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

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15 **Abstract** The possible integration of different sexual ornaments into a composite system, and
16 especially the information content of such ornament complexes, is poorly investigated. Many
17 bird species display complex plumage coloration, but whether this represents one integrated
18 or several independent sexual traits can be unclear. Collared flycatchers (*Ficedula albicollis*)
19 display melanised and depigmented plumage areas, and the spectral features (brightness and
20 UV chroma) of these are correlated with each other across the plumage. In a five-year dataset
21 of male and female plumage reflectance, we examined some of the potential information
22 content of integrated, plumage-level colour attributes by estimating their relationships to
23 previous and current year body condition, laying date and clutch size. Females were in better
24 condition the year before they became darker pigmented, and males in better current year
25 condition were also darker pigmented. Female pigment-based brightness was positively, while

26 male structurally based brightness was negatively related to current laying date. Finally, the
27 overall UV chroma of white plumage areas in males was positively associated with current
28 clutch size. Our results show that higher degree of pigmentation is related to better condition,
29 while the structural colour component is associated with some aspects of reproductive
30 investment. These results highlight the possibility that correlated aspects of a multiple
31 plumage ornamentation system may reflect together some aspects of individual quality,
32 thereby functioning as a composite signal.

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

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37 **Introduction**

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

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

75 The plumage ornamentation of the collared flycatcher (*Ficedula albicollis*) is usually
76 quantified by the size of ornamental white patches (Pärt and Qvarnström 1997; Hegyi et al.
77 2008a). Nevertheless, a recent study revealed that plumage reflectance also varied among
78 individuals, with the brightness and UV chroma of differently coloured plumage areas
79 changing in parallel, thereby forming an integrated signal system which seemed to
80 specifically predict social mating patterns (Laczi et al. 2011). Here we wanted to explore the
81 plumage-level colour variation of female and male collared flycatchers in relation to body
82 condition and patterns of reproduction. We measured the brightness and UV chroma of
83 melanised and depigmented plumage areas, and looked for axes of common variation among
84 these colour measures. We then interpreted these integrated colour axes based on what is
85 known about the mechanisms of colour production in melanised and depigmented areas.

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

88 Associations of ornamentation with reproduction may reflect genetic or hormonally based
89 links, for example, different reproductive tactics by differently ornamented individuals
90 (Duckworth et al. 2003) or differential investment by the partner (Horváthová et al. 2012).
91 Similarly, a simple correlation between ornamentation and body condition does not constitute
92 evidence for condition-dependence. This is particularly true for melanin-based traits which
93 are controlled by pleiotropic genes affecting many other traits as well (Ducrest et al. 2008;
94 Roulin and Ducrest 2013), which can lead to a link between condition and ornaments even
95 under strong genetic control (Almasi et al. 2012). Moreover, non-random habitat occupation
96 in relation to colour also has to be considered (Hegyi et al. 2008b). Nevertheless, specific
97 associations of current ornamentation with previous but not current year condition and
98 reproduction are less likely to arise from genetic reasons or non-random habitat occupation.
99 We therefore compared the observed relationships to those with current year condition and
100 clutch size. We had different predictions for two potential causal backgrounds. In case of
101 pleiotropy or non-random habitat occupation, we expected similar relationships of colour with
102 previous and current condition and reproduction, or weaker effects of previous than current
103 year predictors. In case of condition-dependence, we expected significant effects of life
104 history traits in the previous year, but no or different relationships with traits in the current
105 year. Possible genotype-environment interactions (Piault et al. 2009) do not alter these
106 predictions in the absence of strict environmental periodicity. Additionally, we also
107 considered relationships of ornamentation with past and current laying date, as a well known
108 determinant of reproductive effort and costs (Daan et al. 1990; Török et al. 2004), and as a
109 potential measure of sexual selection on ornamentation in our population (Hegyi et al. 2006,
110 2007a).

111 When considering the condition-dependence of ornamentation, the degree of exaggeration
112 of feather ornaments can be influenced primarily by the actual conditions experienced before
113 and during moult. Various direct effects of moult costs on display traits can be conceived. For
114 example, coloration is negatively influenced by fast moulting (Serra et al. 2007; Griggio et al.
115 2009). Furthermore, it is widely recognized that general nutritional condition during moult
116 may affect colour displays, which show reduced expression in individuals under nutritional
117 stress (McGraw et al. 2002; McGlothlin et al. 2007). In addition, due to the limited resources
118 which are also needed for self-maintenance, trade-offs may arise between the investments into
119 current reproduction versus future colour advertisement, through the effects of reproductive
120 effort on subsequent moulting condition (Griffith 2000; Siefferman and Hill 2005b).

121 In our study species, body condition and reproductive effort during the previous breeding
122 attempt have been shown to predict the sizes of white plumage patches (Gustafsson et al.
123 1995; Török et al. 2003; Hegyi et al. 2008b), which suggests that reproductive effort is
124 relevant to nutritional or physiological state during the subsequent moult (including the winter
125 moult), thereby potentially also affecting the plumage colour traits we measured here. Based
126 on previous studies of the sister species pied flycatcher (*Ficedula hypoleuca*) and other
127 species, we expect birds with lower previous reproductive effort to have lower melanin-based
128 brightness, (i.e. darker melanic areas, Slagsvold and Lifjeld 1992; brighter structurally based
129 intensity, Siefferman and Hill 2005b), and birds in better condition to have higher UV chroma
130 (Siitari and Huhta 2002; Hegyi et al. 2007a). Due to temporal proximity, we expect stronger
131 effects of reproduction and condition during the previous spring on areas moulted in the
132 summer as opposed to the winter moult.

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135 **Materials and methods**

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

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

157 We used a DH-2000 deuterium-halogen light source and an USB-2000 spectrophotometer
158 (Ocean Optics Europe) fitted with a bifurcated micron fibre-optic probe (R400-7; Ocean
159 Optics Europe). The probe was oriented at a 90-degree angle to the feather surface, and the
160 diameter of the measured plumage area was 6 mm. To standardize measuring distance (3 mm)

161 and keep out ambient light, the tip of the probe was fixed in a black plastic sheath.
162 Reflectance data were computed relative to a white WS-1 diffuse reflectance standard (Ocean
163 Optics Europe) and a dark reference (taken while excluding incoming light from the detector).
164 The OOIBase32 software (Ocean Optics Europe) recorded the reflectance spectra in 0.37 nm
165 steps. We recorded two consecutive spectral readings for each plumage region in every bird,
166 removing the probe between the two, and remeasured the standards every 15-20 minutes to
167 calibrate the system. From the reflectance spectra, we calculated two spectral variables,
168 namely brightness (average intensity) from 320 to 700 nm ($R_{320-700}$), and UV chroma ($R_{320-400}$
169 / $R_{320-700}$). Measurement repeatabilities were high for both sexes in all area-specific colour
170 traits ($r > 0.52$, $P < 0.001$). We used the average of the measurements for each area of each
171 individual.

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

179 Analyses were performed in Statistica 8.0 (StatSoft, Inc.). We used an alpha level of 0.05
180 and two-tailed tests. Before the analyses, the spectral variables were standardized (to a mean
181 of zero and SD of one) for year in both sexes, and in the case of males, for age too, because
182 coloration differs between yearling and older males, but not females (Laczi et al. 2011). Both
183 in females and males, we performed separate principal component (PC) analyses for the
184 standardized brightness and the standardized UV chroma of different body areas. The PC axes
185 were rotated using varimax rotation, which did not change the patterns of loadings, but

186 adjusted the axes to explain more variance. In a previous paper (Laczi et al. 2011), we
187 conducted a pooled colour PCA for the two sexes because their trait interrelation patterns
188 were similar regarding both brightness and UV chroma. Although this similarity persisted in
189 the present dataset, we nevertheless decided to do separate PCAs here because the variances
190 of nearly all brightness and UV chroma traits differed markedly between the sexes (Levene
191 tests: wing covert UV chroma $P=0.983$, breast brightness $P=0.138$, all other $P<0.003$;
192 variance higher in males: wing patch brightness and UV chroma, forehead brightness and UV
193 chroma, crown UV chroma and breast UV chroma; variance higher in females: wing covert
194 brightness and crown brightness). As we mentioned previously, measurement repeatabilities
195 were high (r ranging from 0.52 to 0.94), so the different variance implies that the ratio of
196 signal and noise in the measurements is different between males and females, and therefore
197 the information content of the measurements may also differ. Moreover, higher phenotypic
198 variance may also increase the information content of the detected signal for the receiver.

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

206 We tested the relationships of coloration to past reproductive investment by using general
207 linear models (GLMs) with one colour PC as the dependent variable, and laying date and
208 residual clutch size as continuous predictors. When examining relationships of colour axes to
209 past body condition, we used one PC as dependent variable, and past residual body mass and
210 tarsus length as continuous predictors. Residual body mass refers to the residuals of a

211 regression of body mass on tarsus length (as a proxy of body size). Tarsus length was put into
212 the model as an additional predictor because of the potential relationships between colour and
213 body size.

214 When analysing the relationships of coloration to current laying date, clutch size and body
215 mass, we also conducted GLMs. In each case, the four colour PCs were continuous predictors.
216 In one model, laying date was the dependent variable. In the other model, clutch size was the
217 dependent variable, while year was added as a categorical predictor because the mean clutch
218 size of 2012 was significantly lower than that of the other years (Tukey HSD test, all
219 $P < 0.004$). In addition, clutch size was controlled for laying date by including the latter into
220 the model as an additional continuous predictor beside the colour PCs. Finally, in the analyses
221 of current condition, body mass was used as dependent variable and tarsus length and the
222 colour PCs as continuous predictors, so body mass was corrected for body size. All analyses
223 were done separately for the sexes.

224

225

226 **Results**

227

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

235 loaded negatively with the wing coverts and positively with the breast. These two colour axes
236 explained 28.4 and 21.4% of total variance, respectively.

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

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

253 In males, present laying date was negatively related to brightness PC1 ($F_{1,76}=4.49$,
254 $P=0.04$, effect size $r=-0.24$, CI lower=-0.44, CI upper=-0.014; Fig. 3), and showed a negative
255 tendency with UV chroma PC1 ($F_{1,75}=3.03$, $P=0.09$, effect size $r=-0.19$, CI lower=-0.40, CI
256 upper=0.03), implying that structurally brighter males, and possibly also those with higher
257 UV chroma in their dark plumage areas, bred earlier. Present clutch size was positively
258 related to UV chroma PC1 ($F_{1,72}=4.12$, $P=0.04$, effect size $r=0.22$, CI lower=0.002, CI
259 upper=0.43), which means that females mated to males with higher UV chroma in dark

260 plumage areas laid larger clutches relative to their laying date. Present body mass (corrected
261 for body size) correlated negatively with brightness PC2 ($F_{1,79}=7.23$, $P=0.009$, effect size $r=-$
262 0.29, CI lower=-0.48, CI upper=-0.07; Fig. 4), that is, more pigmented males were in better
263 body condition. None of the other relationships was significant ($P>0.11$).

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265

266 **Discussion**

267 The patterns of PC loadings we obtained here for the two sexes separately were very similar
268 to the pooled patterns of the previous paper (Laczi et al. 2011) for brightness, while the
269 differences for UV chroma presumably stem from the separation of two PC axes instead of
270 one. Brightness PC1 in females may indicate the melanin content of the plumage, with birds
271 of lower PC values being more melanised and therefore darker (note that the breast is
272 brownish white in this sex). Brightness PC1 in males (white areas and crown), may represent
273 the structurally based brightness component of the feather (i.e. the thickness of the cortex, the
274 number of scattering elements in the keratin matrix, etc.). Black crown feathers may have
275 entered this PC as they are in the same region as the white forehead feathers and their melanin
276 content seems to vary relatively little in our population (as judged from brightness variation;
277 coefficient of variation, forehead: 9.00, crown: 0.60, wing patch: 7.60, wing coverts: 1.20,
278 breast: 7.32). Brightness PC2 may contain similar information in the two sexes (melanin-
279 based brightness), but with the addition that less melanised, duller (and presumably poorer
280 quality, see below) females may have more abraded and therefore duller white wing patches
281 as well, thereby showing lower brightness contrast in the wing. White patch brightness
282 variation has been suggested to act as a sexual ornament in several species, including females
283 (Hanssen et al. 2006). It is worth to note here that the outer wing coverts bordering the wing
284 patch may have appeared in a separate PC from other contour feathers because they are not

285 replaced in the partial winter moult (see the description of non-breeding plumages in Cramp
286 et al. 1993). In both sexes, UV chroma PC1 largely referred to some (albeit different)
287 melanised plumage areas. UV chroma PC2 in males is easily interpreted as the purely
288 structural colour of white areas. In females, the same PC is more interesting because it
289 suggests an axis of positive correlation between two possible determinants of feather quality:
290 melanisation (less UV in wing coverts) and microstructure (more UV in breast feathers). This
291 correlation can be adaptive if it also appears within the same feather because melanin
292 increases while the structure promoting high UV reflectance likely reduces the structural
293 strength of the feather (Bonser 1995; Prum 2006).

294 Costs of reproduction imply trade-offs between reproductive investment and other life-
295 history traits (Harshman and Zera 2007). In birds, these costs may include, for example,
296 reductions in future survival probability and parasite resistance (e.g. Stjernman et al. 2004),
297 flight ability (Kullberg et al. 2002), or immune function and future fecundity (Hanssen et al.
298 2006). Trade-offs related to offspring production may also involve sexual ornaments
299 (Höglund and Sheldon 1998). However, this possibility is as yet poorly explored in birds (for
300 experiments see Griffith 2000; Siefferman and Hill 2005b). In a Swedish population of our
301 study species, the white forehead patch size of males became smaller in the year after caring
302 for an experimentally enlarged clutch (Gustafsson et al. 1995), while in our population, the
303 white wing patch size change of females was negatively correlated with their previous clutch
304 size (Hegyi et al. 2008b). In the present study, female brightness PC2 was marginally
305 positively related to previous clutch size, i.e. females that laid a larger clutch tended to
306 develop less melanised feathers and a lower wing patch contrast after breeding. Interestingly,
307 it was the colour of the wing feathers which moult only in summer, right after the breeding
308 effort (Cramp et al. 1993) that showed an indication of reproductive costs. Female brightness
309 PC1, on the other hand, was negatively correlated with previous residual body mass, i.e.

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

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

335 2009; Pault et al. 2012). Potential production costs associated with melanin-based plumage
336 ornaments may partly mediate such plasticity (e.g. sensitivity to dietary metal element
337 content, Niecke et al. 2003; McGraw 2006; and physiological stress, Galván and Alonso-
338 Alvarez 2008; Roulin et al. 2008), although wearing costs of melanin-based traits such as
339 intrasexual aggression may also play a role (Senar et al. 1993; Hoi and Griggio 2008).

340 In addition, we found that females with low brightness PC1 (i.e. more melanised
341 individuals) in a given year bred earlier in that year. This could result from their consistently
342 good condition, but brightness PC1 showed little relationship with current condition. It is
343 therefore possible that the pattern with laying date reflects inter- or intrasexual selection
344 favouring darker melanised plumage areas. Such selection could easily be explained by the
345 apparent indicator value of dark plumage in terms of moulting condition and therefore
346 individual quality (see above). Indeed, size of the white wing patch of females has been
347 suggested to play a role in female competition for territories and mates (Hegyi et al. 2007b,
348 2008a, b), so it would not be surprising to find similar relationships for another ornamental
349 attribute of this sex.

350 In contrast to brightness variation, none of the UV chroma PCs in either sex showed
351 any indication of dependence on body condition or breeding investment in the previous year.
352 Nevertheless, males with higher UV chroma in dark plumage areas (high PC1), tended to
353 breed earlier and had higher clutch sizes in the given year, indicating a possible role of UV
354 chroma in sexual selection and differential reproductive investment, although differential
355 arrival and territory quality effects cannot be ruled out. Interestingly, in males, brightness
356 PC1, which also reflected the structural based component of colour variation, was similarly
357 negatively related to current laying date. In the closely related pied flycatcher, males with
358 more pronounced UV chroma on their melanised plumage parts arrived earlier to the breeding
359 site (Siitari and Huhta 2002). In our population, breeding date of a male has been shown to be

360 not only a possible indicator of arrival date from migration, but also a reliable signal of
361 female mate preference (see Hegyi et al. 2007c, 2010). Regarding clutch size, several studies
362 in our population were unable to detect any relationship between the white plumage patch
363 sizes of males and the primary reproductive investment of their mate (Hargitai et al. 2005;
364 Michl et al. 2005; Török et al. 2007), so the positive relationship between UV chroma PC1
365 and residual clutch size is interesting. However, the alternative explanation of differential
366 territory quality cannot be ruled out without further experimental work. Even in the absence
367 of condition-dependence (see Results), UV chroma and the structurally-based component of
368 brightness may reflect differences in the regularity of feather microstructure (Shawkey et al.
369 2003, 2005; Prum 2006), possibly indicating an individual-specific, stable aspect of quality
370 such as developmental stability (Kemp et al. 2006; Prum 2006; Galván 2011).

371 The apparent lack of condition-dependence and sexual selection was especially
372 striking for the UV chroma of the conspicuous white plumage areas of males (comprising UV
373 chroma PC2 in this sex), since the sizes of white patches are sexually selected (Michl et al.
374 2002; Hegyi et al. 2010) while their UV chroma is higher than in females (Laczi et al. 2011).
375 However, phenotypic variation in the UV chroma of white plumage areas is lower in males
376 than in females (see Materials and methods). Males may keep relative UV reflectance at a
377 possible maximum to further increase the conspicuousness of the ornamental white patches,
378 with coloration functioning as a signal amplifier for white patch size (Hasson 1990, 1991). In
379 this case, the variance and information content of white patch UV chroma may be reduced in
380 males. It is interesting that quality indication is characteristic to the UV chroma of dark but
381 not white plumage areas in the sister species pied flycatcher (Siitari and Huhta 2002) and
382 quality-related information in dark feather UV reflectance is also present in some other
383 species (Doucet et al. 2004; Hegyi et al. 2007a).

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

396

397

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410 **References**

411

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

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

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

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

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

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

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

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

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

430 Evans SR, Sheldon BC (2012) Quantitative Genetics of a Carotenoid-Based Color:
431 Heritability and Persistent Natal Environmental Effects in the Great Tit. *Am Nat* 179:79-
432 94

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

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

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

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

441 Griffith SG (2000) A trade-off between reproduction and a condition-dependent sexually
442 selected ornament in the house sparrow *Passer domesticus*. *Proc R Soc Lond B* 267:1115-
443 1119

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

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

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

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

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

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

457 Hasson O (1990) Amplifiers and their role in the evolution of male display. *Evol Ecol* 4:277-
458 289

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

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

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

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

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

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

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

476 Hegyi G, Török J, Tóth L, Garamszegi LZ, Rosivall B (2006) Rapid temporal change in the
477 expression and age-related information content of a sexually selected trait. *J Evol Biol*
478 19:228-238

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

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

482 Hoi H, Griggio M (2008) Dual utility of a melanin-based ornament in bearded tits. *Ethology*
483 114:1094-1100

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

486 Höglund J, Sheldon BC (1998) The cost of reproduction and sexual selection. *Oikos* 83:478-
487 483

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

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

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

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

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

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

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

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

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

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

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

513 Niecke M, Rothlaender S, Roulin A (2003) Why do melanin ornaments signal individual
514 quality? Insights from metal element analysis of barn owl feathers. *Oecologia* 137:153-
515 158

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

518 Piau R, Gasparini J, Bize P, Jenni-Eiermann S, Roulin A (2009) Pheomelanin-Based
519 Coloration and the Ability to Cope with Variation in Food Supply and Parasitism. *Am Nat*
520 174:548-556

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

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

525 Quesada J, Senar JC (2009) Cross-fostering experiments to compare carotenoid- and melanin-
526 based plumage traits and long-term parental effects in post-moulted great tits. *Behaviour*
527 146:1235-1251

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

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

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

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

537 Roulin A, Ducrest A-L (2013) Genetics of colouration in birds. *Semin Cell Dev Biol* 24:594-
538 608

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

542 Senar JC, Camerino M, Copete JL, Metcalfe NB (1993) Variation in Black Bib of the
543 Eurasian Siskin (*Carduelis spinus*) and Its Role as a Reliable Badge of Dominance. *Auk*
544 110:924-927

545 Senar JC, Figuerola J, Domènech J (2003) Plumage coloration and nutritional condition in the
546 great tit (*Parus major*): the roles of carotenoids and melanins differ. *Naturwissenschaften*
547 90:234–237

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

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

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

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

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

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

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

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

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

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

568 Török J, Hargitai R, Hegyi G, Matus Z, Michl G, Péczely P, Rosivall B, Tóth G (2007)
569 Carotenoids in the egg yolks of collared flycatchers (*Ficedula albicollis*) in relation to
570 parental quality, environmental factors and laying order. Behav Ecol Sociobiol 61:541-
571 550

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

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

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

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

581 **Table 1** Summary of loadings of the principal components with the individual spectral
 582 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

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

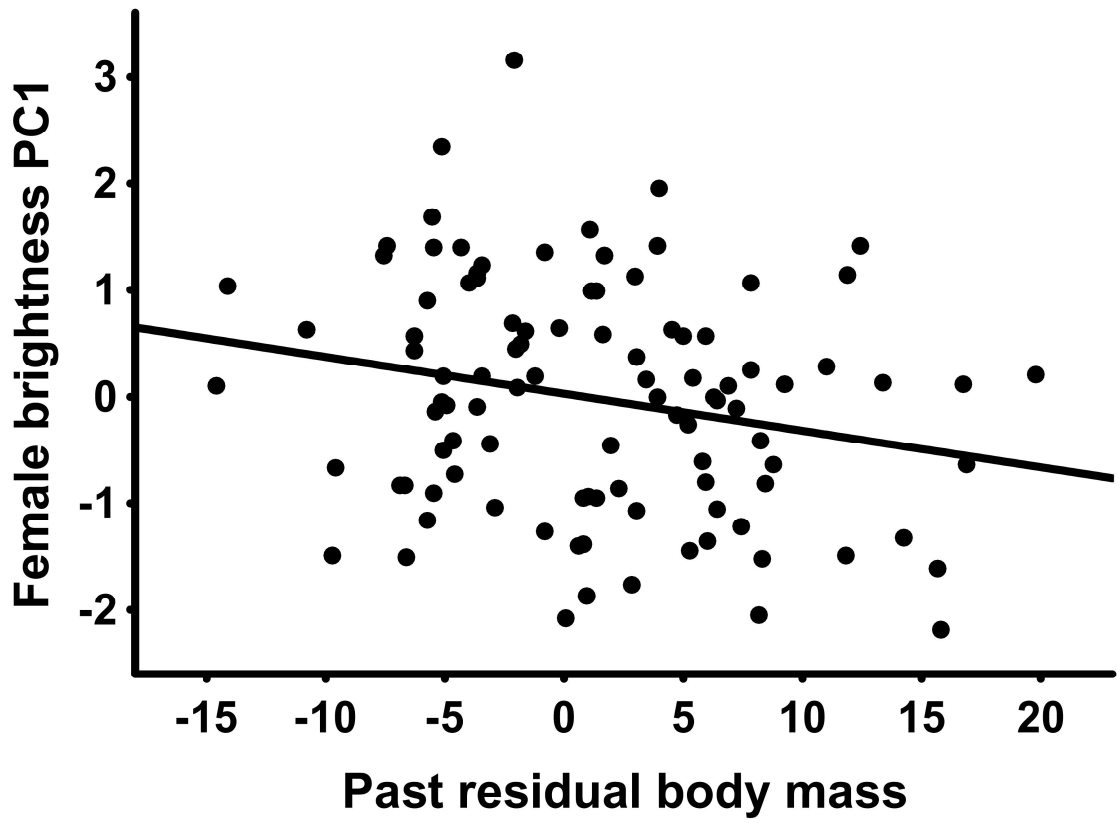
587 **Fig. 1** Relationship between female brightness PC1 and previous year residual body mass in
588 collared flycatchers (residual body mass comes from a regression of body mass on tarsus
589 length)

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

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

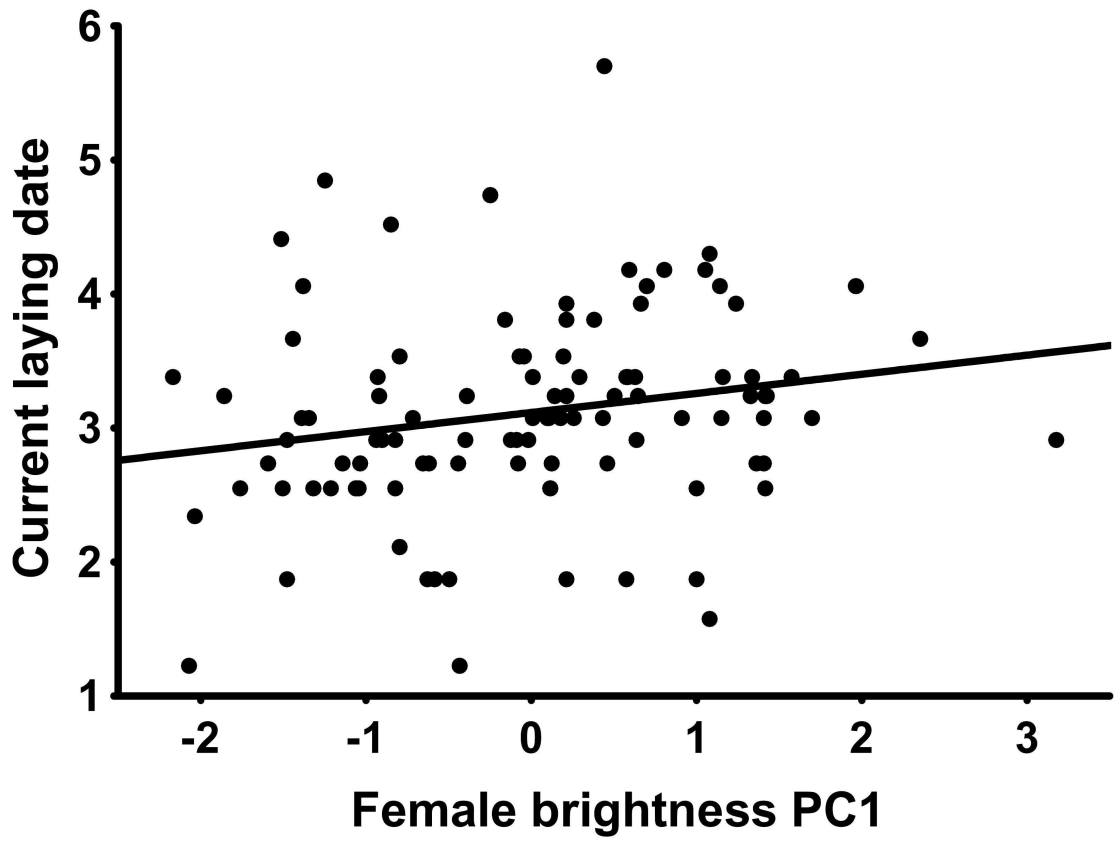
594 **Fig. 4** Relationship between male brightness PC2 and current residual body mass in collared
595 flycatchers

Fig 1



598 Fig 2

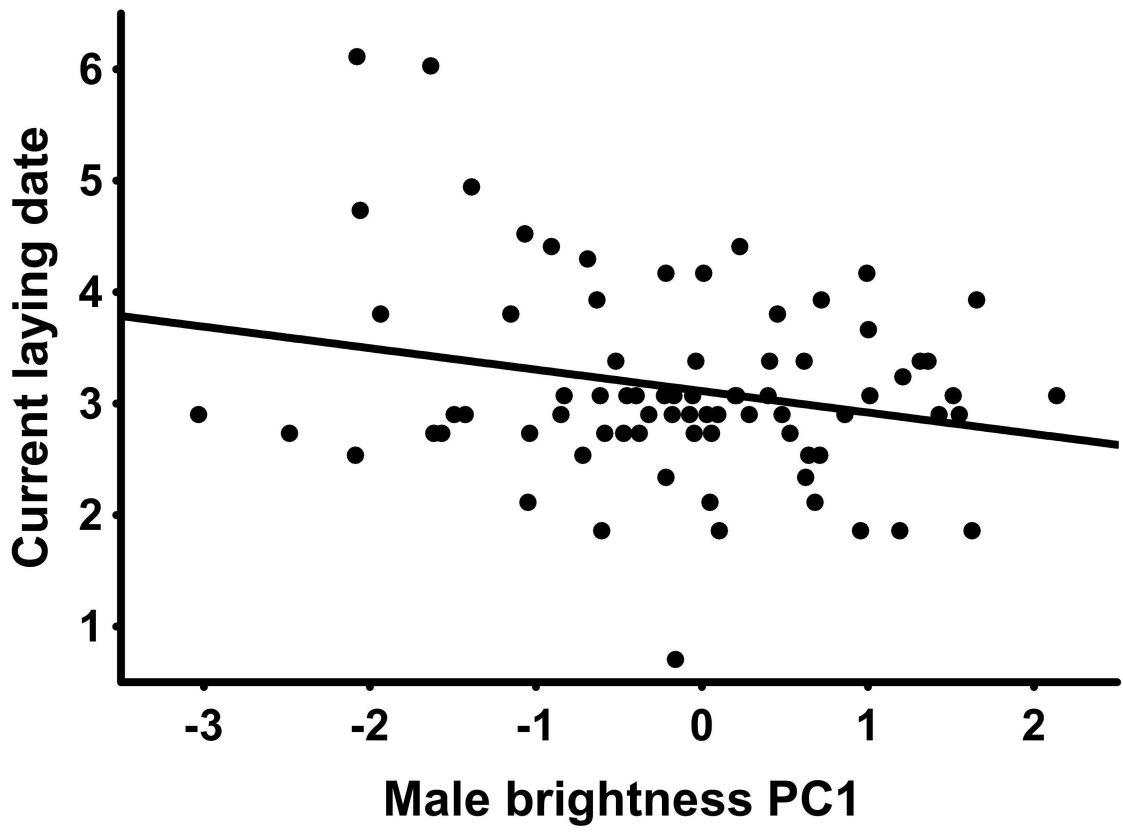
599



600

601 **Fig 3**

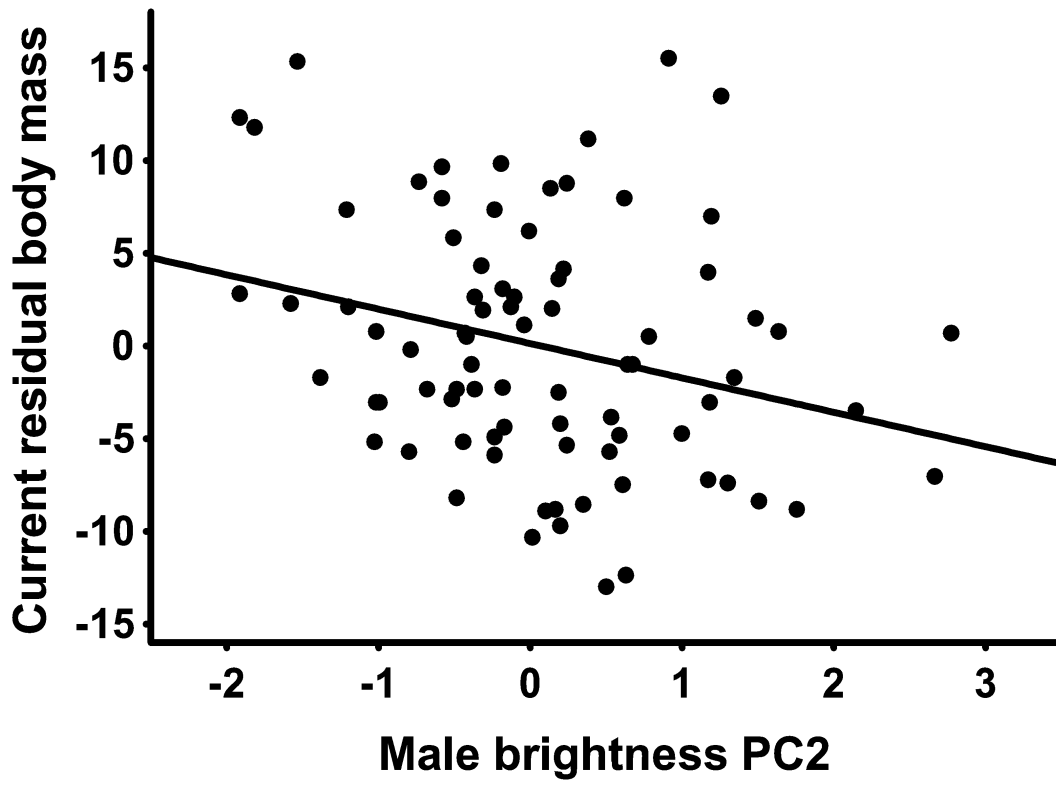
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603

604 Fig 4

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606