methods Part 1 for details). Yearling rectrices therefore reflect body condition at the post-juvenile moult when the rest of the plumage was grown, so they are comparable with the rectrices of older birds. Collected feathers were stored in envelopes in a dark and dry place until processing in the laboratory. Breast stripe area (Figuerola & Senar 2000) was measured in a subset of our birds but it was weakly correlated with plumage reflectance in our population and showed very weak assortative mating so it was omitted from our analysis to preserve sample size. We also did not measure the reflectance of another ornamental trait, the cheek patch (Ferns & Hinsley, 2004) in this study. Inserting additional, possibly unknown ornamental traits could rearrange the results we report here, although this is still the most comprehensive study of great tit plumage reflectance we are aware of. Note that few birds are recaptured between seasons and years in our population (see above). (These were used to test repeatability, see below.) Our comparisons of seasons and years may therefore reflect the fact that we have partly different great tit individuals present in different seasons (residents in spring while residents plus vagrants in autumn). However, absolute differences in average colour among seasons and years were removed from our data before analyses (see below), while any sampling-related differences in colour correlation structure make our results conservative.

SPECTRAL MEASUREMENTS

Reflectance was measured with an Ocean Optics USB2000 spectrometer, using a bifurcated fibre-optic probe and Ocean Optics deuterium-tungsten-halogen light source DH2000. 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. Feathers were placed on top of one another on a piece of black velvet. Three scans were taken for each set of feathers, with frequent calibration using a WS-1 white reflection standard (Ocean Optics Europe) and a black reference (no incoming light to the sensor) during the measurements. Reflectance curves were stored using OOIBase software (Ocean Optics Europe). The repeatability of spectral data acquisition in this system is high (intraclass correlation coefficient $r_I = 0.761$ to 0.969, $N = 948$, all $P < 0.001$; Becker, 1984). Spectral data acquisition was done within a few years of feather collection. As feather samples were stored in a dry, cool and dark place and were not treated with any chemicals, the reflectance characteristics of our samples are unlikely to have substantially changed during the period between collection and measurements (Armenta, Dunn & Whittingham, 2008). Mean reflectance spectra for males and females of each plumage area are shown in Fig. 1.

BODY CONDITION DURING MOULT

Body condition (i.e. nutritional condition) during moult was estimated from the growth rate of tail feathers, that is, ptilochronology (Grubb, 1995; see Hill & Montgomerie, 1994; Keyser & Hill, 1999; Hargitai, Hegyi & Török, 2012 for applications to plumage ornamentation). We restricted these measurements to the autumn data to ensure that we measure the post-moult state of plumage colour, minimizing the confounding effect of differential abrasion. In addition, abrasion also hampers the readability of feather growth bars. The method adapted to the great tit by Senar *et al.* (2003) was used here (also see Hegyi *et al.,* 2007b, 2008). The total width of the first ten visible growth bars (light and dark) from the distal end of the feather was measured by calliper (nearest 0.1 mm), under intense direct illumination. Ten bars indicate body condition over ten days of moult, a long period relative to the total duration of moult. 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 ($r_I = 0.699 \pm 0.030$SE , $N = 302$ in juveniles and $r_I = 0.617 \pm 0.067$SE, $N = 87$ in older birds). The mean of the two sides was used as growth rate in the analyses, except for the case of damaged or missing feathers on one side. Overall 410 of the 425 autumn birds could be measured for ptilochronology, 21 of these on one side of the tail only. The two sides did not differ systematically (paired $t_{388} = 1.208$, $P = 0.228$) so using one side does not cause bias.

STATISTICAL ANALYSIS

We calculated two measures from the spectral data of each plumage area, using the 'tit-visible' range (320 to 700 nm) of the spectra (Hart *et al.,* 2000). The first measure was average reflectance (brightness, $R_{320\text{-}700}$). The second was a measure of the dominant direction of changes in spectral shape in the respective plumage area: UV chroma ($R_{320\text{-}400}/R_{320\text{-}700}$) for the crown and the breast stripe, and yellow chroma $(R_{700}\text{-}R_{450})/R_{700})$ for the yellow breast. These spectral shape measures have previously been validated using principal components analyses (PCA) of raw spectral bands (Hegyi *et al.,* 2007b). Here we calculated derived colour descriptors rather than using a PCA of raw spectral bands. A recent study showed that derived colour variables showed higher correspondence with other colour quantification methods (tristimulus variables, two avian visual models) than spectral band PCs (Evans *et al.*, 2010). We refrained from calculating estimates of the avian visual stimulus (Vorobyev *et al.*, 1998) because great tits use an extreme variety of light environments (from completely open sunlight to forest shade) and the light environment profoundly affects visual stimulus values, thereby making any one of them a misleading estimate of colour in our present case. Correlations between single light environment visual stimulus estimates and raw brightness and chroma of the given plumage area are extremely high (see Supplementary Methods Part 2 for details; see also Delhey & Peters, 2008; Evans *et al.*, 2010).

The six colour variables (brightness and chroma for each plumage area) were first transformed to improve normality of the model residuals (breast stripe brightness log transformed, breast yellow chroma square transformed). Repeated measurements of a given colour variable were then averaged within individuals and the averaged values were compared between sexes, binary ages (yearling or older), years (2006 to 2012, see above) and seasons (autumn vs. spring) in separate general linear models for each colour variable, using all possible interactions (except the three-way interactions of age; General Linear Model module of Statistica 5.5). We used stepwise backward simplification with reintroduction of the removed terms to the final model one by one (Hegyi & Garamszegi, 2011). The results are shown in Table 1. We then extracted standardized residuals (mean of zero, SD of 1) from the final models and used these residuals as area-level colour variables in the following analyses. Using residuals was necessary because different colour traits showed different among-group patterns (Table 1). Therefore, the pooled correlation matrix of raw, uncorrected colour traits does not represent the consistent within-group correlations we detected, and using this matrix to fit overall colour axes would accordingly produce nonsensical results. Residuals were also necessary in the models of assortative mating and repeatability to exclude confounding factors such as year or age. Finally, we used residuals in the analysis of body condition to facilitate comparability with other parts of the paper.

We did not correct our spectral data for date within season. When doing a correction for capture date in the autumn data using second-order polynomials (Delhey *et al.*, 2010), we obtained very similar results to those presented below. We report the uncorrected data here because a similar date-correction in spring would remove a component of individual quality (breeding date) and would therefore confound our results with respect to the information content of coloration.

In the following steps, we compared the correlation matrices of the six standardized (i.e. standard residual) colour traits between sexes, ages, seasons and then years (N = 425 in autumn and N = 433 in spring, see details above under Field methods). Matrix comparisons were done using the common principal component (CPC) method developed by Flury (1988) as implemented in the program CPC (Phillips & Arnold, 1999). This method evaluates a hierarchy of models that represent different degrees of matrix similarity, looking for the number of dominant PC axes (first, first two, first three etc.) that are shared between two data sets. Very small degrees of similarity are termed an “unrelated structure”. Increasing similarity levels correspond to different numbers of common PCs (CPC_1 to CPC_{k-2} where k is the number of input variables). The highest smilarity levels are when all PCs are similar in direction (full CPC), direction and relative importance (matrix proportionality) or direction and absolute importance (matrix equality). The program calculates the Akaike Information Criterion (AIC) as a measure of the relative suitability of these different similarity scenarios (models) given the data. To choose the model best supported by our data, we looked for the simplest model within a difference of AIC = 2 from the model with the lowest AIC. Note that this is not necessarily the model with the lowest AIC. This approach was used consistently when comparing the colour trait correlation matrices of sexes, ages, autumn and spring, or the seven different years. Note that the PCA approach may miss information lying outside the orthogonal axis structure (i.e. among-trait contrasts in our case) but variation outside the axis structure is by definition responsible for a small percent of trait covariation. The PCA approach may also fail if receivers use multiple colour traits hierarchically, but we have no information to build trait hierarchies into our models.

The focus of the present work was on quantifying the correlation structure of colour among plumage areas of the same individuals. Different plumage areas of the same individual, belonging to the same sample, are in practice measured consecutively. Inadvertent fluctuations in measurement quality due to various factors (changing attention levels of measurers, light source drift etc.) necessarily cause autocorrelation among neighbouring measurements, and this autocorrelation may inflate correlations among plumage areas or create artefacts in the correlation matrix. To control for this, we re-measured the samples of 100 randomly chosen breeding individuals (50 males and 50 females) from six different years so that plumage areas were measured separately and in different, completely randomized orders. In this sample, the effect of temporal autocorrelation on the correlations of spectral variables was completely eliminated. We then used CPC to compare the correlation structure in this randomized sample to that in the original measurement order in the same 100 samples.

Based on the results of the matrix comparisons, we then calculated the PCA of individual colour trait residuals using Varimax rotation in the Factor Analysis module of Statistica 5.5 (StatSoft, Inc.). To check the effect of mathematical interdependence, we also ran separate PCAs for brightness and chroma traits. The resulting PC scores (when handling all plumage traits together) were used in three further analyses. First, the within-individual repeatability of univariate colour trait residuals and the PCs was calculated. We had four different types of repeated measurements: from spring to the next spring (*N* = 41; two measurements separated by moult but involving similar degrees of plumage abrasion); from autumn to the next spring ($N = 19$; two measurements separated by a period of plumage abrasion but no moult), between autumn and spring with a moult in between ($N = 19$; both moult and abrasion), and between two autumns ($N = 11$; moult but no abrasion). We have unfortunately too few within-season recaptures and therefore no information on within-season repeatability. Within-individual data were analyzed using general linear models with second measurement as the dependent variable, first measurement as a covariate, and repeat type as a factor, also including their interaction. Sexes were pooled due to the high similarity of their trait correlation matrices (see Results).

Second, relationships with body condition during moult (mean growth bar width) were estimated for univariate residual and composite colour traits in the autumn data. This analysis avoided repeated measures from the same individual ($N = 268$ for males and $N = 142$ for females). We calculated standard residual condition from a model with binary age, year and sex as factors (binary age $F_{1,401} = 4.73$, $P = 0.030$; year $F_{6,401} = 10.48$, $P < 0.001$; sex $F_{1,401} = 29.26$, $P < 0.001$; year x sex interaction was non-significant and removed) and tested the residuals against the residual colour variables using Pearson correlations (age and sex differences in condition-dependence were always non-significant when tested in general linear models; age x condition $F_{1,406} < 2.92$, $P > 0.089$; sex x condition $F_{1,406} < 2.43$, $P > 0.120$). Year comparisons were not done for condition-dependence because the statistical power to detect such patterns was low.

Finally, univariate colour trait residuals and PCs of members of breeding pairs with complete spectral data (only spring data, $N = 175$) were used to estimate assortative mating as one possible measure of sexual selection (Pearson correlations, Basic Statistics module of Statistica 5.5). We also examined the among-year consistency of assortative mating by formal meta-analysis with a ‘random effects model’ that assumes heterogeneity in effect size among samples (Lipsey & Wilson, 2001). Weighted mean effect sizes and their confidence intervals were calculated using Comprehensive Meta-Analysis Version 2 (http://www.meta-analysis.com).

RESULTS

STABILITY OF COLOUR INTEGRATION

In the first step, we compared the correlation matrices of univariate colour trait residuals (i.e. group-standardized colour traits) between the sexes and age groups using CPC. This program uses an information theoretic approach to compare alternative models representing varying numbers of common PC axes (with the remaining, lower order axes being different). The CPC comparison of sexes (Table 2) indicated matrix proportionality, which implies that all PC axes have similar directions and similar relative importance. Concerning age, the first four PC axes of the colour correlation structure were shared between yearling and older birds (Table 2). We then assessed the effect of plumage abrasion on the integration of coloration by comparing the correlation matrices of autumn (little abrasion) and spring (substantial abrasion). The strict sense best model of this CPC comparison indicated that three PCs were shared between the seasons, although the model with one shared PC performed only slightly less well in suitability than the best model (Table 2). This ambiguous result occurred because the first three PCs of the two colour traits were the same in autumn versus spring but PC2 and PC3 “changed places”, although differences in their explained variances were small (results not shown). Our final CPC comparisons focused on among-year differences in trait integration. The ratio of autumn data to spring data was drastically different among years, with the proportion of autumn data among all data of a given year ranging from 19% to 78% (see Methods). Therefore, an among-year comparison of data while pooling autumn and spring would confuse year differences with between-season differences. Consequently, we assessed among-year variation in the correlation matrices separately for autumn and spring (Table 2). The results indicated that correlation patterns were consistent among years, with all PCs shared in both autumn and spring. Among-year means and confidence intervals of correlations for univariate trait pairs (from a random effect meta-analysis) can be seen in Fig. 2 for the two seasons separately.

Finally, to examine possible measurement order effects, we compared in the 100 samples with randomized measurement order the correlation structure of (residual) colour traits to that obtained in the same 100 samples with the original measurement protocol. This revealed a situation very close to matrix equality, i.e. nearly perfect matching (matrix equality AIC = 25.977; matrix proportionality AIC = 27.970; full CPC AIC = 28.112; all other models AIC > 30). Furthermore, using the original protocol, the sample of these 100 data yielded a similar correlation matrix to that in the whole sample (*N* = 858 after eliminating within-individual repeats), again being close to matrix equality (matrix equality AIC = 10.174; matrix proportionality AIC = 12.127; all other models AIC > 17). The latter result shows that the small sample was representative of the whole dataset concerning its correlation structure. We can therefore conclude that the effect of temporal autocorrelation on our correlation matrices was negligible and our results on matrix stability are not measurement artifacts.

MAIN COLOUR AXES

The component loadings of the main PC axes for the pooled dataset (again using residual colour traits) are summarized in Table 3. The first PC linked two plumage areas, correlating positively with the UV chroma of both melanised regions (crown and breast stripe). The second PC described yellow breast coloration, correlating positively with yellow chroma and negatively with brightness. Finally, the third PC was positively related to the brightness of both melanised plumage areas. The proportions of variance explained by the three PCs were very similar and together accounted for 61% of total variance. This PCA included the within-individual repeats for the sake of later repeatability testing. Using one data point per individual yielded almost quantitatively identical results (not shown here).

A separate PCA of brightness and chroma traits to avoid their mathematical interdependence also yielded the same plumage area associations. For both brightness and chroma, the crown and the breast stripe were joined and the breast was treated separately (Supplementary Table 2). This indicates that although PC2 in the pooled PCA (yellow brightness and yellow chroma) could possibly be affected by mathematical dependence and should be viewed as such, other PC axes are not affected by this issue. As PC2 can also be explained by a biological mechanism (lutein absorbance increases chroma and reduces brightness), we use the output of the pooled PCA in the following.

REPEATABILITY AND CONDITION DURING MOULT

In the analysis of within-individual repeatability, there was no significant difference among repeat types in any residual colour trait or PC (four different combinations of abrasion and moult; repeat type x previous value $F_{3,82} < 1.943$, $P > 0.129$). Pearson correlations indicated that repeatability was relatively strong in breast yellow chroma and crown UV chroma, but generally low for the remaining individual traits (Table 4). PC1 had a marginally non-significant repeatability, while the other two PCs were very weakly repeatable.

The only colour traits (residuals or PCs) related to residual body condition during moult in the autumn data (Table 4) were breast yellow chroma and the corresponding PC (PC2). The UV chroma of the two melanized plumage areas showed little trace of condition-dependence. Colour PC1, an indicator of the parallel changes of these two UV chroma traits, was similarly independent of condition.

MATING PATTERNS

Assortative mating for univariate colour trait residuals and colour PCs (spring data only) was generally weak (Table 4). Crown UV chroma and overall UV chroma (PC1) showed the strongest relationships. We also examined within-trait, among-year variation in assortative mating estimates (Fig. 3). The weighted mean estimate significantly differed from zero, indicating consistent positive assortative mating, for crown UV chroma but not for any other individual or composite trait. Overall UV chroma (PC1) gave a combined assortative mating estimate in between those of its two constituent traits. This was not significantly different from zero.

DISCUSSION

Here we examined the stability of phenotypic integration in a system of multiple plumage colour traits. For each of the three plumage areas we considered, we used overall brightness and the single dominant direction of spectral shape variation. This ensured a representative analysis of colour with a reasonable complexity. Of the three plumage areas considered here, our PCAs revealed correlations in brightness and UV chroma between the melanised crown and breast stripe, while the carotenoid containing breast plumage varied independently of the other two areas. Among our PC axes, PC1 and PC3 likely reflect the structural regularity and melanin content of the two melanised areas, while PC2 may mirror the carotenoid content of breast feathers (Bleiweiss, 2004). Notably, a previous study of the same population detected an axis of parallel variation between breast yellow chroma and crown UV chroma (Hegyi *et al.*, 2008). Differences from the present findings may principally stem from the lack of breast stripe reflectance from the previous study, and the different statistical method (spectral band

PCA). As the correlation structure we report here was statistically similar across seven years (see below), we consider our results robust and representative.

The great tit is qualitatively sexually monochromatic, with the basic outline of the ornament complex being the same in the two sexes, but the expression of especially melanin-based coloration clearly differing between males and females (Norris, 1993; Hegyi *et al.*, 2007b). In our data, all colour variables except yellow chroma (see Evans *et al.*, 2010 for a different result) were sexually dichromatic before the calculation of residuals (Fig. 1). Our present analysis nevertheless indicated that the among-trait correlation structure of colour was very similar between males and females. The principal axes of colour variation agreed in both direction and relative importance, suggesting that the genetic or permanent environmental background of colour integration is similar in the two sexes (Price, 1996; Potti & Canal, 2011). Likewise, yearling and older great tits are superficially similar but there are significant changes in some colour variables with age (Table 1). However, the correlation structure of colour was still similar between the two age groups.

Seasonal changes in colour integration may be attributable to two processes: moult and abrasion. Plumage colour traits may diverge in their expression and lose their correlated variation due to different condition-dependence or environmental effects during moult (McGraw *et al.*, 2002; Hill, Doucet & Buchholz, 2005; Hill, Hood & Huggins, 2009; Vágási *et al.*, 2012) or differential abrasion or fading of colour types (Bonser, 1995; Surmacki, Siefferman & Yuan, 2011), plumage regions (Delhey *et al.*, 2010) and even different parts of the same region (Pap *et al.*, 2007). We found that the first three colour axes were shared between autumn and spring, suggesting between-season stability in colour trait interrelation. Sexual ornaments are often used in both mate attraction in the breeding season and competitive contexts outside the breeding season (McGraw, 2004; Reudink *et al.*, 2009). Our results in great tits indicate that paying attention to the same overall aspect of plumage ornamentation throughout the year could be a viable strategy for receivers, either males or females, in this population.

Finally, non-directional among-year variation attributable to environmental conditions is widespread among sexual ornaments (Møller, 1991; Jensen *et al.*, 2006; Evans & Sheldon 2012; Molnár *et al.*, 2012), but among-year variation has rarely been studied for multiple ornaments simultaneously (Chaine & Lyon, 2008a). Different ornamental traits are often affected by different year-specific environmental factors (Hegyi *et al.*, 2007a), and this may in turn limit their utility as components of an integrated signal system. Despite drastic among-year variation in food availability and phenology in our study area (Török *et al.*, 2004; Hegyi, Nagy & Török, 2013), the correlation patterns of colour traits remained nearly identical across years. This stability existed despite the fact that the basic determinants of the different colour traits (effects of year, age, season and their interactions) were different. These results indicate robust integration between the brightness and the UV chroma traits of the two melanised, black plumage areas, while the carotenoid-containing breast plumage did not take part in this integration, perhaps due to its different developmental background. This partial colour integration in the plumage opens a possibility for great tits to use the parallel colour variation of the two melanised areas as a single sexual signal. Although this cannot be definitively assessed without experiments, we could nevertheless tentatively examine whether these potential composite signals (and particularly the UV chroma PC which is the dominant axis of colour trait covariation) could confer specific information on some aspects of individual quality, and whether they could specifically explain variation in assortative mating patterns when compared to the univariate colour traits of different plumage areas.

We first examined within-individual repeatability, which indicates whether a trait can signal stable aspects of individual quality (Tomkins *et al.*, 2004, Wilson & Nussey, 2010). We could not detect significant differences in repeatability between different combinations of moult and abrasion, although the small sample sizes limited the power of this comparison. Overall repeatability was significant only for breast yellow chroma and crown UV chroma. Breast stripe UV chroma had a much smaller repeatability, likely due to looser feather structure and more frequent physical contact with hard surfaces like tree bark or hole entrance, leading to stronger abrasion and soiling than on the crown. For the composite of crown and stripe UV chroma (PC1), repeatability was very similar to the average of the two constituent traits. It therefore seems that considering the two areas together does not provide more reliable information to the receiver in this respect.

We also examined the relationship between body condition during moult and subsequent colour expression. Breast yellow chroma was the only trait for which this correlation was significant, likely due to a relationship between carotenoid availability and body condition during moult (Partali *et al.*, 1987). Note that despite its indicator value, yellow chroma was not subject to significant assortative mating in our population, in contrast to a previous study in Spain (Quesada & Senar, 2009). Melanin-based traits, on the other hand, do not depend so directly on nutritional limitations (but see Talloen, Van Dyck & Lens, 2004; Poston *et al.*, 2005; Bize *et al.*, 2006; Punzalan *et al.*, 2008). Indeed, crown and breast brightness, and their composite (PC3) were largely unrelated to condition during moult. Finally, the condition-dependence of structural plumage colour in birds is debated due to the lack of a clear mechanism (Prum, 2006). Although laboratory experiments suggest nutritional effects on structural colour, these effects largely concerned total reflectance (Siefferman & Hill, 2005a, 2007; Jacot *et al.*, 2010) and only rarely relative UV reflectance (Siefferman & Hill, 2005b; for condition-dependence in non-avian structural colours, see Kemp & Rutowski, 2007; Lim & Li, 2007). In a recent study in blue tits (*Cyanistes caeruleus*) it was further suggested that experimental condition-dependence of structural colour may arise via stress and not body condition (Peters *et al.*, 2011). In our great tits, in agreement with this, neither the UV chroma of black areas, nor the composite colour axis they formed (PC1) correlated with natural body condition during moult to any notable extent. It is important to stress that the UV reflectance of the crown likely has a different developmental mechanism in blue and great tits (involving different feather microstructures), and this mechanism is unknown in detail in both species. Therefore, comparisons must be made with caution.

In sum, despite the robust and stable interrelation between the UV chroma of the crown and the breast stripe in our population, we found no evidence that the “overall” UV chroma of black plumage areas was especially informative when compared to the individual ornaments. Crown and breast UV chroma belong to the same proximate determination pathway as well as the same sensory modality, which may in theory facilitate both developmental and functional integration between them (Hebets & Papaj, 2005). However, an ideal composite ornament should provide emergent or more reliable information than its constituent traits. In our case, the UV chroma of the two black plumage traits is likely produced by a similar mechanism and may therefore provide similar information. Moreover, breast stripe UV chroma seemed to convey less reliable information as judged from its weaker repeatability. In addition, both UV chroma traits were similarly uninformative with respect to body condition during moult. The composite trait, overall UV chroma was similar in condition-independence to its component traits, and its repeatability was halfway between those of the individual traits. Therefore, the two aspects of information content we examined here did not highlight the composite colour traits as particularly informative over the univariate traits. Other measures of information content (e.g. developmental stability, stress tolerance) may have given different results.

The sexual selection perspective reinforced our conclusion on information content that composite traits are “not special”. In the case of mutual sexual selection on ornamentation, specific attention to a composite trait by both sexes may be expected to produce stronger assortative mating patterns for this trait than for its constituents. In our population, breast stripe UV chroma showed weaker mating patterns than crown UV chroma, possibly due to its poorer quality indicator value. Strength of the mating pattern for the composite trait (PC1) was again halfway between those of the two constituent traits, suggesting that great tits pay no special attention to the combined expression of the two traits, or that this attention is not mutual (i.e. involves only one sex). Therefore, despite the stable phenotypic integration between the two UV chroma traits in both sexes, we could not detect functional integration between them in terms of assortative mating. On the other hand, they supported previous results in this population on the importance of crown UV chroma in mutual sexual selection (Hegyi *et al.*, 2007b).

It is important to stress here that the information content and function of sexual signals often differs among populations (Baird, Fox & McCoy, 1997; Dale *et al.*, 1999; Hegyi, Török & Tóth, 2002; Møller *et al.*, 2006). Accordingly, other populations of the great tit may show different patterns of colour trait integration and may show functional integration between some of their colour traits. Moreover, testing other measures of sexual selection (mate choice during the winter, territorial competition in early spring) may have given different results, potentially indicating selection on composite traits, or on yellow chroma, the condition-dependent aspect of plumage colour in this population. Breeding dates do not correlate with either the univariate or the composite colour traits of males or females in our population (results not shown), which reinforces the notion that apparent assortative mating on crown UV chroma was not due to territory quality patterns, but tells little on sexual selection in this particular species. Further studies are needed on this topic, especially given the painful lack of evidence for sexual selection on great tit plumage colour in general.

The possible existence of functional sexual trait complexes is a largely unexplored area in sexual selection research (Hebets & Papaj, 2005). At least three different evolutionary patterns are conceivable with respect to multiple coexisting sexual traits. The first is different information content or function, as discussed above. The second is temporally and spatially fluctuating selection on different traits, as emphasized by some recent reviews (Bussière *et al.,* 2008; Cornwallis & Uller, 2009; Bro-Jørgensen, 2010). Finally, the third possibility is developmental and functional linkage among multiple ornamental traits (Badyaev, 2004). There is apparently great among-species variation in the interrelations and relative roles of multiple ornamental traits (Candolin 2003). In perhaps the most comprehensive empirical study conducted to date, the information content of multiple display traits in lark buntings (*Calamospiza melanocorys*) fluctuated among years of different environmental conditions, and sexual selection on these traits also fluctuated in both strength and direction (Chaine & Lyon, 2008a). Furthermore, in that signal system, different traits conveyed very different information about their bearers (Chaine & Lyon, 2008b). By contrast, in a population of collared flycatchers (*Ficedula albicollis*), mating patterns suggested a special function for the plumage-level parallel variation of colour in mate acquisition (Laczi *et al.*, 2011) and life-history correlates revealed multiple independent, composite colour axes with different information content in the same plumage (Laczi *et al.*, 2013). In our great tit population, we found correlated expression between some colour traits, but no functional integration, at least in terms of assortative mating. The differences from collared flycatchers could be due to the fewer proximate determination pathways of plumage colour (carotenoids are lacking), and the integration of both brightness and chroma traits at the whole plumage level in that species. Future studies on great tits should pay more attention to the hitherto largely unknown role of plumage reflectance in sexual selection (but see Quesada & Senar 2009), with special attention to breast yellow chroma, a highly informative trait (Senar *et al.*, 2003; Jacot *et al.*, 2010; this study), and crown UV chroma, a trait with great sexual dichromatism and a possible role in mutual mate choice (Hegyi *et al.*, 2007b, this study).

Our results convey an important warning for empirical studies of multiple signals. At present, when researchers encounter significant correlations among colour traits, the general reaction (if any) is apparently to combine these into a composite colour measure (Merilä *et al.,* 1999; Siefferman & Hill, 2003, 2005b; but see Jacot *et al.,* 2010 for an exception). Our results suggest that correlated ornaments require more careful treatment. In particular, before considering the traits "together", we must examine whether their composite has any special information to provide to the receiver, and also whether it plays a special role in sexual selection combined to the individual traits. In the absence of special information content or function, the optimal solution is probably to identify the real sexually selected trait "among the trees", and use this in further analyses. This identification may need to be done separately for different contexts (Andersson *et al.,* 2002) and different populations (Dunn *et al.,* 2010) and should ideally be experimental. Any correlative test must pay attention to the statistical problems of multiple testing (Nakagawa & Cuthill, 2007) and collinearity (Graham, 2003). Sexual selection research will greatly benefit from the proper handling of relationships between multiple ornamental traits (Cornwallis & Uller, 2009).

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Table 1. Results from general linear models testing the effects of year, season, sex and age on raw plumage colour traits. We employed backward stepwise model simplification with reintroduction, and adjusted r^2 refers to the final model

	Breast brightness		Breast yellow chroma		Crown brightness		Crown UV chroma		Stripe brightness		Stripe UV chroma	
	F	df	*F*	df	*F*	df	*F*	df	*F*	df	*F*	df
Year	1.90	6, 933	8.43***	6, 933	26.49***	6, 927	39.99***	6, 927	11.24***	6, 926	42.88***	6, 926
Season	86.13***	1, 933	6.71**	1, 933	41.77***	1, 927	606.14***	1, 927	0.30	1, 926	588.15***	1, 926
Sex	51.41***	1, 933	1.08	1, 932	244.29***	1, 927	1390.49***	1, 927	283.53***	1, 926	563.59***	1, 926
Age	0.146	1, 932	13.93***	1, 933	2.35	1, 926	0.13	1, 926	2.58	1, 925	7.79**	1, 926
Year x season	10.29***	6, 933	4.86***	6, 933	74.65***	6, 927	9.73***	6, 927	17.13***	6, 926	32.25***	6, 926
Year x sex	0.89	6, 927	1.20	6, 926	2.06	6, 921	2.21*	6, 927	0.49	6, 920	0.67	6, 920
Year x age	0.89	6, 926	1.14	6, 927	1.51	6, 920	1.24	6, 920	0.60	6, 919	0.54	6, 920
Season x sex	0.00	1, 932	1.93	1, 931	3.43	1, 926	0.29	1, 926	8.85**	1, 926	0.02	1, 925
Season x age	0.99	1, 931	2.66	1, 932	0.01	1, 925	0.61	1, 925	0.02	1, 924	0.54	1, 925
Sex x age	2.72	1, 931	0.87	1, 931	0.02	1, 925	1.70	1, 925	2.46	1, 924	3.62	1, 925
Year x season x sex	1.99	6, 927	1.35	6, 926	10.86***	6, 927	0.87	6, 921	2.65*	6, 926	2.33*	6, 926
Adjusted model r^2	0.188		0.088		0.579		0.764		0.365		0.673	

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Table 2 AIC values from the common principal component (CPC) analyses to assess the similarity of the correlation matrices of our six individual colour traits (group-corrected residuals) between sexes, ages (yearling versus older), seasons (autumn versus spring) and years. The selected models are highlighted by boldface

Model	Sex	Age	Season	Year autumn	Year spring
Equality	18.45	41.44	76.81	161.34	138.59
Proportionality	**20.44**	43.05	78.45	170.84	149.77
Full CPC	23.94	28.65	40.20	**153.66**	**139.16**
4 CPC	25.04	**26.27**	38.36	160.14	142.55
3 CPC	28.36	29.54	**36.39**	163.15	160.08
2 CPC	31.68	34.62	40.86	188.85	177.54
1 CPC	35.98	37.74	38.44	213.76	209.64
Unrelated	42.00	42.00	42.00	252.00	252.00

Table 3 Component loadings (Pearson *r*) and explained variances of the main principal component axes of variation among individual colour traits (group-corrected residuals). Correlations larger than 0.5 are shown in bold

	PC1	PC2	PC3
Breast brightness	-0.177	**-0.765**	0.112
Breast yellow chroma	-0.210	**0.770**	-0.020
Crown brightness	0.032	-0.024	**0.792**
Crown UV chroma	**0.791**	-0.060	-0.013
Breast stripe brightness	-0.079	-0.096	**0.730**
Breast stripe UV chroma	**0.765**	0.033	-0.037
Explained variance	*0.216*	*0.199*	*0.196*
Eigenvalue	*1.294*	*1.193*	*1.175*

Table 4 Within-individual repeatability (all categories pooled), condition-dependence and assortative mating for individual colour traits (group-corrected residuals) and colour PCs. None of the significances disappears after Bonferroni correction (3 x 3 non-independent variables)

	Repeatability						Condition				Assortative mating			
	R_{I}	SE	Pearson *R*	CI lower	CI upper	*N*	Pearson *R*	CI lower	CI upper	*N*	Pearson *R*	CI lower	CI upper	*N*
Breast brightness	-0.025	0.106	-0.028	-0.234	0.180	90	-0.049	-0.145	0.048	410	0.015	-0.134	0.163	175
Breast yellow chroma	0.280	0.098	0.277**	0.074	0.458	90	0.129*	0.032	0.223	410	0.107	-0.042	0.252	175
Crown brightness	-0.037	0.106	-0.042	-0.247	0.167	90	0.022	-0.075	0.119	410	-0.060	-0.206	0.089	175
Crown UV chroma	0.275	0.098	0.280**	0.077	0.460	90	0.068	-0.029	0.164	410	0.194**	0.047	0.333	175
Breast stripe brightness	0.020	0.106	0.022	-0.186	0.228	90	-0.001	-0.098	0.096	410	0.122	-0.027	0.265	175
Breast stripe UV chroma	0.131	0.104	0.137	-0.072	0.335	90	0.011	-0.086	0.107	410	0.122	-0.027	0.266	175
PC1	0.185	0.102	0.182	-0.026	0.345	90	0.041	-0.056	0.138	410	0.188*	0.041	0.327	175
PC2	0.101	0.105	0.100	-0.109	0.301	90	0.120*	0.024	0.214	410	0.049	-0.101	0.195	175
PC3	-0.029	0.106	-0.029	-0.235	0.179	90	0.029	-0.068	0.126	410	0.046	-0.103	0.193	175

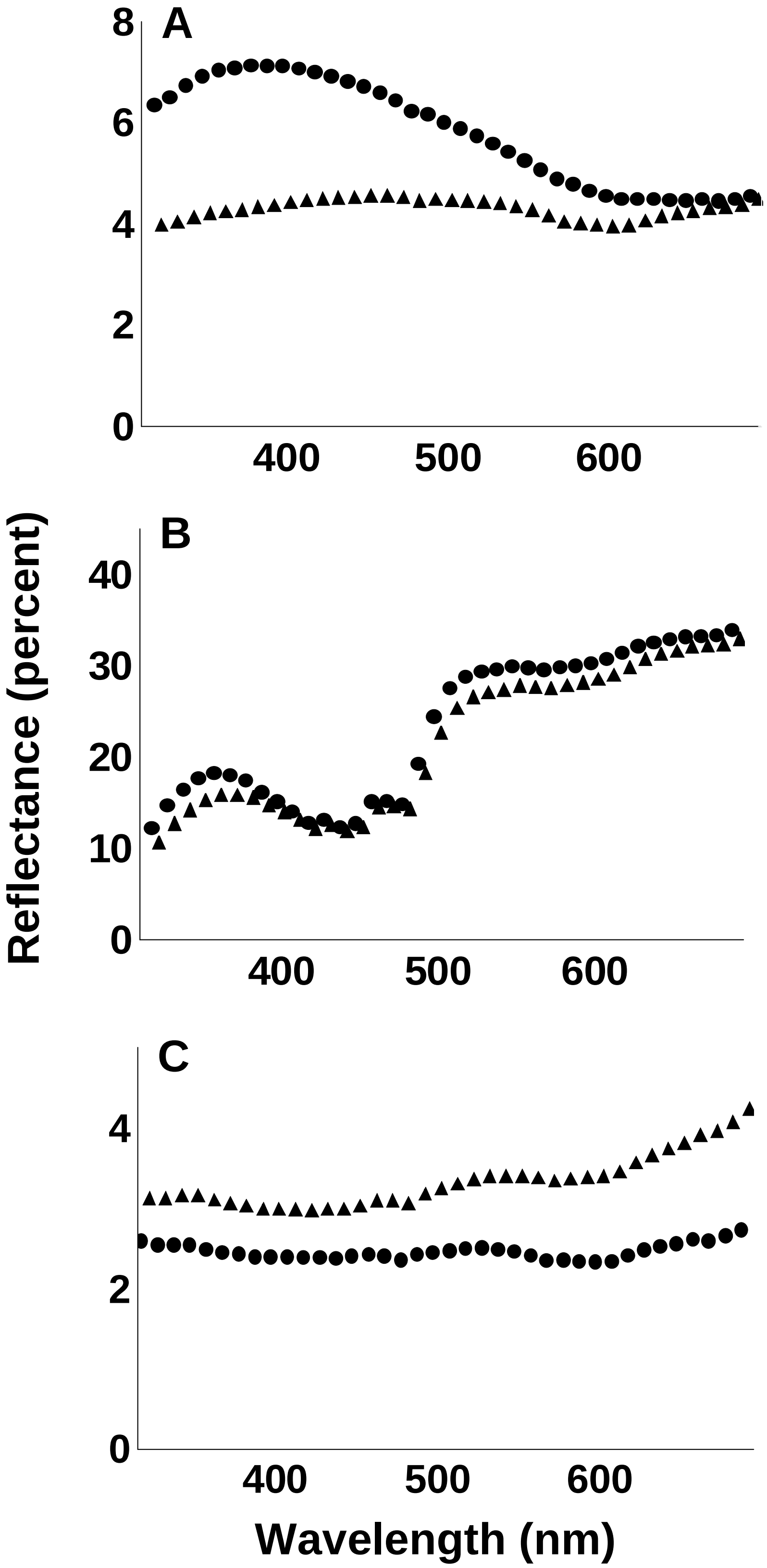
CI, confidence interval; r_{I}, intraclass correlation coefficient; SE, standard error; *, $P < 0.05$; **, $P < 0.01$

Figure 1. Sexual dichromatism in raw reflectance spectra of three plumage areas; A, crown; B, breast; C, breast stripe. Circles refer to males (*N* = 484) while triangles to females (*N* = 374). Mean values are shown. Bars of 95% confidence intervals are smaller than the symbols so they cannot be shown in this figure

Figure 2. Among-year weighted mean correlations (Pearson *r* ± 95%CI) of given individual colour trait pairs (group-corrected residuals) in autumn (filled circles) and spring (horizontal lines). ’AB’ denotes correlation between trait A and trait B. Capital letters refer to breast brightness (A), breast yellow chroma (B), crown brightness (C), crown UV chroma (D), breast stripe brightness (E) and breast stripe UV chroma (F). The dashed line marks zero correlation

Figure 3. Among-year weighted means of assortative mating estimates (Pearson *r* ± 95%CI) for individual (residual) and composite plumage colour traits. Codes of individual colour traits consist of area (B, breast; C, crown; S, breast stripe) and colour variable (BR, brightness; YC, yellow chroma; UV, UV chroma).The dashed line marks zero correlation

966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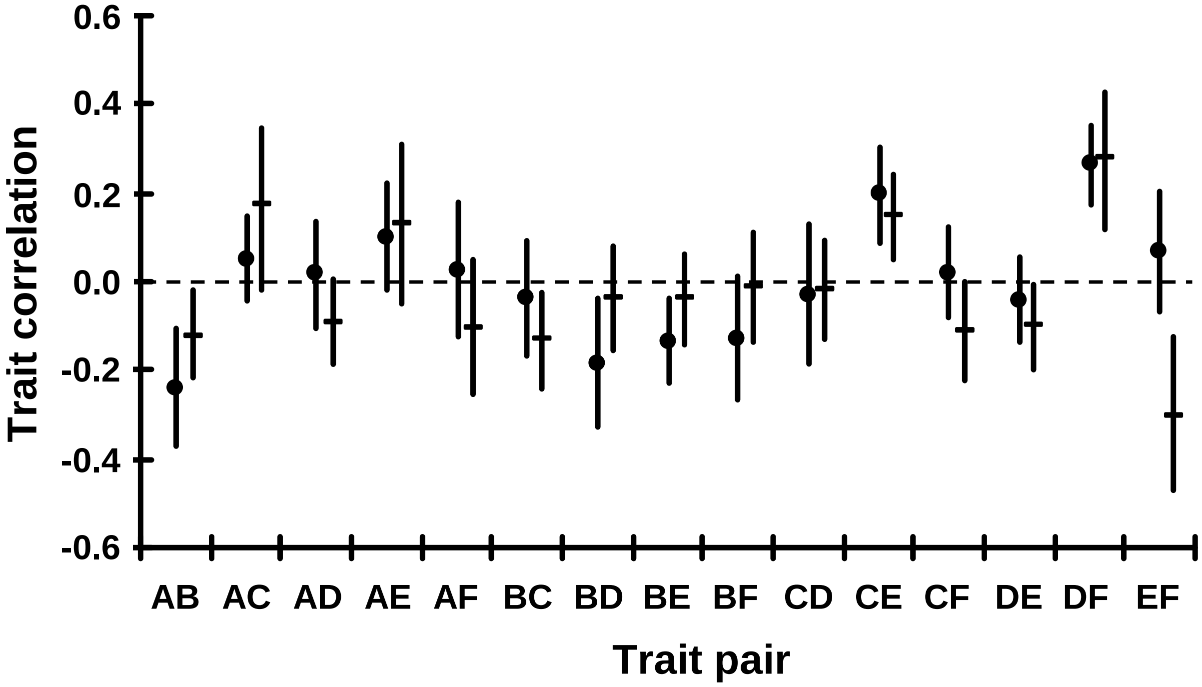
Reflectance (percent)
Wavelength (nm)

967
968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Trait correlation
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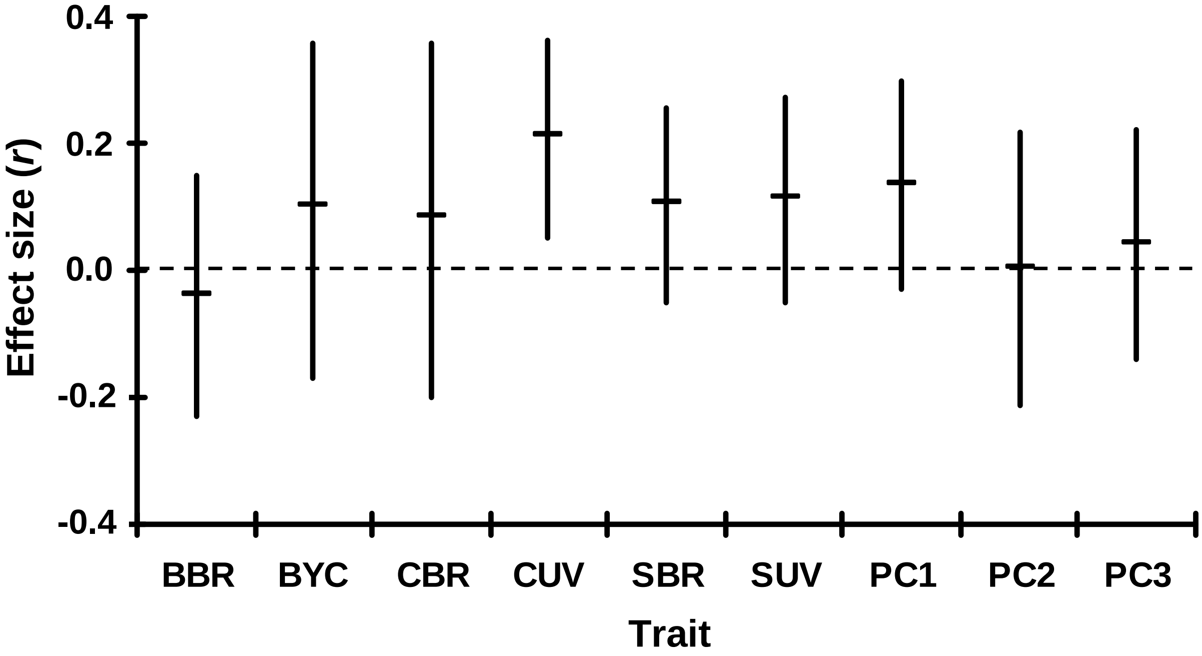
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