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1 **Pigment limitation and female reproductive characteristics influence egg shell**
2 **spottiness and ground colour variation in the house sparrow (*Passer domesticus*)**

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26 **Summary**

27 One of the hypotheses to explain egg colour variation in birds lays in the context
28 of sexual selection where egg colour may signal the female's physical condition and
29 antioxidant capacity. We tested one of the assumptions following from this hypothesis
30 that depositing eggshell pigments should be limited for females. The study was
31 conducted in a house sparrow (*Passer domesticus*) captive population, over several
32 years under constant environmental conditions. This multi brooded species lays eggs
33 which vary in ground colour (biliverdin pigment) and in the intensity and distribution of
34 brownish-red spots (protoporphyrin pigment). Spot darkness, spread and ground colour
35 diminished along the laying sequence, suggesting that the deposition of both pigments
36 was limiting for females at short term. Also the proportion of eggs with biliverdin
37 diminished in consecutive clutches laid by the same female over the breeding season,
38 suggesting a long term cost of biliverdin deposition. On the other hand, spots were
39 darker at the end of the breeding season, indicating that protoporphyrin deposition was
40 probably not limited at long term. This result could indicate a lower capacity of calcium
41 deposition at long term which was compensated for by darker spots. Also female age
42 affected significantly the proportion of bluish eggs and spot patterns. Egg pigmentation
43 decreased with age, indicating that senescing passerine females lay less pigmented eggs.
44 Clutch size, was positively related to the proportion of bluish eggs and to spot patterns
45 (more evenly and darker spotted). These results are in accordance with assumptions for
46 the sexual selection hypothesis.

47

48 **Keywords:** Biliverdin; Protoporphyrin; laying sequence; clutch size; Age

49 **Introduction**

50 The great variation in avian egg-shell pigmentation has always received much attention
51 from evolutionary biologists and numerous adaptive hypotheses on the subject have
52 been proposed in recent decades (reviewed in Underwood and Sealy 2002; Kilner
53 2006). For different bird taxa, this variation has been explained in the context of egg
54 mimicry to face predation risk (e.g. Bakken et al. 1978; Lloyd et al. 2000; Blanco and
55 Bortellotti 2002), to permit egg recognition in the case of brood parasitism (Stokke et al.
56 1999, 2002; Davies 2000; Soler et al. 2000; Lathi 2005, 2006), or more recently also in
57 the context of sexual selection where egg colour may signal the female's physical
58 condition and antioxidant capacity (e.g. Moreno and Osorno 2003, Soler et al. 2005;
59 Siefferman et al. 2006; Hanley et al. 2008).

60 Biliverdin (an antioxidant, responsible for blues and greens) and protoporphyrins
61 (a pro-oxidant, responsible for reds and browns) are the principal pigments responsible
62 for egg colour in birds (Kennedy and Vevers 1976; Miksik et al. 1996; Underwood and
63 Sealy 2002). Moreno and Osorno (2003) suggested that blue green egg pigmentation
64 acts as a sexually selected, condition-dependent signal of female quality. They reasoned
65 that since biliverdin has been shown to have antioxidant properties (Kaur et al. 2003),
66 females should balance the use of biliverdin for protection against free radicals and for
67 deposition into eggshells. The sexual signalling hypothesis proposes that only high-
68 quality females can afford the costs of depositing large amounts of biliverdin during the
69 laying period, a time of high oxidative stress (Moreno and Osorno 2003). Support for
70 this hypothesis however has been ambiguous up till now. A growing number of studies
71 corroborated that the deposition of the antioxidant pigment biliverdin in the eggshell
72 may depend on the higher antioxidant capacity of laying females (Hanley et al. 2008),
73 and be indicative of female quality in terms of immune system (Moreno et al. 2005;

74 Jagannath et al. 2008) or physical condition (Moreno et al. 2006; Siefferman et al. 2006;
75 Krist and Grim 2007; Soler et al. 2008). However, some studies in other bird species did
76 not find such clear support for the signalling function of the biliverdin pigment (Hargitai
77 et al. 2008, Lopez-Rull et al. 2008; Hanley et al. 2008; Hanley and Doucet 2009),
78 stressing the need for more studies on this subject.

79 On the other hand, a possible relation between porphyrin pigmentation (spot
80 patterns) and female physical condition or reproductive parameters has received much
81 less attention up till now (Miksik et al. 1994, 1996; Martínez-de la Puente et al. 2007;
82 Sanz and García-Navas 2009). Protoporphyrins are synthesized in the blood as an
83 intermediate metabolite of haemoglobin, and are transferred via epithelial cells to the
84 shell gland during egg formation (Baird et al. 1975). Due to the pro-oxidant properties
85 of protoporphyrins (Afonso et al. 1999, Shan et al. 2000), it has been proposed that they
86 could either signal female quality because increased pigmentation would indicate
87 oxidative tolerance (Moreno and Osorno 2003) or, alternatively, be an indicator of poor
88 condition, since high levels of protoporphyrins produce physiological stress (Martínez-
89 de la Puente et al. 2007). Surprisingly, there has been found support for both hypotheses
90 in blue tits (*Cyanistes caeruleus*). Martínez-de la Puente et al. (2007) found support for
91 the latter hypothesis since females that laid more spotted eggs showed poorer body
92 condition and higher levels of a stress protein. But Sanz and García-Navas (2009) found
93 support for the sexual selection and signalling hypothesis of spot patterns, since spotting
94 was positively related to some indicators of female quality (tarsus length, clutch size)
95 and paternal investment.

96 Nevertheless, as with any other phenotypic characteristic, the variation in egg
97 pigmentation between females is due to a combination of genetic, environmental and
98 maternal influences and the understanding of the source of variation is important for

99 testing the various hypotheses advanced to explain egg colour variation. For example,
100 both egg shell pigments have been shown to be influenced by environmental factors. In
101 the case of biliverdin, evidence has emerged of the importance of local environmental
102 factors such as dietary intake (e.g. Soler et al. 2008) or rainfall and temperature (Avilés
103 et al. 2007). Previous work showed how also the quantity of protoporphyrins may vary
104 according to diet (e.g. Joseph et al. 1999). More recently, Gosler et al. (2005) have
105 proposed that protoporphyrins are used to compensate for localised shell thinness
106 arising from a lack of calcium in the environment, thereby reducing the permeability
107 and water loss during incubation (Higham and Gosler 2006; Sanz and García-Navas
108 2009). Furthermore, it is also important to distinguish between spot patterns and
109 eggshell ground colour, since the mechanisms determining eggshell ground colour and
110 spot patterns are not necessarily the same (e.g. Underwood and Sealy 2002; Moreno and
111 Osorno 2003; Lopez-Rull et al. 2008).

112 In the present study, we collected data over a four-year period from various
113 clutches laid by the same females in a captive house sparrow (*Passer domesticus*)
114 population, which was kept under the same environmental conditions. This multiple
115 brooded species lays eggs which vary both in ground colour and in the intensity and
116 distribution of brownish-red spots (Seel 1968; Lowther 1988). First, we explored within
117 female constancy in egg shell patterns. Furthermore, the sexual selection hypothesis
118 explaining variation in egg colour and patterns assumes that pigment deposition should
119 be limiting for females (Moreno and Osorno 2003; Morales et al. 2008). We analysed
120 variation in spot patterns and ground colour according to egg laying sequence and a
121 negative relationship would reflect short term limitation in pigment deposition. In
122 addition, we also explored if pigment deposition may be limiting for females along the
123 breeding season (long time span) and analysed egg colouration according to the number

124 of clutches laid by the same female in a breeding season. Finally, we studied if clutch
125 size and female age influenced variation in egg ground colour and spot patterns. In
126 house sparrows, as in many other species, clutch size is a plastic trait that varies with
127 age and that shows significant variation between females (Hatch and Westneat 2007;
128 Nakagawa et al. 2007; Westneat et al. 2009). Since clutch size can be considered to be
129 an indicator of investment in the present reproductive attempt, a positive relationship
130 between clutch size and egg ground colour is predicted (Moreno and Osorno 2003). If
131 spot patterns in the sparrow are indicative for poorer quality females (Martínez-de la
132 Puente et al. 2007), a negative relation between clutch size and spot patterns is
133 expected. Alternatively, if spot patterns are indicative for high quality females, larger
134 clutches should be more spotted (Sanz and García-Navas 2009). With respect to age,
135 most studies have reported that reproductive traits improve after the first year of life
136 (e.g. review in Christians 2002) which could also be reflected in egg pigmentation (e.g.
137 Gosler et al. 2005; Siefferman et al. 2006). However, there is also evidence that elderly
138 females may display poorer physical condition and/or reproductive traits than those of
139 intermediate age (e.g. Clutton-Brock 1988; Newton 1989; Stearns 1992; Robertson and
140 Rendell 2001) and then senescence may also be reflected on egg pigmentation (Moreno
141 et al. 2005).

142

143 **Methods**

144 *The study species*

145 The house sparrow nests in cavities or builds enclosed nests on tree branches and
146 performs between one and seven reproductive attempts during each breeding season in
147 Europe (Cramp and Perrins 1994; Anderson 2006). The species is chiefly monogamous
148 and has a low frequency of extra-pair copulations (Veiga and Boto 2000). The ground

149 colour of house sparrow eggs varies from pure white to bluish and the eggs are covered
150 with spots of diverse size and pigmentation. The distribution of spots varies
151 considerably, from a large patch at the blunt end to a uniform spot pattern across the
152 entire eggshell and patterns of the last-laid egg in a clutch tends to be different from the
153 others (Seel 1968; Yom-Tov 1980; Lowther 1988).

154

155 *Characteristics of the study population*

156 The study population has been kept in a 45m³ aviary in the Science Faculty of Granada
157 University since 1999. Due to significant mortality, especially of fledged juveniles in
158 the post-breeding period, the population size was more or less constant (mean 75 ± SE
159 10 individuals) throughout the study years, with a mean sex ratio of 1:1. The aviary
160 provided 40 nest boxes, of which about 50% were occupied each breeding season. The
161 birds were provided *ad libitum* with water, seed mix, fly maggots, vitamins, powdered
162 calcium, mineral salts, apple, lettuce and nestling food. They were also provided with a
163 sand tray for their habitual dust-baths. Cotton wool and plant material was provided for
164 nest construction during the breeding season. The artificial illumination was regulated
165 by a timer which kept the birds at the same photoperiod as the external environment.

166

167 *Data collection*

168 The study was carried out during the breeding seasons of 2003-2006 inclusive. Daily
169 observations were done from the start of each breeding season to identify breeding
170 females and their nest boxes as well as possible changes in nest box use for consecutive
171 clutches.

172 All birds born in the captive population were colour-ringed as nestlings with a
173 combination of 4 rings to allow individual identification. Hence, the exact age was

174 known for breeding females that were born in captivity. We used minimum ages of
175 females that were not born in captivity, i.e. the number of years they spent in captivity,
176 plus one year. Female age ranged from 1 to 6 years, and was divided in 3 age classes (1
177 = first-time breeders; 2 = middle aged, two and three years old and 3 = advanced age, \geq
178 four years old). Since individuals were not captured again during the course of the
179 study, we do not have information on physical quality variables of breeders. Therefore,
180 possible influences of male quality on female reproductive investment were not taken
181 into account.

182 The nest boxes were examined daily to obtain information on the process of nest
183 building, laying order, clutch size and the number of clutch. Throughout the study there
184 were no instances of two eggs being laid in the same day in any of the nest boxes, so
185 there is practically no chance that any of the eggs in the studied- clutches was deposited
186 by another female (Petrie and Møller 1991; Jackson 1992). Other experiments after
187 clutch completion and during the nestling stage were performed in most of the studied
188 broods, for which the number of clutches laid by a female could not be considered as a
189 reliable possible indicator of female quality.

190

191 *Evaluation of egg characteristics*

192 During the course of the study 206 complete clutches, laid by 33 different females, were
193 photographed against a neutral grey card, incorporating a colour chart, using a Minolta
194 Dimage 7 digital camera. Photographs were taken indoor and under constant light
195 conditions.

196 Assessments of egg characteristics were carried out for each egg from the
197 photographs (n = 830 eggs). Clutches of 19 females were photographed in only one
198 year, either because the birds died during the next winter or because they laid their first

199 clutch in 2006, the final year of study. However, for these females, except for three, we
200 had information on at least two consecutive clutches during the same breeding season.
201 Information from two or more years was available for 14 females. With respect to first-
202 time breeders, we obtained photos of clutches of two females in 2005 and seven in
203 2006. Clutches laid by first-time breeders in 2004 were used in a parallel experiment
204 which did not permit complete clutches to be photographed and so these were not used
205 to contribute first-time breeder data to the present study. Thus, in total we obtained data
206 from 9 first-time breeders and 25 adults females, from which one of the first-time
207 breeders past to adult in the following breeding season.

208 The same observer assessed the photos of each clutch against the following
209 parameters, following the criteria and methodology of Gosler (2000, 2005; see also
210 Sanz and García-Navas 2009) with principal classes: spot intensity (I: scored from 1 for
211 palest spots to 5 for the darkest); average spot size (S: scored from 1 for small spots to 3
212 for large spots) and spot distribution (D: scored from 1 for > 90% spots concentrated in
213 one end, to 5 for an even spot distribution over the surface). Unspotted eggs received a
214 0-quote for all the spot-parameters. Shell ground colour was divided in four classes:
215 white, brownish-white, bluish-white, or bluish. Within the study sample of 830 eggs,
216 69.6 % were white, 1.4 % were brownish-white, 3.8 % bluish-white, and 25.1 % were
217 bluish. Given this very low percentage of brownish-white and bluish-white eggs, these
218 were excluded from further analyses to avoid strong unbalanced models.

219 Repeatability of the observer's assessments was estimated by evaluating the
220 characteristics of 280 eggs from the 63 clutches of 16 females in 2003 a second time 15
221 days after the first (Lessells and Boag 1987) and was highly significant for all variables
222 ($P < 0.001$ in all cases).

223

224 *Statistical analyses*

225 Because some of the variables of eggshell spot patterns were significantly
226 intercorrelated (I and S: $r_{830} = 0.379$, $P < 0.001$; I and D: $r_{830} = 0.033$, $P = 0.337$; S and
227 D: $r_{830} = 0.166$, $P < 0.001$), we used a Principal Component Analysis (PCA) of the
228 correlation matrix of the original I, S and D values (Gosler et al. 2000; Sanz and García-
229 Navas 2009). Two Principal Components explained 80.7 % of the variation in spots
230 pattern. The PC1 describes variation in pigment intensity and spot size and it was
231 referred to as the ‘darkness’ of the egg (see Gosler et al. 2000, 2005; Sanz and García-
232 Navas 2009). Eggs with larger and more intense spots showed higher PC1 values (factor
233 loadings: I: 0.780, D: 0.325, S: 0.842). PC1 expresses 47.5 % of the total variation in I,
234 D and S. The second principal component (PC2) describes the level of spot aggregation
235 and shows higher values for eggs with more widely distributed spots over their surface
236 (factor loadings: I: 0.377, D: 0.928, S: 0.009). PC2 then represents the ‘spread’ of
237 maculation (see Gosler et al. 2000, 2005; Sanz and García-Navas 2009). PC2 expresses
238 a further 33.2 % of the total variation in I, S and D.

239 To test which variables best explained variation in eggshell spot patterns
240 (darkness and spread) General Linear Mixed Models (GLMM) were constructed with
241 PC1 and PC2 as the dependent variables. For ground colour a Generalized Linear
242 Mixed Model (GLIMMIX) was used (binomial distribution: 0 = white ground colour, 1
243 = bluish ground colour, logit link function). In all analyses laying order, clutch size and
244 the number of clutch were considered as covariables. The three age classes (see *Data*
245 *collection*) were included as a fixed factor using Bonferroni adjustment. The variables
246 female identity and year were random factors to determine whether differences existed
247 in egg colour patterns between females and to avoid pseudo replication, and to take into
248 account possible random variation due to study year.

249 The model residuals did not differ from a normal distribution (Kolmogorov-
250 Smirnov: all $P > 0.2$). The degrees of freedom of the GLMM and GLIMMIX models
251 were calculated using the Satterthwaite method (Fai and Cornelius 1996) and were
252 performed with SAS (SAS 1989-96 Institute Inc., Cary, NC, USA; Littell et al. 1996).

253 Furthermore, the same models were included in a Variance Components
254 Analysis to obtain the % of variance explained by female identity in spot darkness and
255 spread (STATISTICA 7.0, StatSoft, Inc. 2004).

256

257 **Results**

258 Female identity had a significant effect on all egg colour characteristics but the
259 year had no significant effect on any of them (Tables 1 and 2). Variance Components
260 Analyses revealed that the variance attributable to female identity in pigment spread
261 was higher (26.6%) than for pigment darkness (12.7%).

262 Spot darkness (PC1) was significantly influenced by egg laying order, clutch
263 size, clutch number and female age (Table 1). The first eggs within a given clutch were
264 darker spotted than the final ones and spot darkness increased with clutch number and
265 clutch size (Table 1). In addition, clutches from older females (> four years) were less
266 dark spotted compared to those of first time breeders (GLMM Bonferroni corrected: $P =$
267 0.005) and middle-aged ones (GLMM Bonferroni corrected: $P < 0.001$, Table 1, Fig.1).
268 Spot darkness did not differ significantly between first time breeders and middle-aged
269 ones (GLMM Bonferroni corrected: $P = 0.76$, Fig.1).

270 On the other hand, pigment spread (PC2) diminished significantly according to
271 laying sequence and clutch number, but increased with clutch size (Table 1). No
272 significant differences existed between age classes (Table 1).

273 Ground colour (white vs bluish) was significantly influenced by laying order,
274 clutch number, clutch size and female age (Table 2). Within a clutch, the last-laid eggs
275 were mainly white ones and the last clutches of the season had more white eggs than the
276 earlier ones. Eggs from small clutches were more white and older females laid a higher
277 proportion of white eggs (first time breeders = 67.9%, middle-aged = 64.8%, older
278 females = 85.0%).

279

280 **Discussion**

281 House sparrow egg colour was significantly determined by female identity. However,
282 the variance in spot patterns attributable to female identity was larger for pigment
283 spread (26.6%) than for pigment darkness (12.7%), suggesting that pigment spread is
284 less flexible within females than pigment darkness. The fact that pigment darkness was
285 affected by female age, but pigment spread was not, supports this idea.

286 Furthermore, and in agreement with the sexual selection prediction, our results
287 indicate that house sparrows females experience certain limitation in pigment deposition
288 for both pigments (ground colour and spots) and at least short term. With respect to
289 ground colour, the last laid eggs within a clutch and the last clutches within the breeding
290 season had a higher proportion of white eggs. Previous findings on free-living bird
291 populations of pied flycatchers (*Ficedula hypoleuca*) and collared flycatchers (*Ficedula*
292 *albicollis*) also pointed at a short time limitation or cost in the deposition of the
293 biliverdin pigment since egg darkness decreased along the laying sequence (Moreno et
294 al. 2005; Krist and Grim 2007). However, other studies found the opposite relationship
295 where last laid eggs were the most bluish ones (Siefferman et al. 2006; Hargitai et al.
296 2008). This suggests different reproductive strategies across species. While a decrease
297 in egg pigmentation indicates female limitation in pigment deposition; an increase in

298 egg pigmentation may indicate higher investment in last laid eggs, facilitating survival
299 of last-hatched young (“brood survival hypothesis”, Slagsvold et al. 1984). Our results,
300 in addition, suggest also a possible cost of maintaining the same level of investment in
301 biliverdin deposition throughout the breeding season (long term) in female house
302 sparrows, even when food is freely available. With respect to spot patterns, results
303 suggest that at least at short term the deposition of protoporphyrin would be limited for
304 females because both spot darkness and spread decreased along the laying sequence.
305 Contrary to these findings, Gosler et al. (2005) found a linear increase through the
306 clutch in pigment darkness in great tits (*Parus major*). However, this increase was not
307 predicted by the observed changes in eggshell mass, egg size or shape (i.e. structural
308 hypothesis: spots compensate for eggshell thinning) and the authors suggest that some
309 additional physiological programme may be operating through the clutch. Although we
310 cannot test eggshell thickness in relation to laying order and pigmentation in the present
311 study, our results support the idea that different mechanisms may operate across species
312 or under different environmental conditions. More studies are necessary to attain more
313 general patterns explaining within clutch egg pigmentation patterns. With respect to
314 long term effects on protoporphyrin deposition, at least three other studies have
315 examined this along the breeding season. In the great tit, pigment darkness was
316 negatively related to laying date (Gosler et al. 2000). In the closely related blue tit,
317 females laid less spotted (% spots), but brighter and more reddish clutches at the end of
318 the breeding season (Martínez de la Puente et al. 2007). Nevertheless, in the same
319 species Sanz and García-Navas (2009) found that laying date had no effect on
320 pigmentation patterns (darkness and spread). So, again, these previous findings point at
321 no general relationship between pigmentation and the course of the breeding season.
322 Still, all these studies were conducted on single brooded species while the present study

323 analyzed the possible limitation in spot patterns in different clutches of the same female
324 during the same breeding season and under constant environmental conditions. Our
325 results showed that first clutches had eggs where spots were more spread, but less dark.
326 Then, given that spots were darker at the end of the breeding season, protoporphyrin
327 deposition was probably not limited at long term. However, the less dark spots and
328 more evenly spread at the beginning at the breeding season could indicate a lower
329 capacity of calcium deposition for females in last clutches; so at long term. This lower
330 capacity could then be compensated for by darker spots (Gosler et al. 2005). However,
331 in the present study we have no information to test possible effects of other egg
332 properties or female condition variables on spot patterns.

333 Female age affected significantly the proportion of bluish eggs and spot patterns:
334 the proportion of bluish eggs and spot darkness declined in females older than four
335 years. Some studies failed to find effects of age on egg ground colour (Hargitai et al.
336 2008) or spot patterns (Sanz and García-Navas 2009). Other studies found that adults
337 compared to first-time breeders laid more pigmented (Siefferman et al. 2006), brighter
338 (Martínez de la Puente et al. 2007) or less dark spotted eggs (Gosler et al. 2005).
339 Nevertheless all these studies distinguished only two age classes and had no data on
340 older age classes to evaluate senescence on egg pigmentation. One study however did
341 evaluate the effect of an older age class on egg pigmentation in pied flycatchers, and
342 also found that older females (> 4 years) laid lighter and less pigmented eggs (Moreno
343 et al. 2005). These results, together with results of the present study, suggest that
344 senescing passerine females lay less pigmented eggs.

345 On the other hand, larger clutches had a higher proportion of bluish eggs and
346 eggs were more evenly and darker spotted compared to small clutches. These findings
347 are in accordance with those of Sanz and García-Navas (2009) in blue tits where larger

348 clutches also had more evenly distributed spots. Since clutch size is likely a reliable
349 indicator of investment in the present reproductive attempt (e.g. Christians 2002), these
350 results rather support the hypothesis that red-brownish pigmentation and bluish
351 pigmentation of eggs reflect a higher maternal investment in sparrows (Moreno and
352 Osorno 2003) and therefore may be involved in the sexual selection theory explaining
353 egg colouration. However, further studies are needed to determine the relationship
354 between female condition/quality and age and clutch size in the house sparrow.

355 In conclusion, both ground colour and spot darkness seem to be limited egg
356 characteristics for house sparrow females, at least short term (along the laying
357 sequence). Also females that laid larger clutches laid more bluish and more evenly and
358 darker spotted eggs and female age had a negative effect on egg pigmentation. These
359 findings are rather in agreement with predictions proceeding from the sexual selection
360 hypothesis explaining egg colour variation in house sparrows.

361

362 **Zusammenfassung**

363 **Grenzen der Pigmenteinlagerung und Merkmale der weiblichen** 364 **Fortpflanzungsorgane beeinflussen Fleckung und Hintergrundfarbvariation von** 365 **Eierschalen in Haussperlingen (*Passer domesticus*).**

366

367 In der vorliegenden Arbeit haben wir Merkmale von Eifärbung innerhalb individueller
368 Weibchen einer in Gefangenschaft gehaltenen Haussperlingspopulation (*Passer*
369 *domesticus*) über mehrere Jahre hinweg untersucht. Ziele waren (1) die Bedeutung der
370 genetischen Einflüsse zu schätzen, (2) zu untersuchen, ob Pigmenteinlagerung
371 eierlegender Weibchen limitiert ist, und (3) ob Indikatoren individueller Qualität von
372 Weibchen (z. B. Gelegegröße, Alter) die Eifärbung beeinflussen. Unsere Ergebnisse
373 zeigen das Vorhandensein einer genetischen Basis, welche die Eifärbung individueller
374 Weibchen bestimmt. Darüber hinaus nahm die Grundfarbe und die Intensität und Größe
375 der Flecken über die Legereihenfolge ab, was auf begrenzte Kapazitäten der
376 Pigmenteinlagerung pro Reproduktionsereignis hinweist. Junge Weibchen und

377 Weibchen mit größeren Gelegen legten dichter gefleckte Eier mit intensiveren und
378 größeren Flecken, sowie einen größeren Anteil Eier mit bläulicher Grundfarbe. Dies
379 kann darauf hindeuten, dass beide Pigmentierungen mit der weiblichen Kondition
380 zusammenhängen. Unsere Ergebnisse zeigen außerdem, dass die Verteilung der Flecken
381 jenes Merkmal von Eiern ist, welches anscheinend am wenigsten von Umweltfaktoren
382 beeinflusst wird.

383

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394

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540 **Table 1.** GLMM models which best explained variation in spots darkness and spots
541 spread. The “Variable Type” column indicates whether a variable was regarded as a
542 covariable (C), fixed factor (F) or random factor (R). F values are given for covariables
543 and fixed factors and Z values for random factors. The sign of the estimator indicates a
544 positive or negative relation between covariables and the dependent variable.

545 **Table 1**

546

	Explanatory variables	Variable type	Estimate	df	F/Z	p
<u>Pigment darkness</u>	Female	R			2.75	0.003
	Year	R			-	-
	laying order	C	-0.13	1,568	31.23	<0.001

	Clutch size	C	0.08	1,569	6.46	0.011
	Clutch number	C	0.05	1,573	5.67	0.017
	Age	F		2,108	26.70	<0.001
<u>Pigment spread</u>	Female	R			3.09	0.001
	Year	R			-	-
	laying order	C	-0.05	1,565	4.29	0.038
	Clutch size	C	0.07	1,589	4.91	0.027
	Clutch number	C	-0.08	1,588	12.83	<0.001
	Age	F		2,135	0.19	0.824

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549 **Table 2.** GLIMMIX model which best explained variation in egg ground colour
550 (binomial distribution variable, logit link function). The “Variable type” column
551 indicates whether a variable was regarded as a covariable (C), fixed factor (F) or
552 random factor (R). F values are given for covariables and fixed factors and Z values for
553 random factors. The sign of the estimator indicates a positive or negative relation
554 relation between covariables and the dependent variable.

555 **Table 2**

556

	Explanatory variables	Variable type	Estimate	df	F/Z	P
<u>Ground colour</u>	Female	R			2.65	0.004
	Year	R			1.03	0.151
	laying order	C	-0.23	1,515	10.24	0.001
	Clutch number	C	-0.28	1,535	11.64	< 0.001
	Clutch size	C	0.29	1,536	7.49	0.006
	Age	F		2,36.4	5.96	0.018

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559 **Figure 1.** The difference in **(a)** spots darkness **(b)** spots spread **(c)** % white eggs
560 between the three age classes (1 = first-time breeders, 2 middle age = two and three
561 years old, 3 advanced age = \geq four years old).

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