

1 Rufous Common Cuckoo chicks are not always female

2 Jaroslav Koleček (<https://orcid.org/0000-0003-1069-6593>),^{1*} Michal Šulc,¹ Radka Piálková,^{1,2} Jolyon
3 Troscianko,³ Milica Požgayová,¹ Marcel Honza¹, Petr Procházka¹ (<https://orcid.org/0000-0001-9385-4547>)
4

5 ¹ The Czech Academy of Sciences, Institute of Vertebrate Biology, Květná 8, CZ-603 65 Brno, Czech
6 Republic

7 ² Faculty of Natural Sciences, University of South Bohemia, Branišovská 31, CZ-370 05 České
8 Budějovice, Czech Republic

9 ³ Centre for Ecology & Conservation, College of Life & Environmental Sciences, University of Exeter,
10 Penryn Campus, Penryn, Cornwall, TR10 9FE, UK

11

12 *Corresponding author.

13 Email: j.kolecek@gmail.com

14 Phone: +420 605 552 978

15

16 Abstract

17 The Common Cuckoo shows two adult plumage morphs – adult male plumage is grey and adult
18 females are either grey or, less frequently, rufous. The situation is less clear in juveniles, as both
19 sexes exhibit variable proportions of grey and rufous colour. We thus describe the patterns related to
20 sex-specific plumage colour variation in a central European Cuckoo population. We genetically
21 determined sex of 91 Cuckoo chicks and using visual classification of photographs we scored juvenile
22 plumage colouration of individual chicks into five classes based upon the increasing proportion of
23 feathers with rufous colour. To verify these scores, we sampled chick feathers and quantified the
24 proportion of rufous colour on individual feathers by digital image analysis. We found that juvenile
25 females had a higher proportion of rufous colour on feathers than juvenile males. However, the
26 difference was marginally non-significant based on visual inspection alone, and some male chicks
27 even showed intensively rufous plumage like those of juvenile females. In contrast, we captured only
28 grey adult males (N = 37) while 5 out of 20 adult females were rufous. The rufous colour of Cuckoo
29 feathers considerably differed from the grey colour and the difference was larger in adults than in
30 juveniles. We show that chicks, unlike adult females, cannot be visually assigned to either of the
31 adult morphs. Therefore, we encourage further investigation of Cuckoo plumage colouration across
32 the species range and to examine the process of plumage maturation. A detailed genetic analysis is
33 necessary to understand the origin of Cuckoo feather colouration.

34 **Keywords:** avian vision; colour dimorphism; *Cuculus canorus*; molecular analysis; plumage
35 colouration; spectral reflectance

36

37 Introduction

38 Colour synthesis is a very complex process that can be affected both genetically and environmentally
39 (Galván and Solano 2016) and that may result in the coexistence of two (i.e. dimorphism, Bond 2007)
40 or more distinct colour forms in one interbreeding animal population (Huxley 1955). This
41 phenomenon, known as intraspecific colour polymorphism, has been observed across many
42 vertebrate species (Galeotti et al. 2003; Gray and McKinnon 2007) and has provided key models for
43 studies in evolutionary biology including sexual selection, speciation, and mimicry (McKinnon and
44 Pierotti 2010). In birds, the most common plumage pigment, melanin, occurs in two forms –
45 eumelanin and pheomelanin (Hill and McGraw 2006). The proportion of eumelanin and pheomelanin
46 determines bird plumage colouration – lighter (yellow to red) phenotypes originate from the

47 increased deposition of pheomelanin, while darker ones (brown to black) result from the increased
48 deposition of eumelanin (McGraw et al. 2005; Hill and McGraw 2006).

49 Excluding sexual dichromatism, seasonal change and age differences, about 334 (3.5%) of all world
50 bird species exhibit plumage colour polymorphism, including 241 species with only two colour
51 plumage morphs (Galeotti et al. 2003). The colour polymorphism occurs most frequently in
52 Strigiformes (owls and nightjars – 33.5% species), Cuculiformes (11.9%) and Galliformes (9.5%,
53 Galeotti et al. 2003). Colour polymorphism has been mainly explored in adult birds, but far less
54 attention has been paid to such a variation in juveniles. In some species, plumage morphs do not
55 change with age and moults (e.g. Ruff *Philomachus pugnax* and Buzzard *Buteo buteo*; Lank et al.
56 1995; Kappers et al. 2017) whereas in others, they differ between juveniles and adults, such as in
57 many raptors (Ferguson-Lees and Christie 2001; Roulin 2004) or cuckoos (Voipio 1953; Cramp 1985).

58 Obligatory parasitic Common Cuckoos *Cuculus canorus* (hereafter Cuckoos) exhibit two typical colour
59 morphs. Adult males are grey, and females either resemble the males (some with rufous tinge on the
60 upper breast and neck, Noh et al. 2016), or occur in a rufous morph (Voipio 1953; Payne 1967).
61 Female polymorphism is also known in other parasitic cuckoos, but is absent in non-parasitic ones
62 (Payne 1967). In coevolutionary arms races between cuckoos and their hosts, variable plumage
63 colour may have evolved as a result of apostatic selection (Payne 1967) or as a counteradaptation to
64 resemble avian predators – unknown or predator-like plumage can reduce the chance of being
65 recognized and attacked by the hosts (Davies and Welbergen 2008; Welbergen and Davies 2011).
66 Alternatively, the females may simply keep their plumage to adulthood as pedomorphic retention
67 (neoteny, Trnka et al. 2015). Nevertheless, the proportions of morphs differ substantially between
68 sites; from those where the rufous females are almost entirely absent (Thorogood and Davies 2012)
69 to sites where they are common (Honza et al. 2006; Table 1).

70 Cuckoo fledglings exhibit far more varied combinations of grey and rufous colour than adults, to the
71 point where the border between the morphs is hardly detectable (Cramp 1985). So far, only Voipio
72 (1953) attempted to evaluate the plumage polymorphism in juvenile Cuckoos; however his study was
73 restricted to a relatively small number of museum specimens. Contrary to Cramp (1985), Voipio
74 (1953) distinguished three juvenile plumage types – grey-brown and rufous type for both sexes and
75 red phase which should occur in females only. However, his material did not contain any specimen of
76 such a red phase and the variability in the fledgling colouration has never been classified in detail.
77 Moreover, it remains unknown whether the fledglings resembling the adult rufous morph are always
78 females.

79 Here, we describe variation in the proportion of grey and rufous plumage colour in a central
80 European Cuckoo population. We used both subjective assessments and objective digital imaging for
81 colour categorisation, and also compare the plumage colouration between males and females in
82 juveniles and adults. In light of the prior research mentioned above, we predict that the rufous
83 colouration will be more frequent in females than in males. Finally, we also discuss a possible genetic
84 background of Cuckoo plumage colouration.

85

86 **Methods**

87 **Fieldwork**

88 The study was carried out from April to July 2016 and 2017 (with some adults recaptured in 2018) in
89 the fishpond area between Mutěnice (48°54'N, 17°02'E) and Lužice (48°50'N, 17°04'E) in south-
90 eastern part of the Czech Republic. To find active nests with Cuckoo chicks, we systematically
91 searched littoral vegetation with numerous territories of Eurasian Reed Warblers *Acrocephalus*
92 *scirpaceus*, Great Reed Warblers *Acrocephalus arundinaceus* and Sedge Warblers *Acrocephalus*

93 *schoenobaenus*, and also occasionally checked the adjacent sites preferred by Marsh Warblers
94 *Acrocephalus palustris*. In April and May of the same years, we also mist-netted 57 adult Cuckoos
95 from the same study site.

96 Sex determination

97 When the chicks were at least 6 days old, a small amount of blood (5–25 μ l) was collected by tarsal
98 venipuncture and stored in 100 μ l of 96% ethanol. Chick sex was later determined in the laboratory
99 by amplifying a part of the W-linked chromo-helicase-DNA binding gene CHD-W (unique to females)
100 and a part of its homologue, the CHD-Z gene, linked to the Z chromosome (occurring in both sexes,
101 Griffiths et al. 1998). After DNA extraction, the avian sex primers P2 and P8 (Griffiths et al. 1998)
102 were used in 10 μ l PCR reactions (for details of PCR conditions see Abraham et al. 2015). The PCR
103 products were separated by electrophoresis for 45–60 min at 7–10 V/cm using 3% agarose gels
104 stained with SYBR[®] Safe (Life Technologies, Invitrogen, Carlsbad, CA). Heterogametic females were
105 characterised by a two-band profile (~350 and ~400 bp), while homogametic males by only a single
106 band (~400 bp). The sex of adults was determined genetically following the same procedure as in
107 juveniles.

108 Classification of plumage colouration

109 To describe and categorize plumage colouration, we photographed 77 out of the 91 Cuckoo chicks (N
110 = 43 originating from the nests of the Eurasian Reed Warbler, N = 32 from Great Reed Warblers and 1
111 each from Marsh and Sedge Warblers) at the age between 9 and 18 days (median = 14). We
112 distinguished five plumage colour classes based upon the proportion of rufous colour present on
113 feathers on a Cuckoo back and upon the shape and size of rufous spots and stripes on individual
114 feathers on the following semi-continuous ordinal scale: 1) Grey – rufous colour missing or present
115 on <5% extent of contour feathers on less than one half of the back, individual rufous spots smudgy
116 and disjunct. 2) Medium grey – rufous colour on 5–10% extent of contour feathers on the major part
117 of the back. Individual rufous spots thin, their length exceeds the width. The spots usually do not
118 form stripes crossing shafts of the feathers. 3) Intermediate – similar to 2, but the disjunct rufous
119 spots are thicker, their length usually equals width. The spots are present on 11–20% extent of
120 almost all back feathers. Sometimes, the spots may cross the whole feather, but in such case their
121 width is similar to 2. 4) Medium rufous – rufous on 21–40% extent of all back feathers, rufous stripes
122 lead across the feather and their width is similar to 3. Some stripes may be interrupted, but the gap
123 thinner than one third of stripe length. 5) Rufous – rufous on >40% extent of all back feathers,
124 uninterrupted stripes cross the feathers and their width is larger than in 3 and 4 (see Electronic
125 Supplementary Material, Fig. S1 for sample photographs representing individual classes). These
126 classes did not coincide with the plumage morphs in Voipio (1953; i.e. grey-brown, rufous and red)
127 and did not depend on age when the chicks were photographed (linear regression: slope = 0.066 \pm
128 0.068 SE, $F_{1,75} = 0.94$, $P = 0.334$).

129 Based on this five-degree classification, one image of each chick was independently assigned to one
130 plumage colour class by the authors (except for J. T.) and Vojtěch Brlík, Radka Poláková, Peter Samaš
131 and Kateřina Sosnovcová (Electronic Supplementary Material, Table S1), who all have field
132 experience with Cuckoo chicks. Each of the ten judges was blind to the nestling sex. Using R (R Core
133 Team 2016) we calculated interclass correlation coefficient (ICC; package 'irr', Gamer et al. 2012) for
134 testing similarity in scores given by individual judges and thus the reliability of the classification. The
135 scores given by individual judges to each chick were highly consistent (ICC = 0.883, $F_{76,693} = 76.6$, $P \ll$
136 0.001, Electronic Supplementary Material, Table S1). Finally, we averaged the obtained plumage
137 colour scores.

138 We distinguished between grey and rufous colour morph in the captured adult Cuckoos. The grey
139 morph is typical of predominantly grey upper parts without contrasting rufous colouration

140 (Electronic Supplementary Material, Fig. S2). The rufous morph resembles the (v) rufous juvenile
141 plumage colour class – i.e. individuals have marked rufous stripes on the upper part of their body
142 (Voipio 1953; Electronic Supplementary Material, Fig. S2). As the differences between the adult
143 morphs are clear-cut, we did not apply any detailed evaluation of feathers or images of plumage
144 colouration.

145 Analysis of feather colouration

146 Because the human classification of chick plumage coloration may be subjective, we also performed
147 a digital analysis of feather colouration and correlated the results obtained from both approaches.
148 We collected feathers from the upper back belonging to 87 out of the 91 Cuckoo chicks (N = 50 chicks
149 found in the nests of Eurasian Reed Warblers, 35 in Great Reed Warblers and 1 each in Marsh and
150 Sedge Warblers) at ages of between 11 and 23 days (median = 14). Feathers were scanned using a
151 flatbed scanner (HP Color LaserJet Pro MFP M176n, resolution: 14028 × 10200; 1200 dpi). Because
152 we expected a certain degree of variability in feather colouration, we always scanned up to six
153 feathers from the same individual, performed further analyses for each feather separately and then
154 averaged the measurements to obtain single values for each individual. Feathers were fastened by a
155 strip of adhesive tape to a white sheet of paper and scanned with a scale. Colour measurements of
156 the feathers were made using ImageJ version 1.51k (ImageJ 2018), by selecting four regions of
157 interest: dark grey feather sections, rufous feather sections, light grey down feather sections and the
158 white background of paper. In total, the mean RGB values of 442 feather sections were measured to
159 create a model training database. These values were then used to create a neural-net multinomial
160 logistic regression model in R (version 3.4.2), using the ‘nnet’ package (version 7.3–12, Venables and
161 Ripley 2002), where feather section types (dark grey, rufous, light grey or background) were the
162 dependent and the RGB values the independent variables. This model was able to classify 99.77% of
163 the 442 training measurements correctly. The resulting model was converted into a JAVA ImageJ
164 plugin script, which transformed all scanned feather images into the four categories based on pixel
165 colour and reported the number of pixels in each group. The proportion of rufous colour on each
166 feather was calculated as number of pixels denoting rufous colour divided by number of pixels
167 denoting rufous and dark grey colour of the feather (see Electronic Supplementary Material, Fig. S3).
168 This proportion did not depend on the age of the chicks (linear regression: slope = -0.763 ± 0.801 SE,
169 $F_{1,85} = 0.91$, $P = 0.343$).

170 In addition, we measured spectral reflectance (300 to 700 nm) of the feathers from eight chicks (two
171 females, six males) and two adult rufous females. We collected three feathers from the upper back of
172 each individual. The feathers from each individual were stacked upon each other and attached to a
173 black sheet of paper with adhesive tape. We used a reflectance spectrophotometer (USB 2000,
174 Ocean Optics, Dunedin, FL), a deuterium and halogen light source (DT-Mini-GS, Ocean Optics), and a
175 quartz optic fibre (QR400-7-UV/VIS-BX, Ocean Optics). The measurements were relative to a
176 standard white reference (WS-1, Ocean Optics) and to darkness. Reference and dark calibration were
177 made prior to the measurement. MŠ performed all measurements under standard light conditions, at
178 the same angle (90°) and same distance from the feather sample. The reflectance of both grey and
179 rufous colours was measured in the central part of the feather (Electronic Supplementary Material,
180 Fig. S3). We performed and averaged three measurements of the grey part and other three measures
181 of the rufous part of the feather sample per each individual. The average values of the grey and
182 rufous from each individual were then averaged to obtain the overall mean reflectance spectra for
183 each colour in juveniles and adults separately.

184

185 Results

186 Ranges of mean plumage colour scores were similar between juvenile Cuckoo males (mean score =
187 2.29 ± 0.16 SE, range: 1.0–4.5) and females (2.82 ± 0.20 SE, 1.0–5.0; Electronic Supplementary
188 Material, Table S1), both scoring around the middle of the scale. Females were marginally non-
189 significantly more rufous than males (Mann-Whitney-Wilcoxon $W = 917$, $P = 0.068$; Fig. 1a). This
190 human classification of chick plumage colouration tightly positively correlated with the proportion of
191 rufous colour measured from individual feathers using image analysis ($r_s = 0.87$, $P < 0.001$; $n = 73$).
192 The proportion was significantly higher in juvenile females (0.7–46.0%, mean = $16.81\% \pm 2.00$ SE, $n =$
193 38) than in juvenile males (0.5–38.2%, mean = $11.07\% \pm 1.42$ SE, $n = 49$; Mann-Whitney-Wilcoxon W
194 = 1197, $P = 0.023$; Fig. 1b).

195 Out of 57 captured adult Cuckoos, all 37 males belonged to the grey morph, while 15 out of 20
196 females were grey and 5 rufous. The rufous colour of Cuckoo back feathers considerably differed
197 from the grey colour (Fig. 2) and the difference seemed to be larger in adults than in juveniles (Fig.
198 2).

199

200 Discussion

201 Our study presents detailed data on colour variation of Cuckoo chicks and provides a simple human
202 visual classification tool that may help to categorize plumage colouration of Cuckoo chicks without
203 using image analysis. Both the digital image analysis as well as visual classification revealed that most
204 Cuckoo chicks exhibited grey or intermediate plumage colouration, but the two distinct colour
205 morphs present in Cuckoo adults are hardly distinguishable in chicks. Regardless of the method used,
206 female chicks were generally more rufous than males. Surprisingly, the intersexual differences in
207 plumage colouration and in the proportion of rufous colour on feathers were quite small in chicks
208 and some males had rather intensely rufous plumage. Yet, such male colouration was not observed
209 in adult Cuckoos where the rufous plumage was recorded only in about a quarter of females mist-
210 netted in our study area, which is similar to the proportion of female chicks belonging to the medium
211 rufous and rufous plumage colour classes.

212 We developed a five-degree classification which covers the large variability of chick plumage
213 colouration. Unlike Voipio (1953), who first attempted to explain patterns in Cuckoo plumage
214 colouration, we doubt that it is possible to reliably visually distinguish any colour plumage morphs in
215 Cuckoo chicks. Instead, individual chicks that we examined exhibited a continuous variability of
216 rufous colour in individual feathers as well as in their plumage (see also Cramp 1985). Hypothetically,
217 the two ‘most rufous’ chick plumage colour classes 4 and 5 might roughly correspond with the rufous
218 morph in adult females (e.g. due to prevailing uninterrupted rufous stripes). In which case, around
219 25% of female chicks would be rufous, which corresponds with the proportion of adult rufous
220 females in the study population. In contrast, 15% of juvenile males and no adult males would be
221 rufous. Thus while we may possibly see a link between the colouration of juvenile and adult females,
222 the process of male plumage maturation is probably different.

223 Despite Voipio’s (1953) statement that the plumage of juvenile males should not be intensely rufous,
224 together with ‘textbook’ images claiming that rufous Cuckoo chicks are females (but see Mann 2014),
225 the male chicks which we investigated fell into all plumage colour classes, and some thereby
226 resembled even the most rufous females. This striking finding could suggest that some male chicks
227 may occasionally retain rufous colour until adulthood (Ringleben 1958; Becker 1989; Busche 2003).
228 Although the proportion of adult rufous females in our study population agrees with their
229 distribution across Europe (see Table 1 for summary), we did not record any adult rufous males. In
230 Germany, adult rufous Cuckoo males should represent about 1% of rufous Cuckoos (Busche 2003)
231 but the mechanisms underlying the origin of this male ‘morph’ remain unknown and may differ from
232 those in females. We may only speculate that such males could perhaps suffer from moult disorder

233 or eumelanin production dysfunction. Moreover, previous evidence comes only from visual
234 observations where the sex was not genetically determined. Therefore, it is possible that even some
235 (rufous) females may rarely exhibit male calls and thus may resemble males (Odom et al. 2014).
236 Evidence will probably remain sporadic, because the most reliable approach – ringing projects
237 targeting the Cuckoo – are scarce and commonly used bird monitoring schemes do not take the
238 Cuckoo morphs into account (e.g. Gregory et al. 2007). Consequently, most observers presumably do
239 not pay much attention to the colour and sex of individual Cuckoos.

240 In birds, plumage colour morphs are known to be genetically determined (Galeotti et al. 2003) and
241 mostly follow Mendelian segregation of several alleles at a limited number of loci (Sinervo and
242 Zamudio 2001; Roulin 2004; McKinnon and Pierotti 2010; Wellenreuther et al. 2014). Plumage
243 polymorphism may also be governed by few genes of major effect (e.g. a supergene sensu Küpper et
244 al. 2016). Cuckoo colour morphs have been known for a long time (Voipio 1953), but the underlying
245 genetic mechanisms remain unknown. A high proportion of the juvenile Cuckoos within the
246 intermediate plumage colour class (possibly heterozygous) as well as a lower proportion of grey and
247 namely rufous Cuckoos (possibly homozygous) may suggest that Cuckoo plumage colouration follows
248 Mendelian inheritance as well (see also Voipio 1953). However, the differences in colouration
249 between most juvenile males and females are weak. Thus we cannot rule out the possibility that
250 interactions among loci or between genes (e.g. non-allelic genes, duplicate genes with cumulative
251 effects with dominance or epistatic genes – recessive or dominant epistasis) or even more complex
252 mechanisms contribute the variation in plumage colouration (Sinervo and Svensson 2002; Carlborg
253 and Haley 2004; Phillips 2008; Rankin et al. 2016). Rufous colouration of adult females and juveniles
254 may further indicate that sex-specific endocrine cascades during development or frequency-
255 dependent selection instead of the complex genetic interactions could determine the sexual
256 differences (Rankin et al. 2016).

257 We have only anecdotal evidence about the plumage colouration in different stages of Cuckoo life –
258 a rufous female chick found at the study site in 2016 (plumage colour score = 5.0) was retrapped
259 there in spring as a rufous adult female in 2017 and also stayed rufous in 2018, when retrapped there
260 again. Similarly, a male recaptured at the study site in 2018 was a grey chick (score = 1.9) ringed
261 there in 2017 (Electronic Supplementary Material, Fig. S2). A possible link between the juvenile and
262 adult Cuckoo colouration was introduced by Trnka et al. (2015) who suggested that the occurrence of
263 the rufous female morph in adults may be caused by neoteny (paedomorphosis), i.e. by the retention
264 of juvenile colouration in the adulthood (Gould 1977; McKinney and McNamara 1991). Such
265 heterochronic plumage ontogeny, in which adult individuals retain juvenile like plumage, has been
266 observed in other bird species (Foster 1987; Berggren et al. 2004). However, individual development
267 of Cuckoo plumage remains unknown.

268 A potential explanation for the evolution of rufous plumage colouration in juvenile Cuckoos concerns
269 the relation between camouflage and the risk of predation (see Wauters et al. 2004). This theory is
270 supported by evidence that, individuals of bird species with a greater proportion of rufous plumage
271 colour have higher relative annual survival rates (Galván et al. 2012; Galván and Møller 2013) except
272 under adverse conditions (Karell et al. 2011). Unfortunately, we do not have enough data to test
273 these assumptions and they remain a perspective for future research.

274 The Cuckoo is well-suited for the study of plumage colouration. Our classification as well as the
275 image analysis showed that juvenile colouration of both sexes was similar. Namely, not only juvenile
276 females, but also juvenile males may exhibit rufous colouration, which, in contrast to adult females,
277 was not observed in adult males. However, the process of colour change during ontogeny, as well as
278 its underlying molecular mechanisms, remains unknown. We thus encourage investigating Cuckoo
279 plumage colouration across the species range in order to compare the patterns and processes of
280 phenotype and genotype variation among populations. Future studies should also test for
281 correlations with behavioural, physiological and fitness traits and explore changes in plumage

282 colouration in particular regions over time. Such research may elucidate whether juvenile and adult
283 colouration differs in response to factors that determine the changes in population size (Roulin
284 2004), and would provide valuable insights into the evolutionary history of colour dimorphism in
285 avian brood parasites, and in animals in general.

286

287 **Acknowledgements**

288 We would like to thank Miroslav Čapek, Václav Jelínek, Lukáš Kulísek, Boris Prudík, Gabriela Štětková
289 and Klára Žabková for help with fieldwork, Vojtěch Brlík, Radka Poláková, Peter Samaš and Kateřina
290 Sosnovcová for assistance in the field as well as for scoring the Cuckoo chicks and Marie Kotasová
291 Adámková for helpful advice on the measurements of spectral reflectance. We are also grateful to
292 two anonymous reviewers for their valuable comments, Petr Suvorov and Veronika Štočková for
293 their support and to the managers of the Hodonín Fish Farm for the permission to conduct the
294 fieldwork on their grounds. This study was supported by the Czech Science Foundation (project 17-
295 12262S) and by the Institutional Research Plan (RVO: 68081766).

296

297 **Compliance with ethical standards**

298 All applicable international, national, and institutional guidelines for the care and use of animals
299 were followed (Czech permit MUHOCJ 34437/2014 OŽP). The authors declare that they have no
300 competing interests.

301

302 **References**

303 Abraham M, Požgayová M, Procházka P, Piálková R, Honza, M (2015) Is there a sex-specific difference
304 between parasitic chicks in begging behaviour? *J Ethol* 33:151–158

305 Arbeitskreis Göttinger Ornithologen (2007) Der Kuckuck in Süd-Niedersachsen – ein Schlawiner mit
306 Problemen. <http://www.ornithologie-goettingen.de/?p=137> (accessed 12 April 2018)

307 Becker L, Dankhoff S (1973) Der Kuckuck (*Cuculus canorus canorus* L.) in der Oberlausitz.
308 *Abhandlungen und Berichte des Naturkundemuseums Görlitz* 48: XIII/1–XIII/9

309 Becker P (1989) Ein rotbraunes Männchen des Kuckucks (*Cuculus canorus*) bei Gronau/Leine.
310 *Beiträge zur Naturkunde Niedersachsens* 42:158

311 Berggren Å, Armstrong DP, Lewis RM (2004) Delayed plumage maturation increases overwinter
312 survival in North Island robins. *Proc R Soc B* 271:2123–2130

313 Berndt RK, Koop B, Struwe-Juhl B (2003) *Vogelwelt Schleswig-Holsteins*, Bd. 5, Brutvogelatlas. 2.
314 Auflage. Wachholtz, Neumünster

315 Bond AB (2007) The evolution of color polymorphism: crypticity, searching images, and apostatic
316 selection. *Ann Rev Ecol Evol Syst* 38:489–514

317 Bračko F (2017) The occurrence of rufous-morph Cuckoo *Cuculus canorus* females in NE Slovenia.
318 *Acrocephalus* 37:61–62

319 Brichetti P, Fracasso G (2006) *Ornitologica italiana*. Identificazione, distribuzione, consistenza e
320 movimenti degli uccelli italiani. Bd. 3. Bologna: Perdisa

- 321 Busche G (2003) Zur Häufigkeit der braunen Färbungsvariante des Kuckucks *Cuculus canorus* in
322 Deutschland. Ornithol Mitt 55:127–131
- 323 Carlborg Ö, Haley CS (2004) Epistasis: too often neglected in complex trait studies? Nat Rev Genet
324 5:618–625
- 325 Cramp S (1985) The birds of the Western Palearctic. Vol. IV. Oxford University Press, Oxford
- 326 Davies NB, Welbergen JA (2008) Cuckoo-hawk mimicry? An experimental test. Proc R Soc B
327 275:1817–1822
- 328 Dittrich W (1982) About the abundance of the Cuckoo (*Cuculus canorus*) in northern Bavaria and the
329 recorded proportion of its hepatic colour phase. Vogelwelt 103:188–189
- 330 Ferguson-Lees J, Christie D (2001) Raptors of the World. Christopher Helm, London
- 331 Foster MS (1987) Delayed maturation, neoteny, and social system differences in two manakins of the
332 genus *Chiroxiphia*. Evolution 41:547–558
- 333 Galeotti P, Rubolini D, Dunn PO, Fasola M (2003) Colour polymorphism in birds: causes and functions.
334 J Evol Biol 16:635–646
- 335 Galván I, Ghanem I, Møller AP (2012) Has removal of excess cysteine led to the evolution of
336 pheomelanin? BioEssay 34:565–568
- 337 Galván I, Møller AP (2013) Pheomelanin-based plumage coloration predicts survival rates in birds.
338 Physiol Biochem Zool 86:184–192
- 339 Galván I, Solano F (2016) Bird integumentary melanins: biosynthesis, forms, function and evolution.
340 Int J Mol Sci 17:520
- 341 Gamer M, Lemon J, Singh IFP (2012) irr: Various Coefficients of Interrater Reliability and Agreement.
342 R package version 0.84
- 343 Gould SJ (1977) Ontogeny and Phylogeny. Harvard University Press, Cambridge
- 344 Gray SM, McKinnon JS (2007) Linking color polymorphism maintenance and speciation. Trends Ecol
345 Evol 22:71–79
- 346 Gregory RD, Vorisek P, Van Strien A, Meyling AWG, Jiguet F, Fornasari L, Reif J, Chylarecki P, Burfield
347 IJ (2007) Population trends of widespread woodland birds in Europe. Ibis 149:78–97
- 348 Griffiths R, Double MC, Orr K, Dawson RJG (1998) A DNA test to sex most birds. Mol Ecol 7:1071–
349 1075
- 350 Heidecke D (1981) Zum Auftreten der rotbraunen Varietät des Kuckucks. Ornithol Rundbr
351 Mecklenburg 24:6–8
- 352 Heyder R (1952) Die Vögel des Landes Sachsen. Akademische Verlag Gesellschaft, Leipzig
- 353 Hill GE, McGraw KJ (2006) Bird Coloration, vol 2. Function and Evolution. Harvard University Press,
354 Cambridge
- 355 Honza M, Šicha V, Procházka P, Ležalová R (2006) Host nest defense against a color-dimorphic brood
356 parasite: great reed warblers (*Acrocephalus arundinaceus*) versus common cuckoos (*Cuculus*
357 *canorus*). J Ornithol 147:629–637

- 358 Huxley J (1955) Morphism in birds. *Acta Int Congr Ornithol* XI:309–328
- 359 ImageJ (2018) *Image Processing and Analysis in Java*. <http://rsb.info.nih.gov/ij> (accessed 26 March
360 2018)
- 361 Kappers EF, Chakarov N, Krüger O, Mueller AK, Valcu M, Kempenaers B, Both C (2017) Classification
362 and temporal stability of plumage variation in Common Buzzards. *Ardea* 105:125–136
- 363 Karell P, Ahola K, Karstinen T, Valkama J, Brommer JE (2011) Climate change drives microevolution in
364 a wild bird. *Nat Commun* 2:208
- 365 Küpper C, Stocks M, Risse JE, dos Remedios N, Farrell LL, McRae SB, Morgan TC, Karlionova N,
366 Pinchuk P, Verkuil YI, Kitaysky A, Wingfield J, Piersma T, Zeng K, Slate J, Blaxter M, Lank D, Burke T
367 (2016) A supergene determines highly divergent male reproductive morphs in the ruff. *Nat Genet*
368 48:79
- 369 Lank DB, Smith CM, Hanotte O, Burke T, Cooke F (1995) Genetic polymorphism for alternative mating
370 behaviour in lekking male Ruff *Philomachus pugnax*. *Nature* 378:59–62
- 371 Lönnberg E (1929) *Svenska Fåglar*. Ivar Baarsen, Stockholm
- 372 Mann C (2014) Common and Oriental cuckoos: photo guide. *Birdwatch* 6/2014:45–51
- 373 Maumary L, Vallotton L, Knaus P (2007) *Die Vögel der Schweiz*. Schweizerische Vogelwarte and Nos
374 Oiseaux, Montmollin and Sempach
- 375 McGraw KJ, Safran RJ, Wakamatsu K (2005) How feather colour reflects its melanin content. *Funct*
376 *Ecol* 19:816–821
- 377 McKinney ML, McNamara K (1991) *Heterochrony: The Evolution of Ontogeny*. Plenum Press, New
378 York
- 379 McKinnon JS, Pierotti ME (2010) Colour polymorphism and correlated characters: genetic
380 mechanisms and evolution. *Mol Ecol* 19:5101–5125
- 381 Noh HJ, Lee JW, Yoo JC (2016) Color morph variation in two brood parasites: common cuckoo and
382 lesser cuckoo. *Ornithol Sci* 15:109–117
- 383 Odom KJ, Hall ML, Riebel K, Omland KE, Langmore NE (2014) Female song is widespread and
384 ancestral in songbirds. *Nat Commun* 5:3379
- 385 Payne RB (1967) Interspecific communication signals in parasitic birds. *Am Nat* 101:363–375
- 386 Phillips PC (2008) Epistasis – the essential role of gene interactions in the structure and evolution of
387 genetic systems. *Nat Rev Genet* 9:855–867
- 388 Quadrelli F (1990) Percentage of “hepatic” Cuckoos, *Cuculus canorus*, in the low Po Plain. *Riv Ital*
389 *Ornitol* 59:313
- 390 R Core Team (2016) R: a language and environment for statistical computing. Vienna: R Foundation
391 for Statistical Computing. <https://www.R-project.org/>
- 392 Rankin KJ, McLean CA, Kemp DJ, Stuart-Fox D (2016) The genetic basis of discrete and quantitative
393 colour variation in the polymorphic lizard, *Ctenophorus decresii*. *BMC Evol Biol* 16:179
- 394 Ringleben H (1958) Ein altes rostrottes Männchen des Kuckucks (*Cuculus canorus*). *J Ornithol* 99:375

- 395 Roulin A (2004) The evolution, maintenance and adaptive function of genetic colour polymorphism in
396 birds. *Biol Rev* 79:815–848
- 397 Sackl P (1985) Zum Auftreten der rotbraunen Farbphase beim Kuckuck, *Cuculus canorus* L., in der
398 mittleren Oststeiermark. *Mitt Abt Zool Landesmus Joanneum* 34:31–33
- 399 Schaanning HTL (1916) Norges Fuglefauna. Cappelen, Kristiania
- 400 Sinervo B, Svensson E (2002) Correlational selection and the evolution of genomic architecture.
401 *Heredity* 89:329–338
- 402 Sinervo B, Zamudio K (2001) The evolution of alternative reproductive strategies: fitness differential,
403 heritability and genetic correlation between the sexes. *J Hered* 92:198–205
- 404 Thorogood R, Davies NB (2012) Cuckoos combat socially transmitted defenses of reed warbler hosts
405 with a plumage polymorphism. *Science* 337:578–580
- 406 Tischler F (1941) Die Vögel Ostpreussens and seiner Nachbargebiete. I. Königsberg (Pr.) u, Berlin
- 407 Trnka A, Grim T (2013) Color plumage polymorphism and predator mimicry in brood parasites. *Front*
408 *Zool* 10:25
- 409 Trnka A, Trnka M, Grim T (2015) Do rufous common cuckoo females indeed mimic a predator? An
410 experimental test. *Biol J Linn Soc* 116:134–143
- 411 Venables WN, Ripley BD (2002) *Modern Applied Statistics with S*. Fourth Edition. Springer, New York
- 412 Voipio P (1953) The hepaticus variety and the juvenile plumage types of the Cuckoo. *Ornis Fennica*
413 30:97–117
- 414 Wauters LA, Zaninetti M, Tosi G, Bertolino S (2004) Is coat-colour polymorphism in Eurasian red
415 squirrels (*Sciurus vulgaris* L.) adaptive? *Mammalia* 68:37–48
- 416 Welbergen JA, Davies NB (2011) A parasite in wolf's clothing: hawk mimicry reduces mobbing of
417 cuckoos by hosts. *Behav Ecol* 22:574–579
- 418 Wellenreuther M, Svensson EI, Hansson B (2014) Sexual selection and genetic colour polymorphisms
419 in animals. *Mol Ecol* 23:5398–5414
- 420 Witherby HF, Jourdain FCR, Ticehurst NF, Tucker BW (1949) *The Handbook of British Birds*. HF & G.
421 Witherby, London

422 **Table 1** Proportions of adult rufous Common Cuckoo females based on published evidence. Asterisk denotes samples considering only females

423

424	Site	Coordinates	N	Rufous females (%)	Source
425	<i>Germany</i>				
426	East Prussia			relatively scarce	Tischler 1941
427	Göttingen	51°33'N 9°56'E		<30*	Arbeitskreis Göttinger Ornithologen 2007
428	Mecklenburg			<1	Heidecke 1981
429	Mindelsee	47°45'N 9°01'E		16.7	Löhrl 1979
430	Northern Bavaria		664	12 (4.7–23.5)	Dittrich 1982
431	Saxony			nowhere frequently	Heyder 1952
432	Sleswick-Holsatia			5–10	Berndt et al. 2003
433	Upper Lusatia		>300	<5	Becker and Dankhoff 1973
434	Entire country			<1–24	Busche 2003
435					
436	<i>United Kingdom</i>				
437	Wicken Fen	52°18'N, 0°17'E		<1*	Thorogood and Davies 2012
438	Entire British Isles			scarce	Whitherby et al. 1949
439					
440	<i>Other countries</i>				

441	Finland		8*	50	Voipio 1953
442	Italy			1.6–1.8	Brichetti and Fracasso 2006
443	Norway			rarer than grey	Schaanning 1916
444	Sweden			rarer than grey	Lönnberg 1929
445	Switzerland			scarce	Maumary et al. 2007
446					
447	<i>Other sites</i>				
448	Apaj (Hungary)	47°09'N 19°05'E	30*	60	Honza et al. 2006
449	Fürstenfeld (Austria)	47°03'N 16°05'E	27	11.1	Sackl 1985
450	Lužice (Czech Rep.)	48°50'N 17°04'E	30*	20	Honza et al. 2006, this study
451	North-east Slovenia		103	6.7	Bračko 2017
452	Po Plain (Italy)		115	1.77	Quadrelli 1990
453	Štúrovo (Slovakia)	47°51'N 18°36'E	8*	37.5	Trnka and Grim 2013

454 **Figure legends**

455 **Fig. 1** Proportion of (a) mean plumage colour scores in 41 male and 36 female Cuckoo chicks (1 –
456 grey, 2 – medium grey, 3 – intermediate, 4 – medium rufous, 5 – rufous) and (b) proportion of rufous
457 colour at individual feathers from the upper back of 49 male and 38 female Cuckoo chicks

458 **Fig. 2** Mean reflectance spectra of grey and rufous colour on back feathers of (a) 8 Common Cuckoo
459 chicks (2 females, 6 males) and (b) 2 adult rufous females. Bars denote standard errors of the mean

460

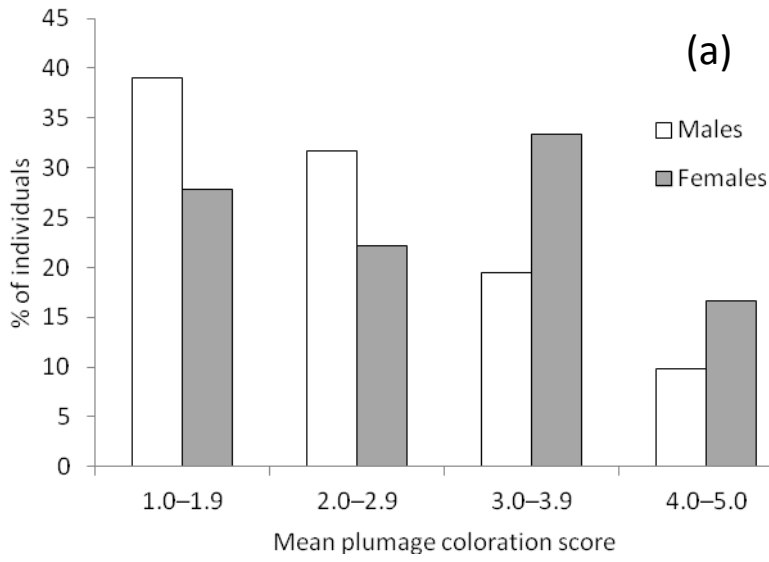
461

462

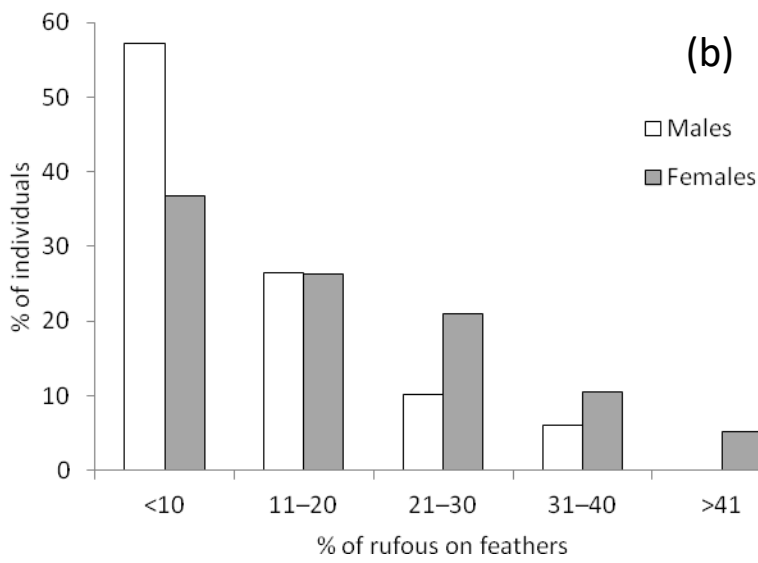
463

464

465

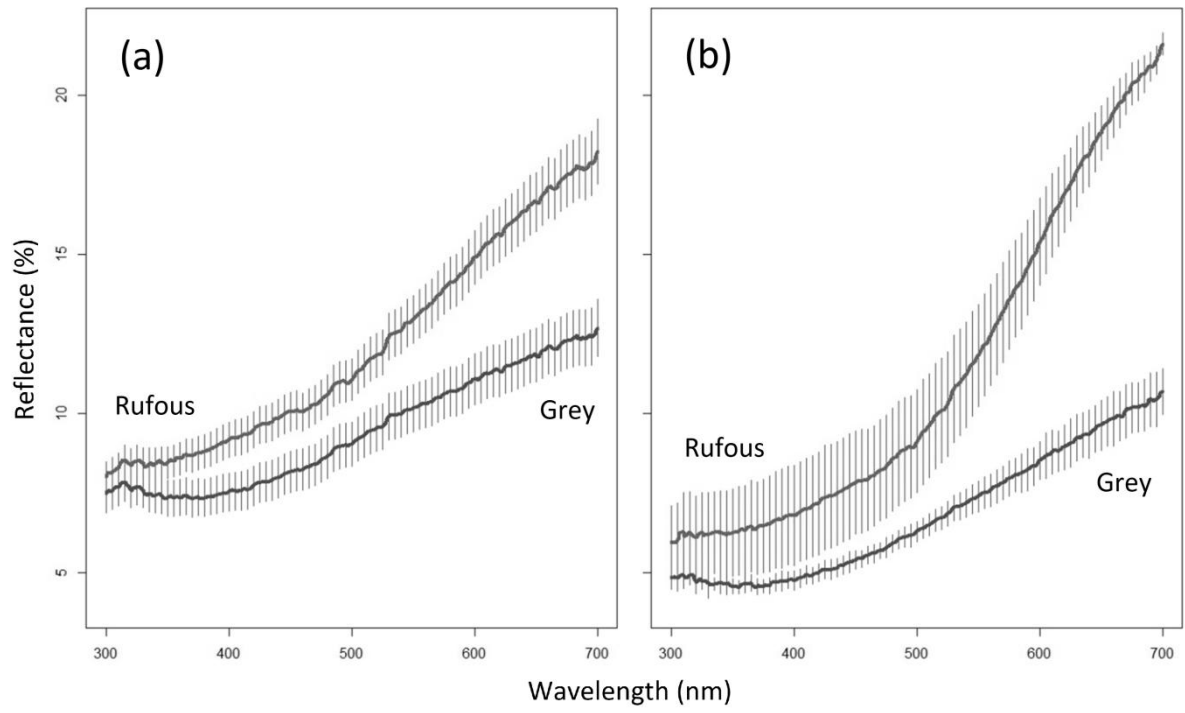


466



467

468 **Fig. 1**



469

470 **Fig. 2**