



POPULATION DIFFERENCES IN THE STRUCTURE AND COLORATION OF GREAT TIT CONTOUR FEATHERS

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3 1 POPULATION DIFFERENCES IN THE STRUCTURE AND COLORATION OF GREAT TIT
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18 7 Short Running title: Feather color and microstructure
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21 **Abstract**

22 Contour feathers cover most of the avian body and play critical roles in insulation, social
23 communication, aerodynamics and water repellency. Feather production is costly, and
24 development of the optimum characteristics for each function may be constrained by limited
25 resources or time; and possibly lead to trade-offs among the different characteristics.

26 Populations exposed to different environmental conditions may face different selective
27 pressures, resulting in differences in feather structure and coloration, particularly in species
28 with large geographic distributions.

29 Three resident populations of great tit *Parus major* L. from different latitudes differed in
30 feather structure and coloration. Individuals from the central population exhibited less dense
31 and longer contour feathers, with a higher proportion of plumulaceous barbs than either
32 northern or southern birds, which did not differ in their feather structure. UV reflectance and
33 brightness of the yellow of the contour feathers of the breast was higher for the southern than
34 for the northern population. Birds with greener plumage (higher hue) had less dense but
35 longer feathers, independently of the population of origin.

36 Differences in feather structure across populations appear to be unrelated to the contour
37 feather color characteristics except for hue. Nutritional and time constraints during molt might
38 explain the pattern of feather structure, whereas varying sexual selection pressure might
39 underlie the coloration patterns observed. Our results suggest that different selective
40 pressures or constraints shape contour feather traits in populations exposed to varying
41 environmental conditions.

42
43 **Keywords** carotenoid-based coloration – feather microstructure - latitudinal variation - *Parus*
44 *major* - sexual selection – UV plumage reflectance - winter acclimatization

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46 Introduction

47 Contour feathers, which cover most of the avian body, provide aerodynamic shape,
48 insulation and surface for visual signals. Plumage is a crucial insulative layer that helps birds
49 in maintaining water and temperature homeostasis (Stettenheim, 2000). Within species,
50 variation in the total contour feather mass has been found both within and among
51 populations as a consequence of a seasonal acclimatization process, presumably related to
52 changes in thermal insulation (Swanson, 1991; Saarela, Klapper & Heldmaier, 1995).
53 Further, populations experiencing varying winter conditions have been found to differ in their
54 thermal conductance and metabolic adjustments (Dawson et al., 1983; Swanson, 1993;
55 Broggi et al., 2004). Contour feather structure is a plastic trait, which varies according to the
56 environmental, nutritional and physiological conditions experienced during molt (Broggi et al.,
57 2011; Vágási et al., 2012). However, although feather structure seems to play an important
58 role in thermal insulation (Middleton, 1986), population variation in contour feather structure
59 and thermal conductance remains unclear (but see Wolf & Walsberg, 2000).

60 Birds strongly rely on visual traits for social communication and plumage is the main trait
61 involved in such information exchange (Savalli, 1995; Hill & McGraw, 2006). Plumage
62 coloration results from the deposition of pigments in feathers (melanins, carotenoids and
63 porphyrins), differences in feather microstructure, as well as a combination of both.
64 Differences in plumage coloration have been intensively studied with respect to individual
65 quality (Hill & McGraw, 2006). In particular, carotenoid-based coloration has been studied as
66 a condition-dependent signal. Since carotenoids cannot be synthesized *de novo* by animals
67 and need to be acquired (Fox, 1976), individuals may be constrained by limited access to
68 such compounds (Olson & Owens, 1998). Carotenoids also play a role as antioxidants and
69 immune-modulators, and individuals balance their physiological use with the signaling
70 properties, making carotenoids good candidates as honest signals of individual quality
71 (Pérez-Rodríguez, 2009). Besides pigments, plumage coloration can arise as a consequence
72 of changes in feather microstructure (structural coloration) producing blue, green, purple and

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3 73 iridescent coloration (Finger, Burkhardt & Dyck, 1992; Prum, 2006). The final appearance of
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5 74 plumage coloration often results from the interaction between pigmented and structural
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7 75 colors (Shawkey & Hill, 2005; D'Alba et al., 2014), and other factors such as the shape of the
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9 76 feathers (Badyaev & Landeen, 2007).

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11 77 Besides serving a signaling function, the development of colorful feathers can have a
12
13 78 physical influence on feather microstructure. For example, deposition of melanin can directly
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15 79 affect the physical properties of the feather by improving resistance to abrasion (Burt, 1986;
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17 80 Roulin et al., 2013), and decrease bacterial and lice-chewing degradation (Burt, 2009;
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19 81 Gunderson et al. 2008; Kose et al., 1999; but see Grande et al. 2004). Recent studies also
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21 82 suggest that populations may adaptively increase feather melanization when exposed to
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23 83 unfavorable conditions for the plumage (Peele et al. 2009). Furthermore, structurally based
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25 84 coloration such as in iridescent feathers is known to impair plumage hydrophobicity (Eliason
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27 85 & Shawkey, 2011). Therefore, understanding microstructure variation in colourful contour
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29 86 feathers requires the consideration of the different kinds of pigments and structural coloration
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31 87 involved.

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34 88 Feather development costs may arise from different selective pressures derived from varying
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36 89 ecological, social or physiological circumstances. Honest advertisement models posit that
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38 90 sexually selected traits are costly to produce, maintain or bear, brightly colored feathers
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40 91 being classic examples of such traits. The cost of pigmented feathers is often ascribed to the
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42 92 value of the pigment itself, but also the physiological cost of producing the pigmented feather
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44 93 and the survival implications of the general appearance of the plumage (e.g. increased
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46 94 predation risk) should be considered as important costs (Hill & McGraw, 2006).

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48
49 95 Plumage is replaced by periodic molts as it wears and deteriorates over time. Environmental
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51 96 and physiological conditions during feather growth are known to affect feather quality
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53 97 (Strochlic & Romero, 2008; Butler, Leppert & Dufty Jr., 2010; Moreno-Rueda, 2010; Pap et
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55 98 al., 2013). Furthermore, molting is energetically costly (Hoye & Buttemer, 2011), and molt
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57 99 speed is known to adversely affect feather structure (Vágási et al., 2012) and the expression

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3 100 of certain plumage ornaments (Vágási, Pap & Barta, 2010). Feather production is a
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5 101 demanding process in terms of time and resources, and molting individuals may be exposed
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7 102 to trade-offs with other costly activities such as reproduction (Bensch et al., 1985; Siikamäki,
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9 103 Hovi & Rätti, 1994), migration (de la Hera, Pérez-Tris & Tellería, 2009) or molting speed
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11 104 (Dawson et al., 2000). In fact, few bird species do overlap breeding and molting on a regular
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13 105 basis, particularly in seasonal environments (Dawson, 2008). Thus, the final characteristics
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15 106 of feathers may depend on the balance between the available energy, the requirements for
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17 107 plumage development (Butler, Rohwer & Speidel, 2008), and the different functions feathers
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19 108 serve. Additionally, as individuals are exposed to different environmental and feeding
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21 109 conditions across their geographic range, selective pressures and/or constraints upon
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23 110 different functions of the birds' plumage may change accordingly.

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26 111 The great tit *Parus major* L. is a resident passerine distributed across Eurasia, with
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28 112 populations subjected to very different ecological conditions such as seasonality and food
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30 113 availability (Sanz, 1998). This has led to maladaptive life-history strategies at the borders of
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32 114 their distribution (e.g. Rytönen & Orell, 2001), where great tits are often confronted with time
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34 115 and nutritional constraints affecting molting phenology and speed (Nilsson & Svensson,
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36 116 1996). Previous studies have shown that great tits from different populations differ in their
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38 117 feather structure, and these differences are likely determined by nutritional constraints
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40 118 (Broggi et al., 2011). We studied population variation in contour feather structure and
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42 119 coloration (carotenoid-based and structural) among three wild European great tit populations
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44 120 located in the southern, middle and northern portions of the species distribution range, which
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46 121 are exposed to very different environmental and ecological conditions, particularly during the
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48 122 non-breeding season. We investigated the relation between different contour feather traits
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50 123 across populations to find out whether feather traits vary in concert or independently, and in
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52 124 accordance with the different ecological/environmental circumstances encountered.

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3 1264 127 **Materials and methods**

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7 128 We captured 61 wild great tits from three locations: 25 in Oulu, Finland (65°N, 25°30'E), 12 in
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9 129 Lund, Sweden (55°40'N, 13°25'E), and 24 in Barcelona, Spain (41°23'N, 2°9'E) hereafter the
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11 130 northern, central and southern populations respectively. Birds from the northern and central
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13 131 populations were sampled from January to March 2001; and birds from the southern
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15 132 population from February to March 2002. Oulu study area consists of mid-boreal forests with
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17 133 winters characterized by average temperatures of about -8°C, minimum day length of less
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19 134 than 4 h and permanent snow cover. Lund study area consists of mixed forests of pine and
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21 135 deciduous trees, with average winter temperatures of about 0°C, minimum day length of 7 h
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23 136 and non-permanent snow cover. The southern study site in Barcelona consists of mixed
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25 137 forests of pine and oaks, winters with an average temperature of about +8°C, minimum day
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27 138 length of 9 h and absence of snow cover.

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30 139 Birds were captured using baited funnel traps (Senar et al., 1997), and a few yellow contour
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32 140 feathers were plucked from an area on the right side of the breast, between the shoulder and
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34 141 the breast black stripe of each individual. All feathers were stored under equal conditions, i.e.
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36 142 dry and dark, for later analyses in the laboratory. Feather structure analyses in the central
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38 143 and northern populations were undertaken in 2004, whereas the feather structure from the
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40 144 southern population and all color measurements were undertaken in 2005 in the Natural
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42 145 History Museum facilities in Barcelona. Age (adult, N=31, or yearling, N=30) and sex (30
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44 146 males and 31 females were determined according to Jenni & Winkler (1994)).

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47 147 All procedures were approved by the ethical committee of the University of Oulu (097/04),
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49 148 Malmö/Lund Animal Care Committee (M126-00), and the Departament de Medi Ambient,
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51 149 Generalitat de Catalunya (2002).

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56 151 *Feather structure analyses*
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3 152 Two contour feathers per individual were analyzed with the help of a stereoscopic
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5 153 microscope with an ocular grid. Structurally, contour feathers are formed by a series of barbs
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7 154 attached on each side of a central rachis, with each barb supporting regular ramifications or
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9 155 barbules (Stettenheim, 2000). To describe contour feather structure we measured six
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11 156 different variables that may explain differences in insulation capacity (Middleton, 1986):
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13 157 density of barbs and barbules from the plumulaceous and pennaceous portions of the
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15 158 feather, proportion of plumulaceous barbs and the total vane length. Details on the
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17 159 measuring procedures of feather structure' variables are provided elsewhere (Broggi et al.,
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19 160 2011). All variables measured were significantly repeatable within an individual (all $P < 0.001$;
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21 161 repeatability between 0.33 and 0.71), as measured by means of a one-way ANOVA with
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23 162 individual as grouping factor. For later analyses, average values of the two measurements
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25 163 were used. All feather structure measurements were done by the same person (A.G.). Data
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27 164 on feather structure from Oulu and Lund wild birds correspond to the same birds as in Broggi
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29 165 et al. (2011). One bird from the Oulu population was excluded from the data set because the
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31 166 feather coloration could not be measured.
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35 36 168 *Plumage color measurements*

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39 169 Coloration of yellow contour feathers from each individual was measured in the laboratory by
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41 170 superimposing all feathers on a black velvet surface (absolute reflectance 0%), replicating
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43 171 the plumage of the bird. This method is repeatable within individuals, and reliably reflects
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45 172 plumage coloration whenever the number of measured feathers are accounted for (details on
46
47 173 the method can be found in Quesada & Senar (2006)). The color of the feathers was
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49 174 measured using the tri-stimulus approach by means of a spectrophotometer Minolta CM-
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51 175 2600d (see Quesada & Senar (2006) for specifications), which provides values of brightness,
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53 176 chroma and hue on the visible scale and reflectance data from 360 to 700 nm. Brightness
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55 177 corresponds to the physical light intensity on a scale from 0 (black) to 100 (white). Chroma
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57 178 (color intensity) is positively correlated with color purity on a scale of 0 for white to 100 for
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3 179 pure color. Hue corresponds to the wavelength of the color and it is expressed in degrees of
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5 180 a circle starting with red, continuing through yellow, green, blue and back to red. In the case
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7 181 of great tits, hue values increase from an orange-yellow to a greenish yellow (Quesada &
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9 182 Senar, 2006). The algorithms to calculate the brightness, chroma and hue variables refer
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11 183 only to the 400-700 nm range and omit the UV region. Since great tit yellow plumage
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13 184 coloration also reflects in the UV (Quesada & Senar, 2006) and given that the maximum
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15 185 peak of absorbency of the fourth cone of vision in the UV range in the closely related blue tit
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17 186 *Cyanistes caeruleus* L. is $\lambda=371$ nm (Hart et al., 2000), we included reflectance at 370 nm as
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19 187 a measure of UV reflection (Prum, 2006). We measured all spectra in reference to a white
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21 188 standard (WS-1, Diffuse Reflectance Standard) (reflectivity over 98%). Dark reference
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23 189 measurements were taken as control for non-specific activity of the sensor in the absence of
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25 190 light. We used the tri-stimulus methodology instead of alternative spectral visual models or
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27 191 PCA methods because this approach is the most appropriate to analyze data from
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29 192 incomplete spectra, without yielding substantial differences in the estimates (Evans et al.
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31 193 2010).

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35 36 195 *Statistical analyses*

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39 196 All variables were normally distributed (tested with Shapiro-Wilk test) and parametric
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41 197 statistics were applied. Feather structure was described by the first factor in a principal
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43 198 component analysis including the six variables measured for feather structure. The rest of
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45 199 the factors had an eigenvalue lower than one.

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48 200 Variation in feather structure was analyzed by linear models with sex, age, population of
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50 201 origin and the respective interactions as fixed effects. Likewise, variation in each color
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52 202 component (chroma, hue, brightness and UV) was analyzed by linear models with sex, age,
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54 203 population of origin and the respective interactions as fixed effects, and feather structure and
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56 204 the number of feathers used in the color analyses as covariates. Residuals from all these

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3 205 models were normally distributed, and the color variables were linearly related to the number
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5 206 of feathers measured as found in previous studies (Quesada & Senar, 2006). We tested the
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7 207 effect of the interactions for all models by comparing each model with a reduced model
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9 208 without the interactions by means of likelihood ratio tests. None of the interactions were
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11 209 significant (all $P > 0.1$), and were finally dropped from the final models. Only the results from
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13 210 final models are shown. All statistical tests were done using IBM SPSS Statistics 20.

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3 2124 213 **Results**

5
6 214 Feather structure variables and color components varied significantly among great tit
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8 215 populations (Table 1 and 2). General contour feather structure was studied by means of a
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10 216 principal component analysis (PCA), with a first factor explaining 57.6% of the variance and
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12 217 an eigenvalue of 3.45. Densities of plumulaceous and pennaceous barbs (0.72 and 0.75
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14 218 respectively), and plumulaceous and pennaceous barbules (0.76 and 0.72 respectively) were
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16 219 positively loaded in the first component of the PCA, while feather length (-0.86) and
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18 220 proportion of plumulaceous barbs (-0.70) were negatively loaded. In summary, high positive
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20 221 values of feather structure correspond to short dense feathers with a low proportion of
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22 222 plumulaceous barbs.

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25 223 Feather structure varied significantly across populations (Table 3; Fig. 1): both northern and
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27 224 southern birds differed from the middle range population by having short and dense feathers,
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29 225 with lower proportion of plumulaceous barbs (Post-hoc Tukey tests, both $P < 0.001$; Table 1).
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31 226 Feathers from southernmost and northernmost birds did not differ from each other (Post-hoc
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33 227 Tukey test, $P = 0.98$; Table 1; Fig. 1). Neither age, sex nor their interactions had any effect on
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35 228 feather structure (Table 3). Feather structure correlated negatively with plumage hue, after
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37 229 correcting for the number of feathers used in measuring the color, so that birds with a more
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39 230 greenish plumage (higher hue) had less dense but longer feathers (Table 3; Fig. 2). Chroma,
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41 231 brightness and UV reflectance were not affected by feather structure (Table 3). Plumage
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43 232 brightness and UV reflectance were higher in the southern than in the northern population
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45 233 (pairwise comparison, $P < 0.05$; Fig. 3). Birds from the central population had intermediate
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47 234 values of brightness not differing from the other two populations (pairwise comparison, both
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49 235 $P > 0.05$); but differed from the northern population in UV reflectance (pairwise comparison,
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51 236 $P = 0.020$). Chroma and hue did not differ among populations (Table 3). Males had higher
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53 237 values of chroma, brightness and UV reflectance than females, whereas hue variation was
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55 238 independent of sex (Table 3).

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241 **Discussion**

242 We found differences between great tit populations in contour feather structure and
243 coloration. Feather structure did not change according to winter severity as the central
244 population had significantly longer feathers with higher percentage of plumulaceous barbs
245 than the two marginal populations. Considering that longer contour feathers with more
246 plumulaceous barbs have lower thermal conductance, and better insulating properties than
247 shorter feathers with a higher proportion of pennaceous barbs (Dove et al., 2007), our results
248 suggest that thermal insulation does not drive contour feather differences among the
249 populations studied. Furthermore, as great tits from southern and northern populations
250 exhibited contour feathers with similar microstructure, both populations living at the
251 distribution margins, and therefore exposed to suboptimal environmental conditions (Sanz,
252 1998) may be constrained in developing optimal feather structure.

253 Broggi et al. (2011) suggested that birds from the central population could be less
254 constrained during feather molt, thereby developing higher quality feathers than those from
255 northern regions. Our results suggest that southern populations could also be constrained
256 during the molting period as they developed feathers of similar structure as in northern
257 populations. Time constraints for molting in the southern population may arise from the
258 higher prevalence of double brooding in great tit populations at low latitudes (Sanz, 1998), or
259 the harshness of summer conditions (Hemborg, Sanz & Lundberg, 2001). Vágási et al.
260 (2012) recently showed a causal link between molt speed and contour feather structure by
261 experimentally increasing molt rate of caged house sparrows *Passer domesticus* L., which
262 developed feathers with similar characteristics to the ones we found in the two marginal
263 populations of great tits (short, dense feathers with low percentage of plumulaceous barbs).
264 Molting late in the season, as observed in populations at higher latitudes (Holmgren, Jönsson
265 & Wennerberg, 2001), is usually compensated for by accelerating molting rate (Dawson,
266 2004), which can in turn decrease feather quality (Dawson et al., 2000; de la Hera et al.,

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3 267 2009). Likewise, great tit breeding success (Sanz, 1998) and yolk carotenoid composition in
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5 268 pied flycatchers *Ficedula hypoleuca* P. (Eeva et al., 2011) present similar non-linear
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7 269 latitudinal patterns as we found for feather structure, which are claimed to result from time
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9 270 constraints (Sanz, 1998) and the mismatch between laying time and caterpillar availability
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11 271 (Rytönen & Orell, 2001; Eeva et al., 2011). Thus, great tits from the northern and southern
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13 272 populations seem to grow suboptimal feathers which may be related to constraints on the
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15 273 length and/or the access to nutrients during the molting period, as compared to birds from
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17 274 the central population. Alternatively, birds from the southern population may be released
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19 275 from the selective pressure for high insulation capacity, and therefore grow an adequate
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21 276 plumage adapted to milder winter conditions.

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24 277 Despite recent demographic studies suggesting that the northern population is a “sink”
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26 278 (Karvonen et al., 2012), Broggi et al., (2005) showed that northern great tits locally adapt
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28 279 their winter metabolism. However, they seem to be unable to develop a highly insulative
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30 280 plumage structure in line with previous results showing higher thermoregulatory costs for
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32 281 great tits from the northern population (Broggi et al., 2004). These results suggest that
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34 282 selective pressure for an optimal feather structure is weak, or otherwise constrained by other
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36 283 more important traits e.g. timing of breeding (Eeva, Veistola & Lehikoinen, 2000; Rytönen &
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38 284 Orell, 2001).

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40 285 Variation in the pattern in feather coloration was mostly unrelated to structure, and only hue
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42 286 exhibited a significant relationship to feather structure. Independent of the population of
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44 287 origin, hue increased with the inferred quality of the contour feathers (negative values of
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46 288 feather structure, Fig. 2). As carotenoid-based hue is related to the ability to acquire food
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48 289 (Senar, Figuerola & Pascual, 2002; Senar et al., 2008) and to general body condition (as
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50 290 inferred from ptilochronology) (Senar, Figuerola & Domenech, 2003), contour feather
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52 291 structure may be a reliable indicator of individual quality in all populations. Higher values of
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54 292 hue are also found in great tit populations inhabiting good quality habitats (Ferns & Hinsley,
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56 293 2008), suggesting that poorer nutritional condition could constrain both signaling properties

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3 294 and structural quality of feathers, thus giving no support for a trade-off between carotenoid-
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5 295 based coloration and structure of great tit feathers.
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7 296 In accordance with previous studies we found that yellow contour feathers of great tits were
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9 297 sexually dichromatic with males having higher values for chroma, brightness and UV (Ferns
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11 298 & Hinsley, 2008; Isaksson et al., 2008), but not hue. Population of origin did not explain the
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13 299 variation in hue or chroma, but we found a decrease in contour feather brightness and UV
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15 300 with increasing latitude. Thus, molt speed does not seem to affect contour feather brightness
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17 301 and UV, as it does with feather structure, contrary to what has been found in other species
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19 302 (Serra et al., 2007; Griggio et al., 2009). Higher values of contour feather brightness and UV
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21 303 in the southern population could be the result of a stronger sexual selection pressure in this
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23 304 population, which may be related to higher population density (Irwin, 2000; Forsman &
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25 305 Mönkkönen, 2003) or higher parasite pressure at lower latitudes (Møller, 1998). However, it
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27 306 should be considered that inter-individual variation in plumage coloration is often
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29 307 considerable i.e. due to sex, age or season (Figuerola & Senar, 2005). Although we
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31 308 controlled for several of these potentially confounding factors, further studies including more
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33 309 populations at different latitudes would be required to properly interpret the latitudinal pattern
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35 310 found in this study.
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38 311 In summary, great tit contour feather structure and coloration differs among the studied
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40 312 populations. The different feather traits do not generally vary in concert although some
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42 313 patterns of co-variation emerge. The results suggest that feather structure could result from
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44 314 constraints during molting among populations at the distribution margins, while coloration
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46 315 may depend on other factors such as a latitudinal decrease on the strength of sexual
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48 316 selection. This study also shows that except for hue, other signaling aspects of carotenoid-
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50 317 based and structural coloration are independent of feather microstructure. Great tits from
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52 318 different populations prioritize the development of certain feather characteristics over others,
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54 319 although the different traits do not seem to interact with each other. Experimental
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3 320 manipulations of feather coloration and/or structure in captivity would be required to further
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5 321 study covariation patterns and possible constraints on the development of feather traits.
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335 **References**

- 336 Badyaev AV, Landeen EA. 2007. Developmental evolution of sexual ornamentation: model
337 and a test of feather growth and pigmentation. *Integrative Comparative Biology* **47**: 221-233.
- 338 Bensch S, Gezelius L, Hasselquist D, Lindström A. 1985. Influence of brood size on moult in
339 female Willow Warblers. *Ornis Scandinavica* **16**: 151-152.
- 340 Bonser RHC. 1995. Melanin and the abrasion resistance of feathers. *The Condor* **97**: 590-
341 591.
- 342 Broggi J, Orell M, Hohtola E, Nilsson J-Å. 2004. Metabolic response to temperature variation
343 in the great tit: an interpopulation comparison. *Journal of Animal Ecology* **73**: 967-972.
- 344 Broggi J, Hohtola E, Orell M, Nilsson J-Å. 2005. Local adaptation to winter conditions in a
345 passerine spreading north: a common garden approach. *Evolution* **59**: 1600-1603.
- 346 Broggi J, Gamero A, Hohtola E, Orell M, Nilsson J-Å. 2011. Interpopulation variation in
347 contour feather structure is environmentally determined in Great Tits. *PLoS ONE*, **6**: e24942.
- 348 Burt EH Jr. 2009 A future with feather-degrading bacteria. *Journal of Avian Biology* **40**: 349-
349 351
- 350 Burt EH Jr. 1986. An analysis of physical, physiological, and optical aspects of avian
351 coloration with emphasis on wood-warblers. *Ornithological Monographs* **38**.
- 352 Butler LK, Rohwer S, Speidel MG. 2008. Quantifying structural variation in contour feathers
353 to address functional variation and life history trade-offs. *Journal of Avian Biology* **39**: 629-
354 639.
- 355 Butler MW, Leppert LL, Dufty AM Jr. 2010. Effects of small increases in corticosterone levels
356 on morphology, immune function, and feather development. *Physiological and Biochemical*
357 *Zoology* **83**: 78-86.

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51
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53
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55
56
57
58
59
60

- 358 D'Alba L, Van Hemert C, Spencer KA, Heidinger BJ, Gill L, Evans NP, Monaghan P, Handel
359 CM & Shawkey MD. 2014. Melanin-Based Color of Plumage: Role of Condition and of
360 Feathers' Microstructure. *Integrative and Comparative Biology* In press.
- 361 Dawson A. 2004. The effects of delaying the start of moult on the duration of moult, primary
362 feather growth rates and feather mass in Common Starlings *Sturnus vulgaris*. *Ibis* **146**: 493-
363 500.
- 364 Dawson A. 2008. Control of the annual cycle in birds: endocrine constraints and plasticity in
365 response to ecological variability. *Philosophical Transactions of the Royal Society B* **363**:
366 1621-1633.
- 367 Dawson A, Hinsley SA, Ferns PN, Bonser RHC, Eccleston L. 2000. Rate of moult affects
368 feather quality: a mechanism linking current reproductive effort to future survival.
369 *Proceedings of the Royal Society of London B* **267**: 2093-2098.
- 370 Dawson WR, Marsh RL, Buttemer WA, Carey C. 1983. Seasonal and geographic variation
371 of cold resistance in House Finches *Carpodacus mexicanus*. *Physiological Zoology* **56**: 353-
372 369.
- 373 de la Hera I, Pérez-Tris J, Tellería JL. 2009. Migratory behaviour affects the trade-off
374 between feather growth rate and feather quality in a passerine bird. *Biological Journal of the*
375 *Linnean Society* **97**: 98-105.
- 376 Dove CJ, Rijke AM, Wang X, Andrews LS. 2007. Infrared analysis of contour feathers: The
377 conservation of body heat radiation in birds. *Journal of Thermal Biology* **32**: 42-46.
- 378 Eeva T, Veistola S, Lehikoinen E. 2000. Timing of breeding in subarctic passerines in
379 relation to food availability. *Canadian Journal of Zoology* **78**: 67-78.
- 380 Eeva T, Ruuskanen S, Salminen J-P, Belskii E, Järvinen A, Kerimov A, Korpimäki E, Krams
381 I, Moreno J, Morosinotto C, Mänd R, Orell M, Qvarnström A, Siitari H, Slater F, Tilgar V,
382 Visser M, Winkel W, Zang H, Laaksonen T. 2011. Geographical trends in the yolk carotenoid
383 composition of the pied flycatcher (*Ficedula hypoleuca*). *Oecologia* **165**: 277-287.

- 1
2
3 384 Eliason CM, Shawkey MD. 2011. Decreased hydrophobicity of iridescent feathers: a
4
5 385 potential cost of shiny plumage. *Journal of Experimental Biology* **214**: 2157-2163.
6
7 386 Evans SR, Hinks AE, Wilkin TA, Sheldon BC. 2010. Age, sex and beauty: methodological
8
9 387 dependence of age- and sex-dichromatism in the great tit *Parus major*. *Biological Journal of*
10
11 388 *the Linnean Society* **101**: 777-796.
12
13 389 Ferns PN, Hinsley SA. 2008. Carotenoid plumage hue and chroma signal different aspects
14
15 390 of individual and habitat quality in tits. *Ibis* **150**: 152-159.
16
17 391 Figuerola J, Senar JC. 2005. Seasonal changes in carotenoid- and melanin-based plumage
18
19 392 coloration in the Great Tit *Parus major*. *Ibis* **147**: 797-802.
20
21 393 Finger E, Burkhardt D, Dyck J. 1992. Avian plumage colors: origin of UV reflection in a black
22
23 394 parrot. *Naturwissenschaften* **79**: 187-188.
24
25 395 Forsman JT, Mönkkönen M, 2003. The role of climate in limiting European resident bird
26
27 396 populations. *Journal of Biogeography* **30**:55-70.
28
29 397 Fox DL. 1976. *Animal bichromes and structural colours*. Berkley, CA: University of California
30
31 398 Press,.
32
33 399 Gunderson AR, Frame AM, Swaddle JP, Forsyth MH. 2008. Resistance of melanized
34
35 400 feathers to bacterial degradation: is it really so black and white? *Journal of Avian Biology* **39**:
36
37 401 539-545.
38
39 402 Grande JM, Negro JJ, Torres MJ. 2004. The evolution of bird plumage colouration: a role for
40
41 403 feather-degrading bacteria? *Ardeola* **51**: 375-383.
42
43 404 Griggio M, Serra L, Licheri D, Campomori C, Pilastro A. 2009. Molt speed affects structural
44
45 405 feather ornaments in the blue tit. *Journal of Evolutionary Biology* **22**: 782-792.
46
47 406 Hart NS, Partridge JC, Cuthill IC, Bennett ATD. 2000. Visual pigments, oil droplets, ocular
48
49 407 media and cone photoreceptor distribution in two species of passerine bird: the blue tit
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 408 (*Parus caeruleus*; L.) and the blackbird (*Turdus merula*; L.). *Journal of Comparative*
4
5 409 *Physiology A* **186**: 375-387.
6
7 410 Hemborg, C., Sanz, J., and Lundberg, A. 2001. Effects of latitude on the trade-off between
8
9 411 reproduction and moult: a long-term study with pied flycatcher. *Oecologia* **129**: 206-212.
10
11 412 Hill GE, McGraw KJ. 2006. *Bird Coloration: Function and evolution*. Cambridge, MA:
13
14 413 Harvard University Press.
15
16 414 Holmgren N, Jönsson P, Wennerberg L. 2001. Geographical variation in the timing of
17
18 415 breeding and moult in dunlin *Calidris alpina* on the Palearctic tundra. *Polar Biology* **24**: 369-
19
20 416 377.
21
22 417 Hoye BJ, Buttemer WA. 2011. Inexplicable inefficiency of avian molt? Insights from an
23
24 418 opportunistically breeding arid-zone species, *Lichenostomus penicillatus*. *PLoS ONE* **6**:
25
26 419 e16230.
27
28
29 420 Irwin DE. 2000. Song variation in an avian ring species. *Evolution* **54**: 998-1010.
30
31
32 421 Isaksson C, Ornborg J, Prager M, Andersson S. 2008. Sex and age differences in
33
34 422 reflectance and biochemistry of carotenoid-based colour variation in the great tit *Parus*
35
36 423 *major*. *Biological Journal of the Linnean Society* **95**: 758-765.
37
38
39 424 Jenni L, Winkler R. 1994. *Moult and aging of European passerines*. London, UK: Academic
40
41 425 Press.
42
43 426 Karvonen J, Orell M, Rytönen S, Broggi J, Belda E. 2012. Population dynamics of an
44
45 427 expanding passerine at the distribution margin. *Journal of Avian Biology* **43**:102-108.
46
47
48 428 Kose M, Møller AP. 1999. Sexual selection, feather breakage and parasites: the importance
49
50 429 of white spots in the tail of the barn swallow (*Hirundo rustica*). *Behavioral Ecology and*
51
52 430 *Sociobiology* **45**: 430-436.
53
54
55 431 Middleton ALA. 1986. Seasonal changes in plumage structure and body composition of
56
57 432 American Goldfinch, *Carduelis tristis*. *Canadian Field Naturalist* **100**: 545-549.
58
59
60

- 1
2
3 433 Møller AP. 1998. Evidence of larger impact of parasites on hosts in the tropics : investment
4 434 in immune function with and outside the tropics. *Oikos* **82**: 265-270.
- 5
6
7 435 Moreno-Rueda G. 2010. Experimental test of a trade-off between moult and immune
8 436 response in house sparrows *Passer domesticus*. *Journal of Evolutionary Biology* **23**: 2229-
9 437 2237.
- 10
11
12
13
14 438 Nilsson J-Å, Svensson E. 1996. The cost of reproduction: A new link between current
15 439 reproductive effort and future reproductive success. *Proceedings of the Royal Society of*
16 440 *London B* **263**: 711-714.
- 17
18
19
20
21 441 Olson VA, Owens IPF. 1998. Costly sexual signals: are carotenoids rare, risky or required?
22 442 *Trends in Ecology and Evolution* **13**: 510-514.
- 23
24
25 443 Pap PL, Vágási CI, Barbos L, Marton A. 2013. Chronic coccidian infestation compromises
26 444 flight feather quality in house sparrows *Passer domesticus*. *Biological Journal of the Linnean*
27 445 *Society* **108**: 414-428.
- 28
29
30
31 446 Peele AM, Burt EH Jr., Schroeder MR, Greenberg RS. 2009. Dark color of Coastal Plains
32 447 Swamp Sparrows may be an evolutionary response to occurrence and abundance of salt
33 448 tolerant, feather-degrading bacilli in its plumage. *Auk* **126**: 531-535.
- 34
35
36
37
38 449 Pérez-Rodríguez L. 2009. Carotenoids in evolutionary ecology: re-evaluating the antioxidant
39 450 role. *BioEssays* **31**: 1116-1126.
- 40
41
42
43 451 Prum RO. 2006. Anatomy, physics and evolution of avian structural colors. In: Hill GE,
44 452 McGraw KJ, eds. *Bird Coloration: Mechanisms and Measurements*. Cambridge, MA:
45 453 Harvard University Press, 295-353.
- 46
47
48
49 454 Quesada J, Senar JC. 2006. Comparing plumage colour measurements obtained directly
50 455 from live birds and from collected feathers: the case of the great tit *Parus major*. *Journal of*
51 456 *Avian Biology* **37**: 609-616.
- 52
53
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53
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57
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- 457 Roulin A, Mangels J, Wakamatsu K, Bachman T. 2013. Sexually dimorphic melanin-based
458 colour polymorphism, feather melanin content, and wing feather structure in the barn owl
459 (*Tyto alba*) *Biological Journal of the Linnean Society* **109**: 562-573.
- 460 Rytkönen S, Orell M. 2001. Great tits, *Parus major*, lay too many eggs: experimental
461 evidence in mid-boreal habitats. *Oikos* **93**: 439-450.
- 462 Saarela S, Klapper B, Heldmaier G. 1995. Daily rhythm of oxygen consumption and
463 thermoregulatory responses in some European winter- or summer-acclimatized finches at
464 different ambient temperatures. *Journal of Comparative Physiology B* **165**: 366-376.
- 465 Sanz JJ. 1998. Effects of geographic location and habitat on breeding parameters of Great
466 tits. *Auk* **115**: 1034-1051.
- 467 Savalli UM. 1995. The evolution of coloration and ornamentation in birds: a review of
468 hypothesis. *Current Ornithology* **12**: 141-190.
- 469 Senar JC, Domènech J, Maria L, Moreno E. 1997. A funnel trap for the capture of tits.
470 *Butlletí del Grup Català d'Anellament* **14**: 17-24.
- 471 Senar JC, Figuerola J, Pascual J. 2002. Brighter yellow blue tits make better parents.
472 *Proceedings of the Royal Society of London B* **269**: 257-261.
- 473 Senar JC, Figuerola J, Domènech J. 2003. Plumage coloration and nutritional condition in
474 the great tit *Parus major*: the roles of carotenoids and melanins differ. *Naturwissenschaften*
475 **90**: 234-237.
- 476 Senar J, Negro JJ, Quesada J, Ruiz I, Garrido J. 2008. Two pieces of information in a single
477 trait? The yellow breast of the great tit (*Parus major*) reflects both pigment acquisition and
478 body condition. *Behaviour* **145**: 1195-1210.
- 479 Serra L, Griggio M, Licheri D, Pilastro A. 2007. Moults speed constrains the expression of a
480 carotenoid-based sexual ornament. *Journal of Evolutionary Biology* **20**: 2028-2034.

- 1
2
3 481 Shawkey MD, Hill GE. 2005. Carotenoids need structural colours to shine. *Biology Letters* **1**:
4 482 121-124.
5
6
7 483 Siikamäki P, Hovi M, Rätti O. 1994. A trade-off between current reproduction and moult in
8 484 the pied flycatcher - An experiment. *Functional Ecology* **8**: 587-593.
9
10
11 485 Stettenheim PR. 2000. The integumentary morphology of modern birds - An overview.
12 486 *American Zoologist* **40**: 461-477.
13
14
15
16 487 Strochlic DE, Romero LM. 2008. The effects of chronic psychological and physical stress on
17 488 feather replacement in European starlings (*Sturnus vulgaris*). *Comparative Biochemistry and*
18 489 *Physiology A* **149**: 68-79.
19
20
21
22
23 490 Swanson DL. 1991. Seasonal adjustments in metabolism and insulation in the dark-eyed
24 491 junco. *The Condor* **93**: 538-545.
25
26
27
28 492 Swanson DL. 1993. Cold tolerance and thermogenic capacity in dark-eyed Juncos in winter:
29 493 Geographic variation and comparison with American tree sparrows. *Journal of Thermal*
30 494 *Biology* **18**: 275-281.
31
32
33
34 495 Tieleman BI, Williams JB. 2000. The adjustment of avian metabolic rates and water fluxes to
35 496 desert environments. *Physiological and Biochemical Zoology* **73**: 461-479.
36
37
38
39 497 Vágási CI, Pap PL, Barta Z. 2010. Haste makes waste: accelerated molt adversely affects
40 498 the expression of melanin-based and depigmented plumage ornaments in House Sparrows.
41 499 *PLoS ONE* **5**: e14215.
42
43
44
45 500 Vágási CI, Pap PL, Vincze O, Benkő Z, Marton A, Barta Z. 2012. Haste makes waste but
46 501 condition matters: molt rate-feather quality trade-off in a sedentary songbird. *PLoS ONE* **7**:
47 502 e40651.
48
49
50
51
52 503 Wolf BO, Walsberg GE. 2000. The role of the plumage in heat transfer processes of birds.
53 504 *American Zoologist* **40**: 575-584.
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4 506 **Table 1** Mean \pm SE of contour feather structure of three great tit populations for different sex
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6 507 and age classes (feather structure data from the central and northern populations from
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8 508 Broggi et al. (2011)). Numbers in brackets indicate sample sizes and different superscript
9
10 509 letters represent statistically significant differences ($P < 0.05$) obtained from linear models with
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12 510 Tukey post-hoc tests for the feather structure variables.

	Southern	Central	Northern
Percentage of plumulaceous barbs (%)			
Adult males	71.3 \pm 0.67 (6)	73.0 \pm 0.73 (6)	71.5 \pm 1.15 (6)
Adult females	71.2 \pm 0.80 (5)	74.3 \pm 3.57 (4)	69.5 \pm 1.85 (4)
Juvenile males	67.2 \pm 1.45 (6)	72.0 (1)	72.0 \pm 1.22 (5)
Juvenile females	69.3 \pm 1.29 (7)	75.0 (1)	69.8 \pm 0.55 (9)
Total	69.7 \pm 0.64 ^a	73.5 \pm 1.16 ^b	70.6 \pm 0.53 ^a
Density of pennaceous barbs (per 1 mm)			
Adult males	1.41 \pm 0.07	1.31 \pm 0.04	1.56 \pm 0.10
Adult females	1.42 \pm 0.09	1.31 \pm 0.14	1.68 \pm 0.08
Juvenile males	1.61 \pm 0.05	1.54	1.59 \pm 0.12
Juvenile females	1.57 \pm 0.03	1.24	1.60 \pm 0.06
Total	1.51 \pm 0.03 ^a	1.32 \pm 0.05 ^b	1.60 \pm 0.04 ^a
Density of plumulaceous barbs (per 1 mm)			
Adult males	3.20 \pm 0.11	2.69 \pm 0.11	3.27 \pm 0.14
Adult females	3.16 \pm 0.15	2.62 \pm 0.19	3.21 \pm 0.14
Juvenile males	3.39 \pm 0.10	3.20	3.06 \pm 1.16
Juvenile females	3.14 \pm 0.08	3.68	3.01 \pm 0.06
Total	3.22 \pm 0.05 ^a	2.71 \pm 0.09 ^b	3.12 \pm 0.06 ^a
Density of pennaceous barbules (per 0.1 mm)			
Adult males	2.10 \pm 0.07	1.88 \pm 0.05	2.34 \pm 0.08
Adult females	2.38 \pm 0.09	1.89 \pm 0.07	2.39 \pm 0.02

Juvenile males	2.08 ± 0.05	2.04	2.31 ± 0.08
Juvenile females	2.20 ± 0.07	1.98	2.27 ± 0.06
Total	2.18 ± 0.04^a	1.90 ± 0.04^b	2.31 ± 0.03^c

Density of plumulaceous barbules (per 0.1 mm)

Adult males	3.05 ± 0.07	2.59 ± 0.08	2.93 ± 0.11
Adult females	3.07 ± 0.08	2.38 ± 0.05	2.91 ± 0.05
Juvenile males	3.01 ± 0.06	2.91	2.81 ± 0.13
Juvenile females	3.01 ± 0.15	2.27	2.86 ± 0.06
Total	3.03 ± 0.05^a	2.52 ± 0.07^b	2.88 ± 0.04^a

Feather length (mm)

Adult males	20.5 ± 0.95	24.4 ± 0.69	18.8 ± 0.84
Adult females	19.5 ± 1.05	25.1 ± 0.53	17.9 ± 0.52
Juvenile males	20.2 ± 0.62	21.8	19.9 ± 1.25
Juvenile females	18.9 ± 0.65	22.3	19.7 ± 0.75
Total	19.7 ± 0.40^a	24.2 ± 0.48^b	19.2 ± 0.44^a

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512

513 Table 2

514 Mean \pm SE for coloration of three great tit populations for different sex and age classes
 515 (feather structure data from the central and northern populations from Broggi et al. (2011)).
 516 Predicted values for each color component are derived from linear models standardized by
 517 the number of feathers used in the measurements. Numbers in brackets indicate sample
 518 sizes and different superscript letters represent statistically significant differences ($P < 0.05$),
 519 obtained from pairwise comparisons for feather coloration components.

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	Southern	Central	Northern
Chroma (%)			
Adult males	22.1 \pm 1.52 (6)	23.6 \pm 1.59 (6)	25.9 \pm 1.59 (6)
Adult females	19.1 \pm 1.58 (5)	20.6 \pm 1.71 (4)	22.8 \pm 1.59 (4)
Juvenile males	21.2 \pm 1.68 (6)	22.7 (1)	25.0 \pm 1.42 (5)
Juvenile females	18.1 \pm 1.59 (7)	19.7 (1)	21.9 \pm 1.31 (9)
Total	20.1 \pm 1.31 ^a	21.7 \pm 1.53 ^a	23.9 \pm 1.15 ^a
Hue (°)			
Adult males	94.1 \pm 0.48	95.0 \pm 0.50	94.3 \pm 0.48
Adult females	93.8 \pm 0.50	94.8 \pm 0.54	94.0 \pm 0.50
Juvenile males	94.1 \pm 0.53	95.0	94.3 \pm 0.45
Juvenile females	93.8 \pm 0.50	94.8	94.0 \pm 0.41
Total	94.0 \pm 0.41 ^a	94.9 \pm 0.48 ^a	94.1 \pm 0.36 ^a
Lightness (%)			
Adult males	61.5 \pm 1.43	58.2 \pm 1.50	56.6 \pm 1.43
Adult females	59.7 \pm 1.49	56.4 \pm 1.76	54.7 \pm 1.23
Juvenile males	62.3 \pm 1.58	59.0	57.4 \pm 1.33
Juvenile females	59.7 \pm 1.49	55.6	53.9 \pm 1.49

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3	Total	60.6 ± 1.23^a	$57.3 \pm 1.43^{a,b}$	55.6 ± 1.08^b
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5	UV (%)			
6				
7	Adult males	21.6 ± 1.04	18.1 ± 1.09	16.3 ± 1.04
8				
9	Adult females	19.8 ± 1.08	16.3 ± 1.17	14.5 ± 1.08
10				
11	Juvenile males	22.4 ± 1.15	18.9	17.1 ± 0.97
12				
13	Juvenile females	20.6 ± 1.09	17.1	15.3 ± 0.90
14				
15	Total	21.1 ± 0.90^a	$17.6 \pm 1.04^{a,b}$	15.8 ± 0.78^b
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For Peer Review

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4 522 **Table 3** Results from the linear models explaining the variation in feather structure and in
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6 523 each color component of great tit contour feathers that is accounted for by population of
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8 524 origin, sex and age. For color components, feather structure and number of feathers (to
9
10 525 account for the variation induced by using different number of feathers for color
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12 526 measurements) were also included as independent factors. Significant effects are shown in
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14 527 bold.

	R ²	df	β	SE	F	P
<hr/>						
Feather structure	0.54					
Population		2, 55			28.12	<0.001
Sex		1, 55			0.00	0.995
Age		1, 55			0.09	0.772
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Chroma	0.49					
Population		2, 53			1.66	0.201
Sex		1, 53			6.23	0.016
Age		1, 53			0.53	0.466
Number of feathers		1, 53	1.38	0.27	26.02	<0.001
Feather structure		1, 53	1.05	0.90	1.36	0.249
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Hue	0.48					
Population		2, 53			0.13	0.882
Sex		1, 53			0.56	0.451
Age		1, 53			0.01	0.994
Number of feathers		1, 53	-0.28	0.83	11.28	<0.001
Feather structure		1, 53	-0.59	0.28	4.47	0.039
<hr/>						
Brightness	0.65					
Population		2, 53			4.16	0.021
Sex		1, 53			5.22	0.026

Age	1,53			0.37	0.547
Number of feathers	1,53	0.95	0.25	14.08	<0.001
Feather structure	1,53	1.20	0.84	2.04	0.159
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UV	0.55				
Population	2,53			7.49	0.001
Sex	1,53			4.64	0.036
Age	1,53			0.78	0.382
Number of feathers	1,53	0.27	0.19	2.08	0.155
Feather structure	1,53	0.31	0.62	0.25	0.619

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5 530 **Fig. 1** Mean \pm SE of contour feather structure derived from the first factor of a principal
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7 531 component analysis (see text) for each great tit population.

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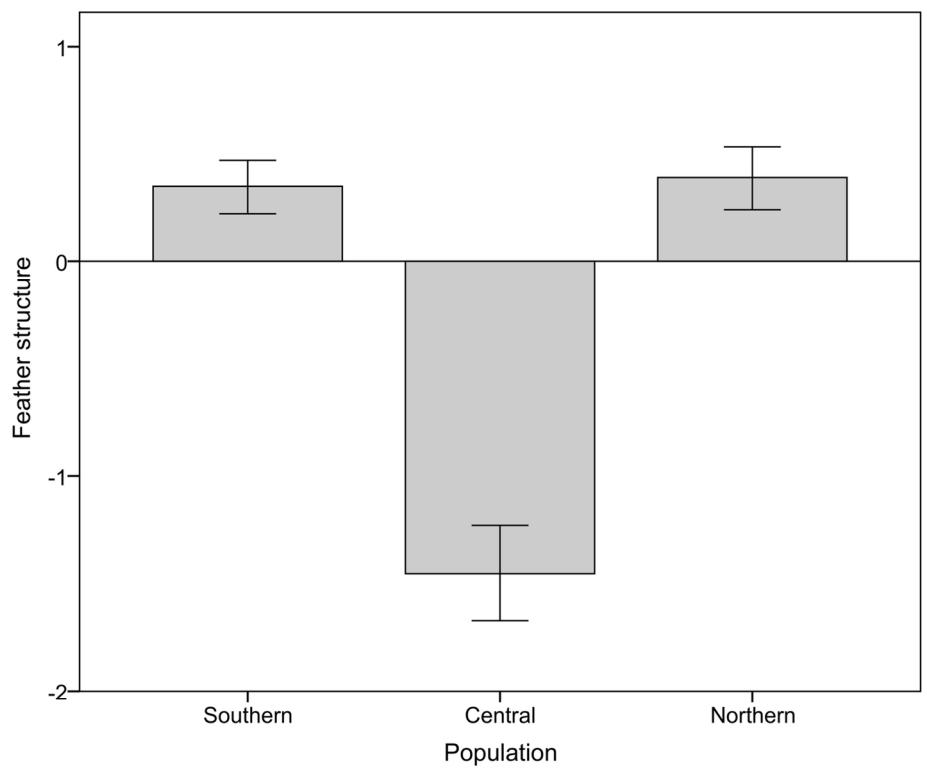
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12 533 **Fig. 2** Relationship between feather structure and hue of great tit contour feathers. Feather
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14 534 structure is derived from the first factor of a principal component analysis (see text) and hue
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16 535 is standardized by the number of feathers used in the measurements.

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21 537 **Fig. 3** Mean \pm SE of each color component of yellow contour feathers for each great tit
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23 538 population, standardized by the number of feathers used in the measurements. * denotes $P <$
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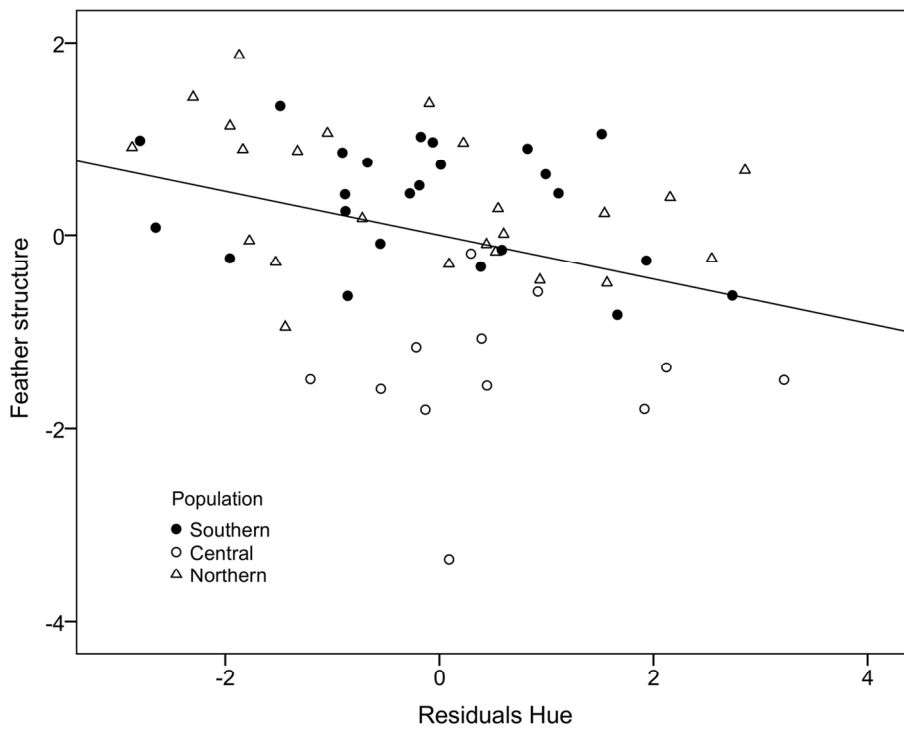
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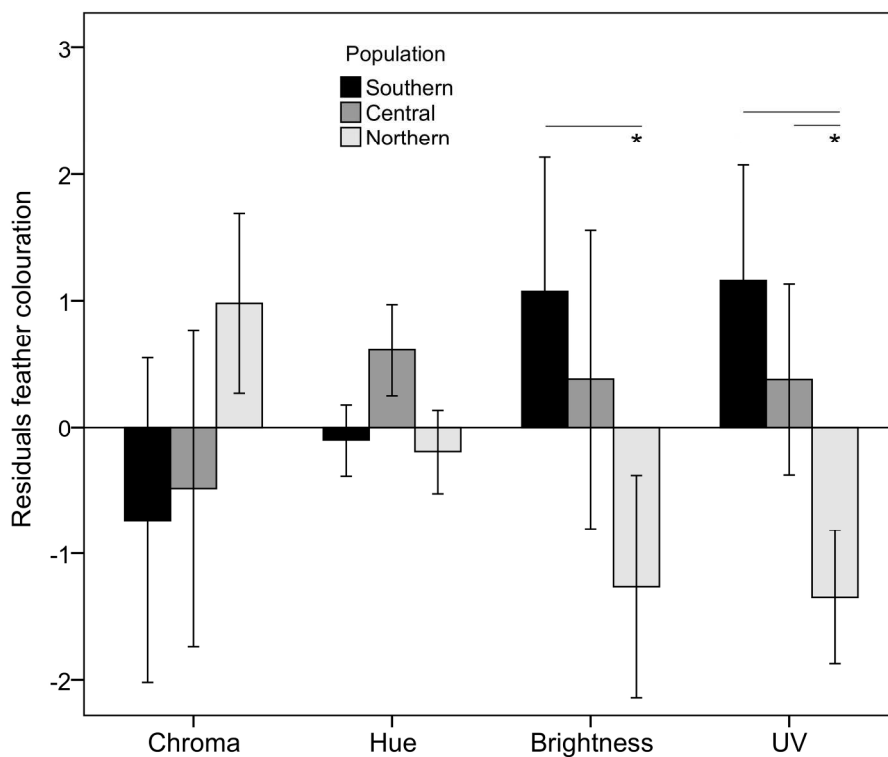
70x58mm (600 x 600 DPI)

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68x56mm (600 x 600 DPI)



112x97mm (600 x 600 DPI)

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