Miklós Laczi [1*] · Gergely Hegyi [1] · Márton Herényi [1] · Dorottya Kiss [1] · Gábor Markó [1,2] · Gergely Nagy [1] · Balázs Rosivall [1] · Eszter Szöllősi [1] · János Török [1]

Integrated plumage colour variation in relation to body condition, reproductive investment and laying date in the collared flycatcher

[1] Behavioural Ecology Group, Department of Systematic Zoology and Ecology, Eötvös Loránd University, Pázmány Péter sétány 1/C, H-1117 Budapest, Hungary.

[2] Department of Plant Pathology, Corvinus University of Budapest, Ménesi út 44, H-1118 Budapest, Hungary.

[*] Corresponding author; e-mail: laczi.miklos@gmail.com

Abstract The possible integration of different sexual ornaments into a composite system, and especially the information content of such ornament complexes, is poorly investigated. Many bird species display complex plumage coloration, but whether this represents one integrated or several independent sexual traits can be unclear. Collared flycatchers (*Ficedula albicollis*) display melanised and depigmented plumage areas, and the spectral features (brightness and UV chroma) of these are correlated with each other across the plumage. In a five-year dataset of male and female plumage reflectance, we examined some of the potential information content of integrated, plumage-level colour attributes by estimating their relationships to previous and current year body condition, laying date and clutch size. Females were in better condition the year before they became darker pigmented, and males in better current year condition were also darker pigmented. Female pigment-based brightness was positively, while male structurally based brightness was negatively related to current laying date. Finally, the overall UV chroma of white plumage areas in males was positively associated with current clutch size. Our results show that higher degree of pigmentation is related to better condition, while the structural colour component is associated with some aspects of reproductive investment. These results highlight the possibility that correlated aspects of a multiple plumage ornamentation system may reflect together some aspects of individual quality, thereby functioning as a composite signal.

Keywords *Ficedula albicollis* · Reflectance · Colour integration · Sexual signals

Introduction

In numerous bird species, plumage ornamentation of both sexes includes differently coloured pigment-based and structurally based ornamental patches. Due to their production and wearing costs, these colour patches are frequently adjusted to the physical condition, physiological state, genetic quality or other attributes of their bearer, thereby providing useful information to the receiver (Andersson 1994; McGraw et al. 2002). In case of ornaments of different developmental origin, their exaggerated expression is usually associated with different costs, and there can be different genetic associations with other phenotypic attributes. As a consequence, such simultaneously displayed ornaments can advertise different aspects of the bearer's quality, as suggested for several species (Hill and McGraw 2006). For example, food deprivation treatment had a negative effect on colour properties of structural blue feathers in the brown-headed cowbird (*Molothrus ater*) and eastern bluebird (*Sialia sialis*), but melanin-based coloration did not change (McGraw et al. 2002; Siefferman and Hill 2005a). In house finches (*Carpodacus mexicanus*), coccidial infection affected the development of carotenoid-based ornaments, but not that of melanin-based ones (Hill and Brawner 1998). A cross-fostering study of great tits (*Parus major*) revealed a predominately quantitative genetic dependence in melanin and environmental dependence in carotenoid coloration (Quesada and Senar 2009). Furthermore, it is even possible that different components of the same colour may relate to partly different aspects of the individual state. In one study of great tits, the chromatic component of yellow feathers was heritable and depended on environmental conditions, while the achromatic component was not heritable (Evans and Sheldon 2012). Another study of this plumage area showed that feather microstructure was under genetic and environmental control, while the carotenoid-based colour component was mainly related to environmental factors (Matrková and Remeš 2012).

Even though multiple ornamental traits often have different proximate origins, it is possible that they advertise partly overlapping information (particularly if they are related to each other), in addition to different aspects of quality. For example, in black-capped chickadee males (*Poecile atricapillus*), spectral properties of white and also black plumage areas predicted the proportion of extra-pair offspring (Doucet et al. 2004), and in the great tit, the short-wavelength part of the reflectance of differently coloured patches uniformly reflected individual condition (Galván 2010). Interestingly, it is possible that multiple traits convey redundant information, but in opposite directions, as found in the barn owl (*Tyto alba*) where more pheomelanic birds are less resistant to oxidative stress, and more eumelanic ones are more resistant (Roulin et al. 2011). In addition to describing the covariation of multiple traits, it is an interesting question how the common (or integrated) variation of traits is related to life-history components, with implications to their information content. Until now, there are only very few investigations of the relationships between life-history components and composite ornamentation (Siefferman and Hill 2003).

The plumage ornamentation of the collared flycatcher (*Ficedula albicollis*) is usually quantified by the size of ornamental white patches (Pärt and Qvarnström 1997; Hegyi et al. 2008a). Nevertheless, a recent study revealed that plumage reflectance also varied among individuals, with the brightness and UV chroma of differently coloured plumage areas changing in parallel, thereby forming an integrated signal system which seemed to specifically predict social mating patterns (Laczi et al. 2011). Here we wanted to explore the plumage-level colour variation of female and male collared flycatchers in relation to body condition and patterns of reproduction. We measured the brightness and UV chroma of melanised and depigmented plumage areas, and looked for axes of common variation among these colour measures. We then interpreted these integrated colour axes based on what is known about the mechanisms of colour production in melanised and depigmented areas.

Finally, we correlated integrated colour expression with primary reproductive investment (clutch size) and body condition in the previous year.

Associations of ornamentation with reproduction may reflect genetic or hormonally based links, for example, different reproductive tactics by differently ornamented individuals (Duckworth et al. 2003) or differential investment by the partner (Horváthová et al. 2012). Similarly, a simple correlation between ornamentation and body condition does not constitute evidence for condition-dependence. This is particularly true for melanin-based traits which are controlled by pleiotropic genes affecting many other traits as well (Ducrest et al. 2008; Roulin and Ducrest 2013), which can lead to a link between condition and ornaments even under strong genetic control (Almasi et al. 2012). Moreover, non-random habitat occupation in relation to colour also has to be considered (Hegyi et al. 2008b). Nevertheless, specific associations of current ornamentation with previous but not current year condition and reproduction are less likely to arise from genetic reasons or non-random habitat occupation. We therefore compared the observed relationships to those with current year condition and clutch size. We had different predictions for two potential causal backgrounds. In case of pleiotropy or non-random habitat occupation, we expected similar relationships of colour with previous and current condition and reproduction, or weaker effects of previous than current year predictors. In case of condition-dependence, we expected significant effects of life history traits in the previous year, but no or different relationships with traits in the current year. Possible genotype-environment interactions (Piault et al. 2009) do not alter these predictions in the absence of strict environmental periodicity. Additionally, we also considered relationships of ornamentation with past and current laying date, as a well known determinant of reproductive effort and costs (Daan et al. 1990; Török et al. 2004), and as a potential measure of sexual selection on ornamentation in our population (Hegyi et al. 2006, 2007a).

When considering the condition-dependence of ornamentation, the degree of exaggeration of feather ornaments can be influenced primarily by the actual conditions experienced before and during moult. Various direct effects of moult costs on display traits can be conceived. For example, coloration is negatively influenced by fast moulting (Serra et al. 2007; Griggio et al. 2009). Furthermore, it is widely recognized that general nutritional condition during moult may affect colour displays, which show reduced expression in individuals under nutritional stress (McGraw et al. 2002; McGlothlin et al. 2007). In addition, due to the limited resources which are also needed for self-maintenance, trade-offs may arise between the investments into current reproduction versus future colour advertisement, through the effects of reproductive effort on subsequent moulting condition (Griffith 2000; Siefferman and Hill 2005b).

In our study species, body condition and reproductive effort during the previous breeding attempt have been shown to predict the sizes of white plumage patches (Gustafsson et al. 1995; Török et al. 2003; Hegyi et al. 2008b), which suggests that reproductive effort is relevant to nutritional or physiological state during the subsequent moult (including the winter moult), thereby potentially also affecting the plumage colour traits we measured here. Based on previous studies of the sister species pied flycatcher (*Ficedula hypoleuca*) and other species, we expect birds with lower previous reproductive effort to have lower melanin-based brightness, (i.e. darker melanic areas, Slagsvold and Lifjeld 1992; brighter structurally based intensity, Siefferman and Hill 2005b), and birds in better condition to have higher UV chroma (Siitari and Huhta 2002; Hegyi et al. 2007a). Due to temporal proximity, we expect stronger effects of reproduction and condition during the previous spring on areas moulted in the summer as opposed to the winter moult.

Materials and methods

The collared flycatcher is an insectivorous, long-distance migratory passerine. Birds moult twice in a year. The first moult period is in summer, after breeding and before migration, and it is complete in mature individuals and partial in offspring. The second, partial moult happens in Africa, in winter, during which birds do not replace their primaries and part of the secondaries (Svensson 1992).

Data were collected in our long-term study plots in the Pilis Mountains, near Szentendre, Hungary (47°43’N, 19°01’E), between 2008 and 2012. Nests were checked regularly to determine the laying date of the first egg (hereafter laying date) and clutch size. Birds were captured in the nest-box during the nestling feeding period. We measured tarsus length using a calliper (to the nearest 0.1 mm), and body weight using a Pesola spring balance (to the nearest 0.1 g). With respect to age, birds were classified as yearlings or adults (>1 year) based on the darkness of primaries and wing patch size in males (Svensson 1992), and ringing data in females (unknown first breeders classified as yearlings; Hegyi et al. 2008b). We took reflectance spectra of a subset of the birds we caught, and we could use data of 102 females and 80 males in the analyses presented here. If we measured an individual in more than one year, we used only one, randomly chosen data point. We recorded the reflectance of the pigmented crown and wing coverts, the depigmented wing patch, the breast (brownish white in females, white in males), and the forehead (melanised in most females, depigmented in males). Among these, the wing patch and the wing coverts are moulted in summer, while the other three areas in winter (Cramp et al. 1993). During the period of our data collection, the birds had not started their summer moult yet.

We used a DH-2000 deuterium-halogen light source and an USB-2000 spectrophotometer (Ocean Optics Europe) fitted with a bifurcated micron fibre-optic probe (R400-7; Ocean Optics Europe). The probe was oriented at a 90-degree angle to the feather surface, and the diameter of the measured plumage area was 6 mm. To standardize measuring distance (3 mm) and keep out ambient light, the tip of the probe was fixed in a black plastic sheath. Reflectance data were computed relative to a white WS-1 diffuse reflectance standard (Ocean Optics Europe) and a dark reference (taken while excluding incoming light from the detector). The OOIBase32 software (Ocean Optics Europe) recorded the reflectance spectra in 0.37 nm steps. We recorded two consecutive spectral readings for each plumage region in every bird, removing the probe between the two, and remeasured the standards every 15-20 minutes to calibrate the system. From the reflectance spectra, we calculated two spectral variables, namely brightness (average intensity) from 320 to 700 nm ($R_{320\text{-}700}$), and UV chroma ($R_{320\text{-}400}$ / $R_{320\text{-}700}$). Measurement repeatabilities were high for both sexes in all area-specific colour traits ($r>0.52$, $P<0.001$). We used the average of the measurements for each area of each individual.

We measured the white patch sizes of birds but did not use them in the present analyses for two reasons. First, as most females do not have a forehead patch, the inclusion of white patch sizes would make the results incomparable between the sexes. Second, presumably due to differential abrasion and soiling, white patch sizes correlate with the spectral attributes of the respective patch, but not with those of other plumage areas (Laczi et al. 2011, our unpublished data), so they do not take part in the plumage-level colour integration examined in this paper.

Analyses were performed in Statistica 8.0 (StatSoft, Inc.). We used an alpha level of 0.05 and two-tailed tests. Before the analyses, the spectral variables were standardized (to a mean of zero and SD of one) for year in both sexes, and in the case of males, for age too, because coloration differs between yearling and older males, but not females (Laczi et al. 2011). Both in females and males, we performed separate principal component (PC) analyses for the standardized brightness and the standardized UV chroma of different body areas. The PC axes were rotated using varimax rotation, which did not change the patterns of loadings, but adjusted the axes to explain more variance. In a previous paper (Laczi et al. 2011), we conducted a pooled colour PCA for the two sexes because their trait interrelation patterns were similar regarding both brightness and UV chroma. Although this similarity persisted in the present dataset, we nevertheless decided to do separate PCAs here because the variances of nearly all brightness and UV chroma traits differed markedly between the sexes (Levene tests: wing covert UV chroma P=0.983, breast brightness P=0.138, all other P<0.003; variance higher in males: wing patch brightness and UV chroma, forehead brightness and UV chroma, crown UV chroma and breast UV chroma; variance higher in females: wing covert brightness and crown brightness). As we mentioned previously, measurement repeatabilities were high (r ranging from 0.52 to 0.94), so the different variance implies that the ratio of signal and noise in the measurements is different between males and females, and therefore the information content of the measurements may also differ. Moreover, higher phenotypic variance may also increase the information content of the detected signal for the receiver.

In the analyses, we used deviations of the onset of egg-laying from the median laying date of the respective year that were then converted to positive by adding to each value the overall minimum value + 0.5 (to avoid zeros), and then square root transformed. As clutch size, we used the number of eggs corrected for transformed laying date (because of the effect of laying date on clutch size, r=-0.36, P<0.001, n=473). This correction was done by the introduction of laying date into the model where clutch size was the dependent variable, and by the calculation of regression residuals where clutch size was the independent variable.

We tested the relationships of coloration to past reproductive investment by using general linear models (GLMs) with one colour PC as the dependent variable, and laying date and residual clutch size as continuous predictors. When examining relationships of colour axes to past body condition, we used one PC as dependent variable, and past residual body mass and tarsus length as continuous predictors. Residual body mass refers to the residuals of a regression of body mass on tarsus length (as a proxy of body size). Tarsus length was put into the model as an additional predictor because of the potential relationships between colour and body size.

When analysing the relationships of coloration to current laying date, clutch size and body mass, we also conducted GLMs. In each case, the four colour PCs were continuous predictors. In one model, laying date was the dependent variable. In the other model, clutch size was the dependent variable, while year was added as a categorical predictor because the mean clutch size of 2012 was significantly lower than that of the other years (Tukey HSD test, all $P<0.004$). In addition, clutch size was controlled for laying date by including the latter into the model as an additional continuous predictor beside the colour PCs. Finally, in the analyses of current condition, body mass was used as dependent variable and tarsus length and the colour PCs as continuous predictors, so body mass was corrected for body size. All analyses were done separately for the sexes.

Results

The results of the PCAs are summarized in Table 1. In females, the first brightness PC loaded positively with the brightness of the forehead, crown and breast. Here, a lower PC value means lower brightness which may result from more melanin. Brightness PC2 loaded positively with the pigmented wing coverts (suggesting lower melanin deposition in the case of higher PC value) and negatively with the white wing patch. These two PCs explained 31.8 and 21.8% of total variance, respectively. UV chroma PC1 loaded positively with the UV chroma of forehead and crown, and less strongly with that of the wing patch. UV chroma PC2 loaded negatively with the wing coverts and positively with the breast. These two colour axes explained 28.4 and 21.4% of total variance, respectively.

In males, brightness PC1 loaded positively with the wing patch (white), forehead (white in males), crown (dark) and breast (white). This axis likely represents structurally based brightness variation, i.e. the higher the amount of scatterers, the higher the brightness and the PC value. Brightness PC2 loaded only with the wing coverts (dark), so here a higher PC value may refer to lower melanin deposition. These two PCs explained 28.7 and 20.3% of total variance, respectively. UV chroma PC1 loaded positively with the wing coverts and crown (dark areas), while UV chroma PC2 with the wing patch, forehead and breast (white areas). These two PCs explained 28.4 and 23.0% of total variance, respectively.

In females, we found a marginally positive relationship between past residual clutch size and brightness PC2 ($F_{1,98}$=3.61, P=0.060, effect size r=0.19, CI lower=-0.008, CI upper=0.38) which implies that females laying larger clutches subsequently tended to grow less contrasting wings. Past residual body mass was negatively related to brightness PC1 ($F_{1,99}$=5.32, P=0.02, effect size r=-0.23, CI lower=-0.40, CI upper=-0.03; Fig. 1), i.e. females with high body condition developed darker plumage after the breeding season. Present laying date correlated positively with brightness PC1 ($F_{1,100}$=4.28, P=0.04, effect size r=0.20, CI lower=0.01, CI upper=0.38; Fig. 2), which means that darker females bred earlier.

In males, present laying date was negatively related to brightness PC1 ($F_{1,76}$=4.49, P=0.04, effect size r=-0.24, CI lower=-0.44, CI upper=-0.014; Fig. 3), and showed a negative tendency with UV chroma PC1 ($F_{1,75}$=3.03, P=0.09, effect size r=-0.19, CI lower=-0.40, CI upper=0.03), implying that structurally brighter males, and possibly also those with higher UV chroma in their dark plumage areas, bred earlier. Present clutch size was positively related to UV chroma PC1 ($F_{1,72}$=4.12, P=0.04, effect size r=0.22, CI lower=0.002, CI upper=0.43), which means that females mated to males with higher UV chroma in dark plumage areas laid larger clutches relative to their laying date. Present body mass (corrected for body size) correlated negatively with brightness PC2 ($F_{1,79}$=7.23, *P*=0.009, effect size *r*=-0.29, CI lower=-0.48, CI upper=-0.07; Fig. 4), that is, more pigmented males were in better body condition. None of the other relationships was significant (*P*>0.11).

Discussion

The patterns of PC loadings we obtained here for the two sexes separately were very similar to the pooled patterns of the previous paper (Laczi et al. 2011) for brightness, while the differences for UV chroma presumably stem from the separation of two PC axes instead of one. Brightness PC1 in females may indicate the melanin content of the plumage, with birds of lower PC values being more melanised and therefore darker (note that the breast is brownish white in this sex). Brightness PC1 in males (white areas and crown), may represent the structurally based brightness component of the feather (i.e. the thickness of the cortex, the number of scattering elements in the keratin matrix, etc.). Black crown feathers may have entered this PC as they are in the same region as the white forehead feathers and their melanin content seems to vary relatively little in our population (as judged from brightness variation; coefficient of variation, forehead: 9.00, crown: 0.60, wing patch: 7.60, wing coverts: 1.20, breast: 7.32). Brightness PC2 may contain similar information in the two sexes (melanin-based brightness), but with the addition that less melanised, duller (and presumably poorer quality, see below) females may have more abraded and therefore duller white wing patches as well, thereby showing lower brightness contrast in the wing. White patch brightness variation has been suggested to act as a sexual ornament in several species, including females (Hanssen et al. 2006). It is worth to note here that the outer wing coverts bordering the wing patch may have appeared in a separate PC from other contour feathers because they are not replaced in the partial winter moult (see the description of non-breeding plumages in Cramp et al. 1993). In both sexes, UV chroma PC1 largely referred to some (albeit different) melanised plumage areas. UV chroma PC2 in males is easily interpreted as the purely structural colour of white areas. In females, the same PC is more interesting because it suggests an axis of positive correlation between two possible determinants of feather quality: melanisation (less UV in wing coverts) and microstructure (more UV in breast feathers). This correlation can be adaptive if it also appears within the same feather because melanin increases while the structure promoting high UV reflectance likely reduces the structural strength of the feather (Bonser 1995; Prum 2006).

Costs of reproduction imply trade-offs between reproductive investment and other life-history traits (Harshman and Zera 2007). In birds, these costs may include, for example, reductions in future survival probability and parasite resistance (e.g. Stjernman et al. 2004), flight ability (Kullberg et al. 2002), or immune function and future fecundity (Hanssen et al. 2006). Trade-offs related to offspring production may also involve sexual ornaments (Höglund and Sheldon 1998). However, this possibility is as yet poorly explored in birds (for experiments see Griffith 2000; Siefferman and Hill 2005b). In a Swedish population of our study species, the white forehead patch size of males became smaller in the year after caring for an experimentally enlarged clutch (Gustafsson et al. 1995), while in our population, the white wing patch size change of females was negatively correlated with their previous clutch size (Hegyi et al. 2008b). In the present study, female brightness PC2 was marginally positively related to previous clutch size, i.e. females that laid a larger clutch tended to develop less melanised feathers and a lower wing patch contrast after breeding. Interestingly, it was the colour of the wing feathers which moult only in summer, right after the breeding effort (Cramp et al. 1993) that showed an indication of reproductive costs. Female brightness PC1, on the other hand, was negatively correlated with previous residual body mass, i.e.

females with more melanised plumage were in better physical condition during the past breeding season. Therefore, poor body condition at nestling rearing seems to indicate not only costs of reproduction but also a physiological state that persists up to the winter moult in Africa and thereby affects the melanisation of other contour feather tracts (brightness PC1). These results together suggest that multiple integrated signals of physiological condition may coexist in the same species, which underscores the importance of properly identifying signal traits in a complex ornamentation system (Cornwallis and Uller 2010). Relationships between female ornamentation and previous year condition and clutch size were not reproduced for current year predictors (effect sizes were two or three times smaller for the current year, details not shown here). Specific effects of past but not current condition and clutch size make general female attributes (pleiotropy, early maternal effects, non-random habitat use etc.) a less likely explanation for the detected patterns than condition-dependence. However, causality needs experimental verification.

In males, brightness PC2 was negatively related to current body mass, that is, more melanised individuals were in better condition. This relationship was weaker but similar in magnitude for the previous year, so it may easily reflect a genetic correlation or general environmental effects such as territory quality. In conclusion, the darkness of melanised plumage areas may be a long-term signal of condition and therefore individual quality in both sexes, but possibly for different reasons. Melanin-based plumage ornaments usually appear to be under strong genetic control (e.g. Slagsvold and Lifjeld 1992; Roulin and Dijkstra 2003; Roulin 2004; Saino et al. 2013). Furthermore, some studies revealed no condition-dependence in melanic ornaments (McGraw and Hill 2000; McGraw et al. 2002; Senar et al. 2003; Siefferman and Hill 2005a). However, findings in several bird species suggest that these traits can change with parasite infection, physical condition and environmental constraints (Veiga and Puerta 1996; Griffith et al. 1999; Fitze and Richner 2002; Jawor et al. 2004; Vergara et al.

2009; Piault et al. 2012). Potential production costs associated with melanin-based plumage ornaments may partly mediate such plasticity (e.g. sensitivity to dietary metal element content, Niecke et al. 2003; McGraw 2006; and physiological stress, Galván and Alonso-Alvarez 2008; Roulin et al. 2008), although wearing costs of melanin-based traits such as intrasexual aggression may also play a role (Senar et al. 1993; Hoi and Griggio 2008).

In addition, we found that females with low brightness PC1 (i.e. more melanised individuals) in a given year bred earlier in that year. This could result from their consistently good condition, but brightness PC1 showed little relationship with current condition. It is therefore possible that the pattern with laying date reflects inter- or intrasexual selection favouring darker melanised plumage areas. Such selection could easily be explained by the apparent indicator value of dark plumage in terms of moulting condition and therefore individual quality (see above). Indeed, size of the white wing patch of females has been suggested to play a role in female competition for territories and mates (Hegyi et al. 2007b, 2008a, b), so it would not be surprising to find similar relationships for another ornamental attribute of this sex.

In contrast to brightness variation, none of the UV chroma PCs in either sex showed any indication of dependence on body condition or breeding investment in the previous year. Nevertheless, males with higher UV chroma in dark plumage areas (high PC1), tended to breed earlier and had higher clutch sizes in the given year, indicating a possible role of UV chroma in sexual selection and differential reproductive investment, although differential arrival and territory quality effects cannot be ruled out. Interestingly, in males, brightness PC1, which also reflected the structural based component of colour variation, was similarly negatively related to current laying date. In the closely related pied flycatcher, males with more pronounced UV chroma on their melanised plumage parts arrived earlier to the breeding site (Siitari and Huhta 2002). In our population, breeding date of a male has been shown to be not only a possible indicator of arrival date from migration, but also a reliable signal of female mate preference (see Hegyi et al. 2007c, 2010). Regarding clutch size, several studies in our population were unable to detect any relationship between the white plumage patch sizes of males and the primary reproductive investment of their mate (Hargitai et al. 2005; Michl et al. 2005; Török et al. 2007), so the positive relationship between UV chroma PC1 and residual clutch size is interesting. However, the alternative explanation of differential territory quality cannot be ruled out without further experimental work. Even in the absence of condition-dependence (see Results), UV chroma and the structurally-based component of brightness may reflect differences in the regularity of feather microstructure (Shawkey et al. 2003, 2005; Prum 2006), possibly indicating an individual-specific, stable aspect of quality such as developmental stability (Kemp et al. 2006; Prum 2006; Galván 2011).

The apparent lack of condition-dependence and sexual selection was especially striking for the UV chroma of the conspicuous white plumage areas of males (comprising UV chroma PC2 in this sex), since the sizes of white patches are sexually selected (Michl et al. 2002; Hegyi et al. 2010) while their UV chroma is higher than in females (Laczi et al. 2011). However, phenotypic variation in the UV chroma of white plumage areas is lower in males than in females (see Materials and methods). Males may keep relative UV reflectance at a possible maximum to further increase the conspicuousness of the ornamental white patches, with coloration functioning as a signal amplifier for white patch size (Hasson 1990, 1991). In this case, the variance and information content of white patch UV chroma may be reduced in males. It is interesting that quality indication is characteristic to the UV chroma of dark but not white plumage areas in the sister species pied flycatcher (Siitari and Huhta 2002) and quality-related information in dark feather UV reflectance is also present in some other species (Doucet et al. 2004; Hegyi et al. 2007a).

To summarize, we found robust parallel variation in the spectral properties of different plumage areas of collared flycatchers. The main axes of variation linked plumage areas in a meaningful way, when considering patterns of development (i.e. spatial proximity within the plumage), moult, and visible colour differences between the sexes. Our results further suggest the possible presence of multiple sexually selected composite colour axes of different information content in the same plumage, even though the colour of our study species has only two proximate origins (melanin-based and structurally based). Our results illustrate the information potentially gained by treating correlated colour variation in multiple plumage areas together. If such meaningful correlated variation is detected in a system, it is also important to conduct experimental manipulations of individual condition to confirm the causality of the observed parallel changes, and to look for mechanistic explanations for the observed clustering of ornamental traits.

Acknowledgements

We are grateful to A. Roulin and anonymous reviewers for the comments of the manuscript. We wish to thank G. Blázi, R. Főző, L.Z. Garamszegi, E. Szász and A. Szegedi for help with the fieldwork. This research was funded by a Bolyai fellowship to G.H., OTKA grants PD72117 and K101611 to G.H., PD75481 and F68295 to B.R. and K75618 to J.T., the Eötvös Loránd University (PhD scholarship) to M.L., the Erdők a Közjóért Alapítvány and the Pilis Park Forestry. During all stages of this work, G.M. was supported by TÁMOP-4.2.1./B-09/1-KMR-2010-0005 and TÁMOP-4.2.2./B-10/1-2010-0023. This research was conducted in agreement with the current laws of Hungary.

References

Almasi B, Roulin A, Korner-Nievergelt F, Jenni-Eiermann S, Jenni L (2012) Coloration signals the ability to cope with elevated stress hormones: effects of corticosterone on growth of barn owls are associated with melanism. J Evol Biol 25:1189-1199

Andersson (1994) Sexual Selection. Princeton University Press, Princeton

Bonser RHC (1995) Melanin and the abrasion resistance of feathers. Condor 97:590-591

Cornwallis CK, Uller T (2010) Towards an evolutionary ecology of sexual traits. 25:145-152

Cramp S, Perrins CM, Brooks DJ (1993) The birds of the Western Palearctic, Volume 7: Old World flycatchers to shrikes. Oxford University Press, Oxford

Daan S, Dijkstra C, Tinbergen JM (1990) Family planning in the kestrel (*Falco tinnunculus*): the ultimate control of laying date and clutch size. Behaviour 114:83-116

Doucet SM, Mennill DJ, Montgomerie R, Boag PT, Ratcliffe LM (2004) Achromatic plumage reflectance predicts reproductive success in male black-capped chickadees. Behav Ecol 16:218-222

Duckworth RA, Badyaev AV, Parlow AF (2003) Elaborately ornamented males avoid costly parental care in the house finch (*Carpodacus mexicanus*): a proximate perspective. Behav Ecol Sociobiol 55:176-183

Ducrest A-L, Keller L, Roulin R (2008) Pleiotropy in the melanocortin system, coloration and behavioural syndromes. Trends Ecol Evol 23:502-510

Evans SR, Sheldon BC (2012) Quantitative Genetics of a Carotenoid-Based Color: Heritability and Persistent Natal Environmental Effects in the Great Tit. Am Nat 179:79-94

Fitze PS, Richner H (2002) Differential effects of a parasite on ornamental structures based on melanins and carotenoids. Behav Ecol 13:401-407

Galván I (2010) Plumage coloration can be perceived as a multiple condition-dependent signal by Great Tits *Parus major*. Ibis 152:359-367

Galván I (2011) Feather microstructure predicts size and colour intensity of a melanin-based plumage signal. J Avian Biol 42:473-479

Galván I, Alonso-Alvarez C (2008) An Intracellular Antioxidant Determines the Expression of a Melanin-Based Signal in a Bird. PLoS ONE 10:e3335

Griffith SG (2000) A trade-off between reproduction and a condition-dependent sexually selected ornament in the house sparrow *Passer domesticus*. Proc R Soc Lond B 267:1115-1119

Griffith SC, Owens IPF, Burke T (1999) Environmental determination of a sexually selected trait. Nature 400:358-360

Griggio M, Serra L, Licheri D, Campomori C, Pilastro A (2009) Moult speed affects structural feather ornaments in the blue tit. J Evol Biol 22:782-792

Gustafsson L, Qvarnström A, Sheldon BC (1995) Trade-offs between life-history traits and a secondary sexual character in male collared flycatchers. Nature 375:311-313

Hanssen SA, Folstad I, Erikstad KE (2006) White plumage reflects individual quality in female eiders. Anim Behav 71:337-343

Hargitai R, Török J, Tóth L, Hegyi G, Rosivall B, Szigeti B, Szöllősi E (2005) Effects of environmental conditions and parental quality on inter- and intraclutch egg-size variation in the Collared Flycatcher (*Ficedula albicollis*). Auk 122:509-522

Harshman LG, Zera JA (2007) The cost of reproduction: the devil in the details. Trends Ecol Evol 22:80-86

Hasson O (1990) Amplifiers and their role in the evolution of male display. Evol Ecol 4:277-289

Hasson O (1991) Sexual displays as amplifiers: Practical examples with an emphasis on feather decorations. Behav Ecol 2:189-197

Hegyi G, Garamszegi LZ, Eens M, Török J (2008a) Female ornamentation and territorial conflicts in collared flycatchers (*Ficedula albicollis*) Naturwissenschaften 95:993-996

Hegyi G, Rosivall B, Szöllősi E, Hargitai R, Eens M, Török J (2007b) A role for female ornamentation in the facultatively polygynous mating system of collared flycatchers. Behav Ecol 18:1116-1122

Hegyi G, Rosivall B, Szöllősi E, Hargitai R, Eens M, Török J (2008b) Phenotypic plasticity in a conspicuous female plumage trait: information content and mating patterns. Anim Behav 75:977-989

Hegyi G, Szigeti B, Török J, Eens M (2007a) Melanin, carotenoid and structural plumage ornaments: information content and role in great tits. J Avian Biol 38:698-708

Hegyi G, Szöllősi E, Jenni-Eiermann S, Török J, Eens M, Garamszegi LZ (2010) Nutritional correlates and mate acquisition role of multiple sexual traits in male collared flycatchers. Naturwissenschaften 97:567-576

Hegyi G, Török J, Garamszegi LZ, Rosivall B, Szöllősi E, Hargitai R (2007c) Dynamics of multiple sexual signals in relation to climatic conditions. Evol Ecol Res 9:905-920

Hegyi G, Török J, Tóth L, Garamszegi LZ, Rosivall B (2006) Rapid temporal change in the expression and age-related information content of a sexually selected trait. J Evol Biol 19:228-238

Hill GE, Brawner III WR (1998) Melanin-based plumage coloration in the house finch is unaffected by coccidial infection. Proc R Soc Lond B 265:1105-1109

Hill GE, McGraw KJ (eds) (2006) Bird Coloration. Harvard University Press, Cambridge

Hoi H, Griggio M (2008) Dual utility of a melanin-based ornament in bearded tits. Ethology 114:1094-1100

Horváthová T, Nakagawa S, Uller T (2012) Strategic female reproductive investment in response to male attractiveness in birds. Proc R Soc Lond B 279:163-170

Höglund J, Sheldon BC (1998) The cost of reproduction and sexual selection. Oikos 83:478-483

Jawor JM, Gray N, Beall SM, Breitwisch, R. (2004) Multiple ornaments correlate with aspects of condition and behaviour in female northern cardinals, *Cardinalis cardinalis*. Anim Behav 67:875-882

Kemp DJ, Vukusic P, Rutowski RL (2006) Stress-mediated covariance between nano-structural architecture and ultraviolet butterfly coloration. Funct Ecol 20:282-289

Kullberg C, Houston DC, Metcalfe NB (2002) Impaired flight ability – a cost of reproduction in female blue tits. Behav Ecol 13:575-579

Laczi M, Török J, Rosivall B, Hegyi G (2011) Integration of spectral reflectance across the plumage: implications for mating patterns. PLoS ONE 6:e23201

Matrková J, Remeš V (2012) Environmental and Genetic Effects on Pigment-Based vs. Structural Component of Yellow Feather Colouration. PLoS ONE 7:e36640

McGlothlin JW, Duffy DL, Henry-Freeman JL, Ketterson ED (2007) Diet quality affects an attractive white plumage pattern in dark-eyed juncos (*Junco hyemalis*). Behav Ecol Sociobiol 61:1391-1399

McGraw KJ (2006) Dietary mineral content influences the expression of melanin-based ornamental coloration. Behav Ecol 18:137-142

McGraw KJ, Hill GE (2000) Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. Proc R Soc Lond B 267:1525-1531

McGraw KJ, Mackillop EA, Dale J, Hauber ME (2002) Different colours reveal different information: how nutritional stress affects the expression of melanin- and structurally based ornamental plumage. J Exp Biol 205:3747-3755

Michl G, Török J, Griffith S, Sheldon BC (2002) Experimental analysis of sperm competition mechanisms in a wild bird population. Proc Natl Acad Sci USA 99:5466-5470

Michl G, Török J, Péczely P, Garamszegi LZ, Schwabl H (2005) Female collared flycatchers adjust yolk testosterone to male age, but not to attractiveness. Behav Ecol 16:383-388

Niecke M, Rothlaender S, Roulin A (2003) Why do melanin ornaments signal individual quality? Insights from metal element analysis of barn owl feathers. Oecologia 137:153-158

Pärt T, Qvarnström A (1997) Badge size in collared flycatchers predicts outcome of mate competition over territories. Anim Behav 54:893-899

Piault R, Gasparini J, Bize P, Jenni-Eiermann S, Roulin A (2009) Pheomelanin-Based Coloration and the Ability to Cope with Variation in Food Supply and Parasitism. Am Nat 174:548-556

Piault R, van den Brink V, Roulin A (2012) Condition-dependent expression of melanin-based coloration in the Eurasian kestrel. Naturwissenschaften 99:391-396

Prum RO (2006) Anatomy, Physics, and Evolution of Structural Colors. In: Hill GE, McGraw KJ (eds) Bird Coloration Vol. 1., 1st edn. Harvard University Press, Harvard, pp 295-353

Quesada J, Senar JC (2009) Cross-fostering experiments to compare carotenoid- and melanin-based plumage traits and long-term parental effects in post-moulted great tits. Behaviour 146:1235-1251

Roulin A (2004) Proximate basis of the covariation between a melanin-based female ornament and offspring quality. Oecologia 140:668-675

Roulin A, Almasi B, Meichtry-Stier KS, Jenni L (2011) Eumelanin- and pheomelanin-based colour advertise resistance to oxidative stress in opposite ways. J Evol Biol 24:2241-2247

Roulin A, Almasi B, Rossi-Pedruzzi A, Ducrest AL, Wakamatsu K, Miksik I, Blount JD, Jenni-Eiermann S, Jenni L (2008) Corticosterone mediates the condition-dependent component of melanin-based coloration. Anim Behav 75:1351-1358

Roulin A, Dijkstra C (2003) Genetic and environmental components of variation in eumelanin and phaeomelanin sex-traits in the barn owl. Heredity 90:359-364

Roulin A, Ducrest A-L (2013) Genetics of colouration in birds. Semin Cell Dev Biol 24:594-608

Saino N, Romano M, Rubolini D, Teplitsky C, Ambrosini R, Caprioli M, Canova L, Wakamatsu K (2013) Sexual Dimorphism in Melanin Pigmentation, Feather Coloration and Its Heritability in the Barn Swallow (*Hirundo rustica*). PLoS ONE 8:e58024

Senar JC, Camerino M, Copete JL, Metcalfe NB (1993) Variation in Black Bib of the Eurasian Siskin (*Carduelis spinus*) and Its Role as a Reliable Badge of Dominance. Auk 110:924-927

Senar JC, Figuerola J, Domènech J (2003) Plumage coloration and nutritional condition in the great tit (*Parus major*): the roles of carotenoids and melanins differ. Naturwissenschaften 90:234–237

Serra L, Griggio M, Licheri D, Pilastro A (2007) Moult speed constrains the expression of a carotenoid-based sexual ornament. J Evol Biol 20:2028-2034

Shawkey MD, Estes AM, Siefferman L, Hill GE (2003) Nanostructure predicts intraspecific variation in ultraviolet-blue plumage colour. Proc R Soc Lond B 270:1455-1460

Shawkey MD, Estes AM, Siefferman L, Hill GE (2005) The anatomical basis of sexual dichromatism in non-iridescent ultraviolet-blue structural coloration of feathers. Biol J Linn Soc 84:259-271

Siefferman L, Hill GE (2003) Structural and melanin coloration indicate parental effort and reproductive success in male eastern bluebirds. Behav Ecol 14:855-861

Siefferman L, Hill GE (2005a) Evidence for sexual selection on structural plumage coloration in female eastern bluebirds (*Sialia sialis*). Evolution, 59:1819-1828

Siefferman L, Hill GE (2005b) Male eastern bluebirds trade future ornamentation for current reproductive investment. Biol Lett 1:208-211

Siitari H, Huhta E (2002) Individual color variation and male quality in pied flycatchers (*Ficedula hypoleuca*): a role of ultraviolet reflectance. Behav Ecol 13:737-741

Slagsvold T, Lifjeld JT (1992) Plumage color is a condition-dependent sexual trait in male pied flycatchers. Evolution, 46:825-828

Stjernman M, Råberg L, Nilsson J (2004) Survival costs of reproduction in the blue tit (*Parus caeruleus*): a role for blood parasites? Proc R Soc Lond B 271:2387-2394

Svensson L (1992) Identification Guide to European Passerines. Märstatryck, Stockholm

Török J, Hargitai R, Hegyi G, Matus Z, Michl G, Péczely P, Rosivall B, Tóth G (2007) Carotenoids in the egg yolks of collared flycatchers (*Ficedula albicollis*) in relation to parental quality, environmental factors and laying order. Behav Ecol Sociobiol 61:541-550

Török J, Hegyi G, Garamszegi LZ (2003) Depigmented wing patch size is a condition-dependent indicator of viability in male collared flycatchers. Behav Ecol, 14:382-388

Török J, Hegyi G, Tóth L, Könczey R (2004) Unpredictable food supply modifies costs of reproduction and hampers individual optimization. Oecologia 141:432-443

Veiga JP, Puerta M (1996) Nutritional constraints determine the expression of a sexual trait in the house sparrow, *Passer domesticus*. Proc R Soc Lond B 263:229-234

Vergara P, Fargallo JA, Martínez-Padilla J, Lemus JA (2009) Inter-annual variation and information content of melanin-based coloration in female Eurasian kestrels. Biol J Linn

Soc 97:781-790

Table 1 Summary of loadings of the principal components with the individual spectral variables

	brightness		UV chroma	
	PC1	**PC2**	**PC1**	**PC2**
Female				
wing patch	0.37	**-0.67**	**0.47**	-0.11
wing coverts	0.22	**0.80**	0.29	**-0.69**
forehead	**0.76**	0.07	**0.77**	-0.11
crown	**0.74**	0.00	**0.69**	0.21
breast	**0.54**	-0.01	0.24	**0.73**
Male				
wing patch	**0.70**	0.01	0.08	**0.65**
wing coverts	0.03	**0.95**	**0.85**	0.05
forehead	**0.51**	-0.26	-0.12	**0.67**
crown	**0.52**	0.17	**0.82**	-0.06
breast	**0.64**	0.15	0.06	**0.52**

The principal component analyses were performed separately for sexes, and also for brightness and UV chroma variables. Spectral variables were standardized for year in females, and for year and age in males. Values approaching or exceeding the lower threshold of large effect size (0.5) are shown in bold.

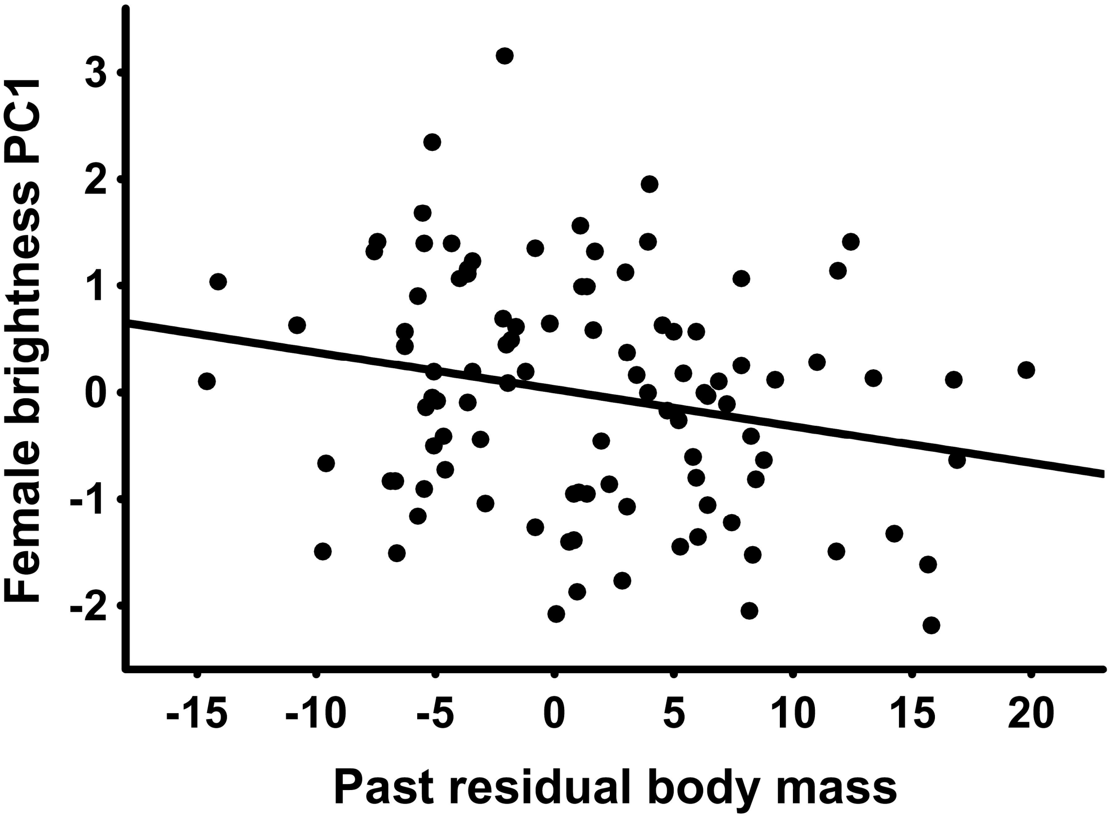
Fig. 1 Relationship between female brightness PC1 and previous year residual body mass in collared flycatchers (residual body mass comes from a regression of body mass on tarsus length)

Fig. 2 Relationship between female brightness PC1 and current laying date in collared flycatchers (laying date was adjusted to the yearly median and square-root transformed)

Fig. 3 Relationship between male brightness PC1 and current laying date in collared flycatchers (laying date was adjusted to the yearly median and square-root transformed)

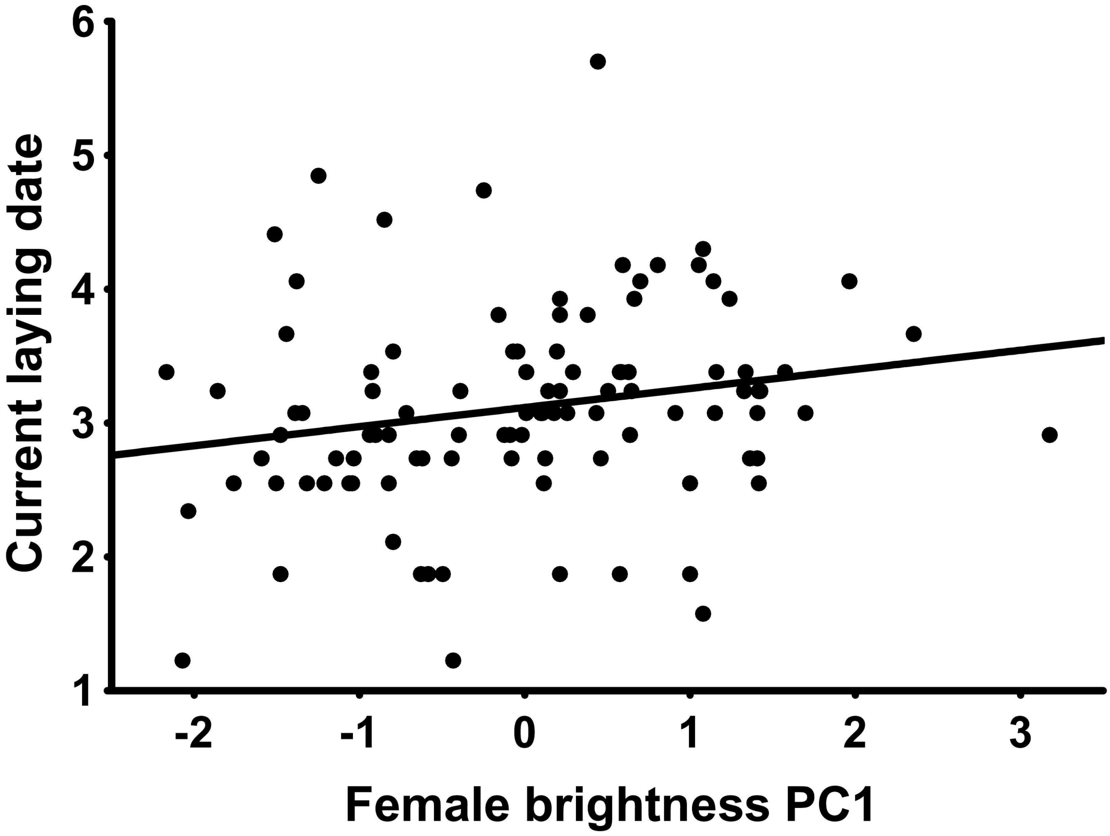
Fig. 4 Relationship between male brightness PC2 and current residual body mass in collared flycatchers

Fig 1


Female brightness PC1
3
2
1
0
-1
-2
-15
-10
-5
0
5
10
15
20
Past residual body mass

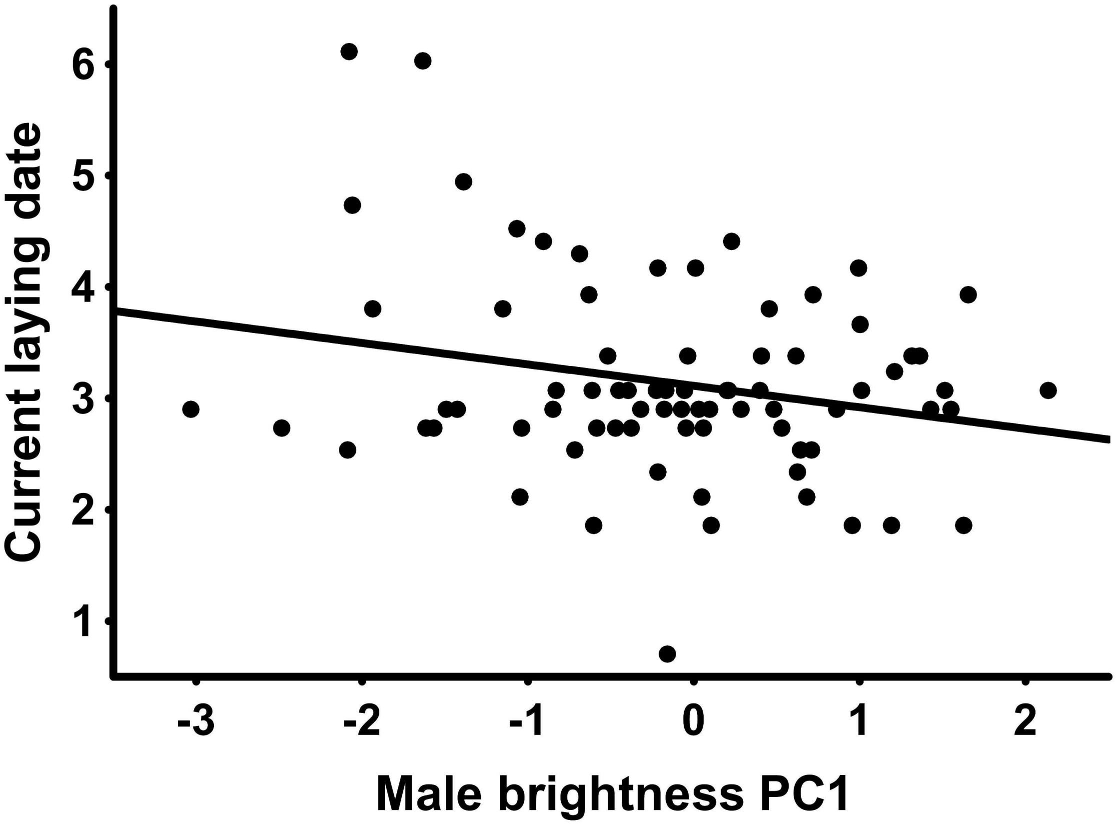
598 **Fig 2**

599



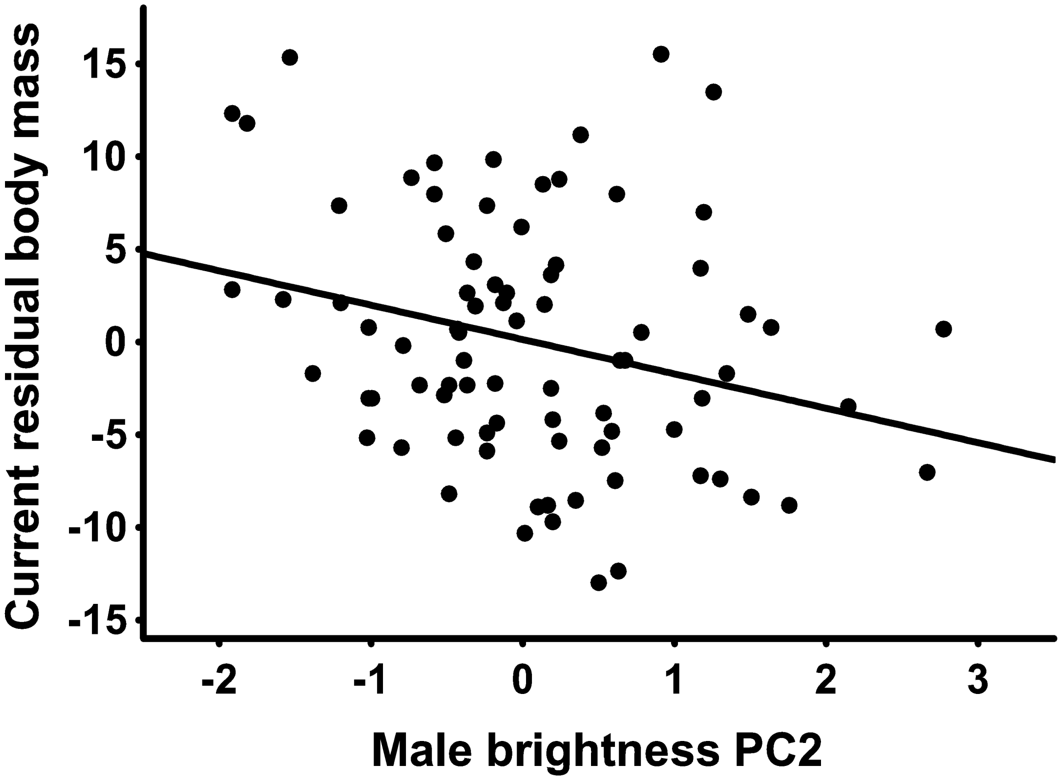
600

Fig 3



604 **Fig 4**

605



606