Egg spotting pattern in common cuckoos and their great reed warbler hosts: a century perspective

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The characteristics of common cuckoo (*Cuculus canorus*) and host eggs are widely thought to have coevolved over time, but few studies have tested this prediction. We compared cuckoo eggs with those of its primary host, the great reed warbler (*Acrocephalus arundinaceus*) from four time periods spanning >100 years (between 1900 and 2014), and studied if cuckoo eggshell patterns better resembled those of their hosts over time. We used image analysis to compare five eggshell pattern variables, relating to marking size, diversity, contrast, coverage, and distribution on the egg surface. Each feature showed different temporal trends. All but one of these variables ("dispersion" of spots among egg regions) were species-specific and differed between hosts and parasites. The magnitude of change was greater for hosts than

33 cuckoos, which could be a consequence of host eggs' more intensive and variable spottiness.

34 Specifically, the proportion of the egg surface covered with pattern increased marginally over

35 time, and the dispersion of spotting became more even over the egg surface. Egg marking

36 contrast showed a decreasing trend, with species differences also decreasing, suggesting

37 better mimicry. Our results suggest multidirectional evolution of eggshell components in this

38 system, with potential implications for mimicry and rejection over time.

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41   INTRODUCTION

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43 Brood parasitic birds lay their eggs in the nests of other avian species, and these foster parents

44 incubate the foreign eggs and feed and rear the foreign nestlings (Rothstein, 1990). During

45 long-term coevolutionary arms races among brood parasites and their hosts, selection

46 pressures drive adaptations of the brood parasites and counter-adaptations of hosts (Davies,

47 2000). The common cuckoo (*Cuculus canorus*; hereafter "cuckoo") is a well-studied brood

48 parasite (Schulze-Hagen, Stokke & Birkhead, 2009), comprising several different host-races

49 (often called "gentes"), in which each female cuckoo specializes on a particular host species.

50 Many hosts have evolved the ability to recognize alien eggs and eject them from their nests,

51 or desert their clutch entirely, and so many female cuckoos deceive host parents through

52 laying eggs that mimic those of their host, making recognition of foreign eggs more difficult.

53   The eggshells of many bird species are highly variable in colour and spotting among

54 individuals (Underwood & Sealy, 2002; Cassey et al., 2010b; Spottiswoode & Stevens, 2010;

55 Hauber, 2014), and brood parasitism is one of the most important selective pressures in

56 modifying these phenotypic traits (Øien, Moksnes & Røskaft, 1995; Soler & Møller, 1996;

57 Kilner et al., 2006). The hosts' main anti-parasite defence mechanism, egg rejection

58 behaviour, is a complex process (e.g. Pozgayová et al., 2011; de la Colina et al., 2012), where

59 background coloration plays a key role in foreign egg recognition by hosts (e.g. Avilés, 2008;

60 Cassey et al., 2008). In contrast, the function of spottiness has less frequently been studied

61 compared to colour, but hosts also likely use various characters of spotting in egg recognition,

62 for example in identifying foreign eggs (Moskát et al., 2008a), or looking for signatures

63 indicating own egg identity (Stoddard, Kilner & Town, 2014), especially those encoded at the

64 blunt pole of the eggs (e.g. Polaciková & Grim, 2010).

65   Most studies on cuckoo-host coevolution have concentrated on 'snapshots' of the arms

66   race, covering a duration of only a few years, or with highly variable sampling periods. Even

67   when studies are based on eggs stored in museum collections (e.g. Avilés & Møller, 2003;

68   Stoddard & Stevens, 2010), datasets are rarely suitable for detecting longer-term trends (but

69   see Spottiswoode & Stevens, 2012). As such, there is a pressing need for long-term studies on

70   the coevolution of egg colour (e.g. Cherry & Bennett, 2001; Antonov et al., 2010) and pattern

71   mimicry (Medina et al., 2016; Brulez et al., 2016). Previous modelling has predicted that

72   cuckoos should over time benefit from increasing the resemblance of their own eggs to those

73   of the host, and that hosts should be under selection to escape parasitism (Takasu, 2003).

74   Therefore, the selection pressure derived from brood parasitism may promote host eggs in a

75   given population to change phenotypes over time (Takasu et al., 2009; Spottiswoode &

76   Stevens, 2012). Consequently, we cannot simply expect a continuous improvement in cuckoo

77   egg mimicry to host egg phenotypes in a host-brood parasite relationship, but instead might

78   expect more of a Red Queen scenario of both parties evolving changes in phenotype over

79   time; the host to escape cuckoo mimicry, with the cuckoo in turn under selection to keep up.

80   In Hungary, the great reed warbler (*Acrocephalus arundinaceus*) is the main host of

81   the cuckoo in wetland habitats (Moskát & Honza, 2002). The eggs laid by great reed warbler-

82   specific cuckoos in Hungary are often cited as one of the most impressive cases of mimicry of

83   spotted, non-plain coloured cuckoo eggs found (e.g. Southern, 1954; Davies & Brooke, 1991;

84   Moksnes & Røskaft, 1995). The high cuckoo parasitism on great reed warblers has been

85   documented in detail from the Hungarian population (Zölei, Bán & Moskát, 2015), with eggs

86   collected from the early 1900's available for study in museums, offering an exceptional

87   possibility for revealing how cuckoo egg mimicry has changed over a more than 100-year

88   period. Here, we used digital images of the eggs of cuckoos and great reed warblers to

89   evaluate how the pattern of spottiness, as an important component of egg mimicry (Stoddard

90   & Stevens, 2010), changed from the 1900's until the present day. In order to quantify egg

91   pattern mimicry between brood parasite and its host, we used the method of Stoddard &

92   Stevens (2010), based on digital image analysis of several components of egg patterns (Table

93   1). We hypothesized that cuckoos have been under selection pressure to improve their

94   eggshell pattern match to that of their hosts, and so we investigated trends in eggshell pattern

95   components during approximately the last hundred years. We predicted that mimicry (defined

96   as the numerical difference between the egg parameters studied) has improved continuously

97   during this period. As an alternative hypothesis, we predicted that the arms race between the

98   cuckoo and great reed warbler was in a steady state during the studied period, resulting in a

99   stable relationship or small fluctuations in egg phenotypes. We predicted no significant

100   change in similarity of cuckoo and host eggs in this case.

101

102   METHODS

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104   DATA COLLECTION

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106   We photographed parasitized clutches of great reed warblers collected from the central

107   regions of Hungary contained in the egg collection of the Natural History Museum (Tring,

108   Hertfordshire, UK) and the Mátra Museum (Gyöngyös, Hungary, a branch of the Hungarian

109   Natural History Museum, Budapest). We studied only spotting pattern of eggs stored in dark

110   places, under museum conditions, not exposed to direct sunlight. Changes in colours over a

111   long time could be more relevant when eggshell colours rather than spottiness are studied, as

112   previous studies showed some changes in the background colour of eggshells, mostly in terms

113   of blue-green chroma (Cassey et al., 2010a; Navarro & Lahti, 2014). In addition, we took

114   pictures in the field of parasitized clutches in central Hungary, *ca*. 50 km south of Budapest,

115   in the vicinity of the village Apaj (47°07'N; 19°06'E). All clutches were photographed on a

116   Kodak Grey Card with a digital camera Olympus E-510 (Olympus Imaging Corp., Tokyo,

117   Japan). Kodak Colour Control Patches were also placed near the eggs on each photo as a

118   reference. We took pictures of clutches containing one cuckoo egg (single parasitism). All

119   eggs were collected from 1901 to 1969 and field images were taken in 2014. Four stages are

120   characterized in this approximately 100-year period: (i) the "1900s" (1901-1903, n = 16), (ii)

121   the "1930s" (1934-1939, n = 16), (iii) the "1960s" (1960-1969, n = 12) and (iv) "2014" (n =

122   20). We photographed complete clutches (4, 5 or 6 eggs), but randomly chose one host egg

123   per clutch for the following analyses (Fig. 1). To prevent using more than one clutch from the

124   same female, we included different sites and years, and in the recent dataset we took care not

125   to use replacement clutches built after predation. Altogether, 128 eggs from 64 nests were

126   measured and used in the analyses (64 cuckoo and 64 host eggs). All clutches were used in

127   the analysis; no preliminary selection was applied on the similarity of cuckoo eggs to host

128   eggs (in order to attempt to identify cuckoo gentes) so that we could represent the available

129   morphs of cuckoo eggs, similarly to other studies on spotting pattern (e.g. Stoddard &

130   Stevens, 2010). In general, interclutch variation of great reed warblers is high in our study

131   area (Moskát & Honza, 2002), and there are corresponding cuckoo egg morphs for most of

132 the common and some of the rarer egg types, but cuckoo eggs belonging to other cuckoo

133 gentes may also occur (c.f. Lovászi & Moskát, 2004).

134

135 PATTERN ANALYSIS

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137 Quantifying spottiness, at least as revealed through human scoring, seems to be an important

138 aspect of cuckoo-host coevolution (Moksnes & Roskaft, 1995). Although some previous

139 studies have used computer programs, e.g. NIH-Image or Scion Image for Windows, for

140 image analysis of eggshell spottiness (Soler J, Soler M, Møller, 2000; Moskát, Szentpéteri &

141 Barta, 2002), these were influenced by subjective decisions by humans and based on

142 uncalibrated images. However, images from most cameras require calibrating before they

143 should be used, to control for differences in light conditions and the camera-specific response

144 to light intensity (see Stevens et al., 2007). Here, we first linearized our images to correct for

145 the camera's non-linear response to light levels (radiance), and normalised the images with

146 regards to the grey standard to control for light conditions, thus deriving images

147 corresponding to reflectance information (Stevens et al., 2007). We then applied the method

148 used by Stoddard & Stevens (2010), based on digital image analysis to quantify several

149 aspects of egg pattern. All calibrations and image analyses were carried out by self-developed

150 computer programmes written in MATLAB (The MathWorks, Inc., MA, USA) and its

151 associated Image Processing toolbox. As with previous work, we took the mediumwave

152 ('green') image channel for analysis because this most closely approximates to avian

153 achromatic vision, which likely primarily underlies pattern perception (see Spottiswoode &

154 Stevens, 2010). Before quantifying the eggs' pattern, we rescaled each image to 50 pixels/mm

155 in ImageJ (Abramoff, Magelhaes & Ram, 2004), because our photographs were taken at

156 slightly variable distances (Spottiswoode & Stevens, 2010). We used the length of the yellow

157 segment of Kodak Colour Control Patches as a reference for this.

158 During the analyses, each calibrated image of an egg was filtered into a set of new

159 images using Fast Fourier Transformation and seven octave-wide, isotopic band-pass filters

160 (Barbosa et al., 2008; Stoddard & Stevens, 2010; Spottiswoode & Stevens, 2010). These new

161 images include information regarding pattern at different spatial scales, which can be thought

162 of as acting analogous to a set of sieves, each image capturing information for different sized

163 markings from smaller sizes (high spatial frequency) to larger sizes (low spatial frequency).

164 These seven filtered images or 'granularity bands' (Barbosa et al., 2008) contain information

165 about the relative importance of different marking sizes in contributing to the overall pattern

166   (marking filter size, referred to as "filter size"). From each of the seven granularity bands we

167   calculated the overall pattern 'energy' (total energy), as the sum of the squared pixel values in

168   each image divided by the number of pixels in the image (Chiao et al., 2009; Stoddard &

169   Stevens, 2010). The total energy of the spectrum corresponds to the overall amplitude of the

170   spectrum, and provides a measure of pattern contrast of the egg markings (Stoddard &

171   Stevens, 2010; Spottiswoode & Stevens, 2010). This variable expresses how contrasting the

172   markings are against the egg background colour ("total energy"; see Table 1). These seven

173   values produce a 'granularity spectrum', where the maximum energy value corresponds to the

174   filter size containing the highest energy, and thus the dominant marking filter size ("filter

175   size"). We also calculated the proportion that this maximum value contributes to the total

176   energy of the spectrum ("proportion energy"), which indicates the importance of the dominant

177   marking size to the overall egg pattern. A high value shows that the egg pattern is dominated

178   by just one or a few filter sizes (Stoddard & Stevens, 2010; Spottiswoode & Stevens, 2010).

179   In addition, we calculated the relative proportion of three main egg regions covered by

180   markings, as opposed to the base colour (simply referred as pattern "coverage"). Methods

181   again closely followed Stoddard & Stevens (2010). First, we thresholded each image into a

182   binary format, whereby markings are encoded by one (1.00) and background egg colour

183   encoded by a zero (0.00). From this, the proportion of the total pixel values that corresponded

184   to a marking was calculated. This value shows the overall proportion of the egg that is

185   covered with markings (Stoddard & Stevens, 2010; Spottiswoode & Stevens, 2010). We also

186   calculated these metrics for three regions of the egg surface, corresponding approximately to

187   the top (narrow), middle, and base (wider) thirds of the egg. The standard deviation of pattern

188   coverage among the bottom, middle, and top regions of an egg is a measure of pattern

189   dispersion (referred as "dispersion" in analyses), which indicates how concentrated the pattern

190   is at the bottom of the egg. As we revealed differences among egg regions (see Results), we

191   therefore considered the egg regions separately in our linear models, and called this division

192   as "topology". Previous studies failed to find differences among the markings of eggshell

193   regions and averaged measurements for the overall egg surfaces (e.g. Stoddard & Stevens,

194   2010). Other studies looking for markings of the eggshell in cuckoo hosts revealed the

195   importance of the bottom region (blunt pole) over the top region (sharp pole), as hosts

196   primarily used the information content of the blunt pole for recognition (Polaciková et al.,

197   2007, 2010, 2011; Polaciková & Grim, 2010). While there are other potential methods that

198   could be used to quantify egg pattern mimicry, including a recent computer science approach

199   of Stoddard et al. (2014), the metrics we use here have been shown to effectively predict egg

200   rejection behaviour in experimental studies in other parasite-host systems and are broadly

201   based on principles of early spatial vision processing (Spottiswoode & Stevens, 2010, 2011;

202   Stevens, Troscianko & Spottiswoode, 2013).

203

204   DATA ANALYSIS

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206   *Comparison of spottiness*

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208   Response variables (see below) were tested among years (factor with 4 levels: 1900, 1930,

209   1960 and 2014), species (factor with 2 levels: GRW as great reed warbler, C as cuckoo) and

210   topology (i.e. egg regions, factor with 3 levels: bottom, middle and top). We also included

211   their interactions, and the estimation was done using the ordinary least square mean method.

212   The following response variables were used (and their abbreviations): (1) proportion energy,

213   (2) filter size, (3) the common logarithm of filter size (log filter size), (4) coverage, (5) total

214   energy and (6) dispersion. These responses were tested by a single-argument ANOVA

215   (permutational ANOVA), where the number of iterations was taken to generate exact P values

216   (Box, 1988). The description of identification of overall trends by time series analyses in the

217   eggshell's pattern is available in the ESM section no. 1. We used two datasets in the analyses,

218   in the first case all parameters were considered according to the species (great reed warbler,

219   'GRW' and cuckoo, 'C') , while in the second case the egg parameters were defined as a

220   differences between the species, thus the effectiveness of the mimicry can be tested. We refer

221   to this dataset as 'mimicry data' in the text below.

222   All analyses were carried out in R 3.1. 2 (R Core Team, 2014) using the package

223   lmPerm for permutational ANOVA (Wheeler, 2010) and TTR for time series analyses (Ulrich,

224   2013).

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226

227   RESULTS

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229   COMPARISON OF SPOTTINESS

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231   In the first step of our analyses we compared egg parameters among the top, middle, and

232   bottom regions of the eggs. As we found differences among them (c.f. topology effects in

233   Table 2), we could not combine all regions of the eggs into one operation unit for subsequent

234   analyses. Permutational ANOVA revealed different effects for all five variables measured

235   (Table 2). All variables, except filter size (even when log transformed), showed a year effect.

236   Topology effect (the effects of egg regions) was revealed for coverage and contrast.

237   Compound effect ('species x topology') was significant for proportion energy and contrast.

238   The variable proportion energy slightly increased over time with consistently higher values

239   for great reed warblers than cuckoos, while dispersion showed the opposite trend (Table 2 and

240   Fig. 2).

241   We found increasing trends for the variables coverage and total energy. However, the

242   values of these parameters were consistently higher for great reed warblers than cuckoos

243   (Table 2 and Fig.2). The metrics coverage and total energy showed similar trends for both of

244   the two species (Fig. 2). Based on the above mentioned issues for egg's topology, we suggest

245   that the source of differences is the species itself rather than any topological differences

246   between egg's regions.

247   We also analyzed mimicry (the difference between the two species' values for each

248   variable) for only those pattern metrics where significant effects were revealed above.

249   Interestingly, most variables did not show a year effect – only total energy showed a

250   significant year effect, a decreasing trend ($p = 0.004$), and differences among egg parts

251   (topology: $p = 0.008$; Table 3 and Fig. 3). As the difference decreased between cuckoo and

252   host eggs regarding this variable, this means that mimicry improved with regards to total

253   energy.

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255   ESTIMATING TRENDS IN TIME

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257   We found a clear and distinctive seasonal pattern in the egg parameters based on the

258   decomposition of time series between 1900 and 2014. The optimum ARIMA model proved to

259   be the most useful, with 2, 2, and 5 parameters for the eggs' data. The residuals indicated

260   small variation around the mean zero; none of them was greater than the double of the

261   standard deviations, thus this model provided the best fit. The autocorrelation of the residuals

262   was not significantly different from zero as a set, and had a constant variance, thus confirming

263   the adequacy of the model for all egg parameters. The decomposition of time series data

264   (summarized in ESM as SFigs. 1-4) revealed that (those parameters which were affected

265   significantly by the year) the parameter proportion energy showed consistent changes in its

266   pattern over the period studied, while total energy showed a decreasing trend in time for both

267 species. For the mimicry data, this latter variable also showed a similar decreasing trend (Fig.

268 4). As we defined mimicry as the difference between host and parasitic eggs, this suggests that

269 mimicry was improving with time.

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272   DISCUSSION

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274 Here, we compared egg pattern components of cuckoo-host egg mimicry at four stages over a

275 period of more than 100 years. Such data are scarce in studies of the evolutionary biology

276 between hosts and brood parasites. We evaluated five parameters of eggshell spottiness of

277 great reed warblers, cuckoos, and calculated their resemblance (indicative of 'mimicry') over

278 time. Although great reed warbler and cuckoo eggshell patterns are widely thought to have

279 coevolved, each of the five characters showed different trends, except filter size (equating to

280 the main egg marking size), which did not show any changing pattern with time. In general,

281 the magnitude of these changes was greater for great reed warblers than cuckoos, which could

282 be a consequence of their more intensive and variable spottiness (see similar results in

283 Spottiswoode & Stevens, 2012 for another host-brood parasite relationship). These results

284 could be explained by stronger selection pressures on hosts to evolve signatures by which

285 they can recognise their eggs than on cuckoos to match their eggs (M.I. Cherry, pers. comm.).

286 Interestingly, a recent study on cuckoos and great reed warblers in Hungary pointed to the

287 stability of this host-brood parasitic relationship regarding parasitism rate and hosts' responses

288 to parasitism in the last 70 years (Zölei et al., 2015). In the present study, most variables

289 showed no difference between species, with the exception of coverage and total energy. For

290 the latter two characters, our results provided support for the assumption that brood parasitism

291 is a selective force in cuckoo-host coevolution and egg phenotypes. Hosts of brood parasites

292 only use some aspects of egg appearance in guiding their egg rejection behaviour at any one

293 time, and those features used might be the ones that differ most between parasites and hosts

294 (e.g. Spottiswoode & Stevens, 2010, 2011). Therefore, it may be that at different stages (time

295 periods) of the arms race different aspects of egg appearance are used in rejection and

296 therefore under selection. Other traits may not be under selection at the same time.

297   Although we do not know exactly when this parasitic relationship started, our host-

298 brood parasite system seems to be a relatively well-stabilized one as the main characteristics,

299 such as high parasitism rate, high rate of multiple parasitism, and mid-level rejection rate,

300   seem to be consistent over time (see Zölei et al., 2015). The study by Zölei et al. (2015) also

301   suggests that despite the unusually and permanently high parasitism rate found in our study

302   area for at least the last 70-80 years, our host-brood parasite system is in dynamic

303   evolutionary equilibrium, where the ratio of hosts accepting or rejecting parasitic eggs is in

304   equilibrium (Lotem & Nakamura, 1998), and the recognition error rate is low (Stokke et al.

305   2016). This could explain the lack of one universal trend in the changes of eggshell spotting

306   characters revealed in the present study. Newly established cuckoo-host relationships are

307   expected to be rather unstable, as both empirical and modeling studies have revealed. For

308   example, they may show well-documentable changes in time, either in parasitism rate (Takasu

309   et al., 1993; Barabás et al., 2004), egg appearance (Avilés et al., 2006), or egg discrimination

310   of hosts (Røskaft et al., 2002, 2006). Sometimes, phenotypic changes can be detectable within

311   a shorter period when strong selection pressures affect coevolutionary changes (Spottiswoode

312   & Stevens, 2012), and phenotypic traits may oscillate around fixed points in the phenotypic

313   space and result in discrete categories of egg phenotypes (Takasu, 2003, 2005).

314   Our results revealed multidirectional changes in coevolutionary adaptations in

315   eggshell spottiness, which is in accordance with previous results that eggshell pigments are

316   likely to have several functions (Maurer, Portugal & Cassey, 2011), suggesting

317   multidirectional evolution of different eggshell components (Gosler, Higham & Reynolds,

318   2005). For example, eggshell pigments may protect embryos from solar radiation (Lahti,

319   2008). Furthermore, while some eggshell characteristics are heritable, others may also be

320   affected by the temporary and local availability of chemical and dietary components

321   necessary for pigment synthesis (Hubbard et al., 2010). Rainfall (Avilés et al., 2007),

322   temperature (Honza, Procházka & Pozgayová, 2012; Hargitai et al., 2016), food type (Moreno

323   et al., 2006; Hargitai, Herényi & Török, 2008), and light exposure (Navarro & Lahti, 2014)

324   also could be factors leading to changes in population trends of egg appearance. Berkowic et

325   al. (2015) revealed that cuckoo and host egg sizes also changed during a longer period. Their

326   study pointed to climatic factors influencing host-brood parasite egg morphs primarily being

327   driven by coevolution. The complexity of coevolution of host and brood parasitic eggs is also

328   demonstrated by Cherry, Bennett & Moskát (2007a). They revealed a weak matching process

329   of host and cuckoo eggs by cuckoos when they lay; i.e. cuckoos less frequently selected host

330   nests where host eggs differed from their own egg type. In this way cuckoos were selective

331   for clutch appearance when they laid, and consequently, they slightly increased the matching

332   efficiency of their eggs (see also Honza et al., 2014 for a similar result in the same host

333   species). However, regarding the unusually high parasitism rate in our study area (frequently

334 over 50%, Zölei et al., 2015, instead of the normal 5-10%, Davies, 2000), this weak selection

335 process cannot always prevent host clutches from receiving poorly-matched cuckoo eggs (see

336 Fig. 1).

337　　　　Although the eggs of some of the host species and their corresponding cuckoo eggs are

338 immaculate blue, pale blue, or white (e.g. Yang et al., 2014), the eggs of most species are

339 speckled with dark spots (Moksnes & Roskaft, 1995). Such eggshell pigments have a role for

340 coding information regarding the ownership of the eggs (Stevens, 2013), as signatures for

341 individual recognition of eggs by nest owners (Stoddard et al., 2014), or the lack of spotting

342 may also be indicative on the parasitic status of the eggs (de la Colina et al., 2012). Selection

343 may favour the development of these egg signatures (Swynnerton, 1918), which may be of

344 help in the discrimination of own eggs (Moskát & Hauber, 2007). Typically, enhanced egg

345 discrimination is observed in hosts of avian brood parasites and brood parasitism is an

346 important factor in the evolution of such traits (Stokke, Moksnes & Røskaft, 2002; Kilner,

347 2006; Igic et al., 2012; Thorogood & Davies, 2013). For example, in an experimental study on

348 great reed warblers' egg discrimination, Moskát et al. (2008a) revealed that density of small

349 spots (speckling) affected recognition of parasitic eggs. However, large spots, either in low or

350 high density, only moderately enhanced egg rejection, except when the whole eggshell

351 surface is covered with these spots, at which point rejection rates abruptly increased (Moskát

352 et al., 2008b; see also Hauber et al., 2014 for similar results on two thrush species, *Turdus*

353 spp.). Interestingly, cuckoo eggs have smaller spot sizes than great reed warbler eggs,

354 something that is clearly visible to humans at first sight and which was also supported by our

355 analyses. This may increase the deception of the parasitic eggs because small spots have

356 increased importance in egg recognition by hosts (see above). As such, the higher importance

357 of small speckling mimicry over that of larger spots suggests a role of functional mimicry,

358 which is related to great reed warblers' pattern recognition mechanisms (Moskát et al.,

359 2008a,b). Stoddard and Stevens (2010) also suggested that cuckoos may develop more

360 general characteristics (which is illustrated by high consistency of egg parameter values for

361 cuckoos) in egg mimicry rather than highly specialized characters. Good potential examples

362 for these jack-of-all-trade eggs are the garden warbler (*Sylvia borin*)-type cuckoos in Europe,

363 which appears in nests of several host species (Lovászi & Moskát, 2004), and the Horsfield's

364 bronze-cuckoo *Chalcites basalis* in Australia (Feeney et al., 2014). However, in Japan,

365 cuckoo eggs with highly specialized complex lining pattern characters were also found

366 (Nakamura, Kubota & Suzuki, 1998; Takasu et al., 2009; Moskát et al., 2012).

367   Spottiswoode and Stevens (2012) revealed in an African finch-cuckoo finch

368 relationship that egg spot characteristics appeared to be evolving independently from another

369 in a 40 years period. Interestingly, our conclusion in the common cuckoo-*Acrocephalus* host

370 system is also similar, in that several egg characteristics showed different types of change in a

371 greater than 110 year period. We suggest further analyses on changes of egg spottiness in a

372 wide range of brood parasite-host relationships. Egg collections in museums offer a good

373 basis for comparison with recent states of coevolutionary processes among hosts and brood

374 parasites.

375   The present study concentrated on changes of eggshell spottiness in time. Previous

376 studies on Hungarian great reed warblers and cuckoos investigated aspects of eggshell

377 coloration in natural parasitism (e.g. Cherry, Bennett & Moskát, 2007b; Igic et al., 2012). A

378 future study may reveal the long-term changes of eggshell coloration in this system. For

379 example, colour space modeling approaches on eggshell coloration of chaffinches (*Fringilla*

380 *coelebs*) and bramblings (*F. montifringilla*), being ancient or rarely parasitized hosts of the

381 cuckoo in Fennoscandia, revealed that importance of site fidelity versus nomadity of host

382 species, which could also lead to evolutionary changes in host eggshells, representing

383 different evolutionary trajectories (Vikan et al., 2011). Although the basic mechanism

384 affecting eggshell appearance seems to be coevolutionary interactions in brood parasitism,

385 future studies should also focus on other potential effects that could influence changes in egg

386 appearance in each party (e.g. immigration at a metapopulation scale, rainfall, solar effects,

387 and so on). Furthermore, studies on the mechanisms of how hosts use key features of eggshell

388 spotting (signatures) for egg recognition are also badly needed.

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ACKNOWLEDGEMENTS

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629   seven decades. *Journal of Avian Biology* **46:** 570–576.

630   Table 1. Description of the variables used for pattern analysis.

631

632

Name of variables	Description
Proportion energy	Expresses the dominance of the main marking size. (It can be defined as the measure of the importance of the main marking size in relation to the overall egg pattern; a high value of it indicates that the egg pattern is dominated by this spot size.)
Filter size	The dominant marking filter size reveals which filter size has the most energy, i.e. what is the most important marking size.
Total energy	The overall amplitude of the spectrum, which is essentially how contrasting the markings are against the egg background colour.
Coverage	The proportion of the egg/region (upper, middle, and base) covered with markings.
Dispersion	Degree of how concentrated the markings are across the different egg regions. (The standard deviation of pattern coverage in the three egg regions.)

633
634

635

636 Table 2. Summary table for the permutational ANOVA test for egg parameters. Legends for
637 the independent variables: year (1900s, 1930s, 1960s, and 2014), species (great reed warbler
638 and common cuckoo), topology (the top, middle and bottom regions of eggs). Significant
639 terms (p < 0.05) are shown in bold.

640

responses	explanatories	d.f.	SS	MS	iteration	P
Proportion energy						
	Year	**1**	**0.081**	**0.082**	**5000**	**<0.001**
	Species	**1**	**0.014**	**0.014**	**3167**	**0.031**
	year:species	1	<0.001	<0.001	51	0.863
	Topology	2	0.005	0.002	509	0.473
	year:topology	2	0.007	0.004	936	0.405
	species:topology	**2**	**0.017**	**0.008**	**2359**	**0.041**
	year:species:topology	2	0.004	0.002	51	0.980
	Residuals	372	1.096	0.003		
Filter size						
	Year	1	2.56	2.555	51	1.000
	Species	**1**	**8.17**	**8.167**	**4864**	**0.020**
	year:species	1	0.14	0.139	51	1.000
	Topology	2	6.90	3.448	51	1.000
	year:topology	2	1.36	0.681	69	0.841
	species:topology	2	7.77	3.885	2395	0.063
	year:species:topology	2	3.52	1.761	1478	0.063
	Residuals	372	519.55	1.397		
Log filter size						
	Year	1	0.073	0.073	51	0.804
	Species	**1**	**0.199**	**0.198**	**5000**	**0.017**
	year:species	1	0.009	0.009	239	0.297
	Topology	2	0.071	0.036	1232	0.337
	year:topology	2	0.036	0.018	852	0.437
	species:topology	2	0.170	0.085	941	0.166
	year:species:topology	2	0.068	0.034	81	0.988
	Residuals	372	12.274	0.033		
Coverage						
	Year	**1**	**0.110**	**0.109**	**4157**	**0.024**
	Species	**1**	**1.035**	**1.035**	**5000**	**<0.001**
	year:species	1	0.007	0.007	65	0.615

Topology	**2**	**1.538**	**0.769**	**5000**	**<0.001**
year:topology	2	0.048	0.024	2921	0.121
species:topology	2	0.002	0.001	51	1.000
year:species:topology	2	0.023	0.012	154	0.870
Residuals	372	6.332	0.017		

Total energy

Year	**1**	**396596**	**396596**	**5000**	**<0.001**
Species	**1**	**4268584**	**4268584**	**5000**	**<0.001**
year:species	1	131042	131042	51	1.000
Topology	**2**	**265843**	**132922**	**5000**	**<0.001**
year:topology	**2**	**83547**	**41773**	**2181**	**0.044**
species:topology	**2**	**207637**	**103818**	**5000**	**<0.001**
year:species:topology	2	5431	2716	191	0.613
Residuals	372	6399311	17202		

Dispersion

Year	**3**	**0.26**	**0.08**	**5000**	**<0.001**
Species	1	0.01	0.01	342	0.22
year:species	3	0.03	0.01	534	0.63
Residuals	120	1.76	0.01		

641

642 Table 3. Summary table for the permutational ANOVA test for egg mimicry (the difference

643 between the two species' values for each variable). See Table 1 for the explanation of variable

644 names. Legends for the independent variables: year (1900s, 1930s, 1960s, and 2014), species

645 (great reed warbler and common cuckoo), topology (the top, middle and bottom regions of

646 eggs). Significant terms ($p < 0.05$) are shown in bold.

647

Responses	explanatories	d.f.	SS	MS	iteration	P
Proportion energy						
	Year	3	0.030	0.013	179	0.363
	Topology	2	0.027	0.005	592	0.174
	year:topology	6	0.754	0.006	541	0.638
	Residuals					
Coverage						
	Year	3	0.144	0.048	2189	0.198
	Topology	2	0.003	0.001	51	0.000
	year:topology	6	0.056	0.009	251	0.916
	Residuals	132	4.153	0.031		
Total energy						
	Year	**3**	**316611**	**105537**	**5000**	**0.036**
	Topology	**2**	**229311**	**114656**	**5000**	**0.008**
	year:topology	6	239254	39876	4060	0.182
	Residuals	132	3333508	25254		
Filter size						
	Year	3	1.690	0.565	136	0.889
	Topology	22	9.120	4.563	792	0.246
	year:topology	6	3.100	0.516	179	1.000
	Residuals	132	345.83	2.619		
Log filter size						
	Year	3	0.035	0.012	53	0.943
	Topology	2	0.156	0.078	319	0.241
	year:topology	6	0.071	0.012	201	0.935
	Residuals	132	8.007	0.061		

672

673   Legend to figures

674

675   Fig. 1 Common cuckoo eggs found in Hungary in great reed warbler nests with a randomly-

676   selected host egg from the same nest. For each study period five clutches are shown. (Scale

677   was not adjusted. Photo credit Miklós Bán and Csaba Moskát)

678

679   Fig. 2 Changes in eggshell characters over time. On the graphs, the full circles show the

680   mean, while the white space between the circles and the vertical lines represent the standard

681   error for the mean, while the vertical lines denote the standard deviation of the samples.

682   Variables: proportion energy, coverage, total energy, and dispersion, C: common cuckoo

683   eggs, GRW: great reed warbler eggs, B: bottom region, M: middle region, and T: top region

684   of eggs. (See for more details in Methods.) The two species were combined where no

685   difference was revealed.

686

687   Fig. 3 Changes in eggshell total energy for mimicry between common cuckoos and great reed

688   warblers over time. Mimicry was defined as the absolute difference between the two species

689   egg characters. On the graphs, the full circles shows the mean, while the white space between

690   the circles and the vertical lines represent the standard error for the mean, while the vertical

691   lines denote the standard deviation of the samples. (Letters B, M, and T denotes the bottom,

692   middle, and top regions of eggs, respectively)

693

694   Fig. 4 Seasonal decomposition of time series data for total energy of mimicry between

695   common cuckoos and great reed warblers between 1900 and 2014. (Mimicry was defined as

696   absolute difference between the two species egg characters.) The data denote the original

697   structure of time series data, the season indicates the within-year variation patterns, whereas

698   the trend denotes the global (between-year) patterns in the data set. The remainder shows
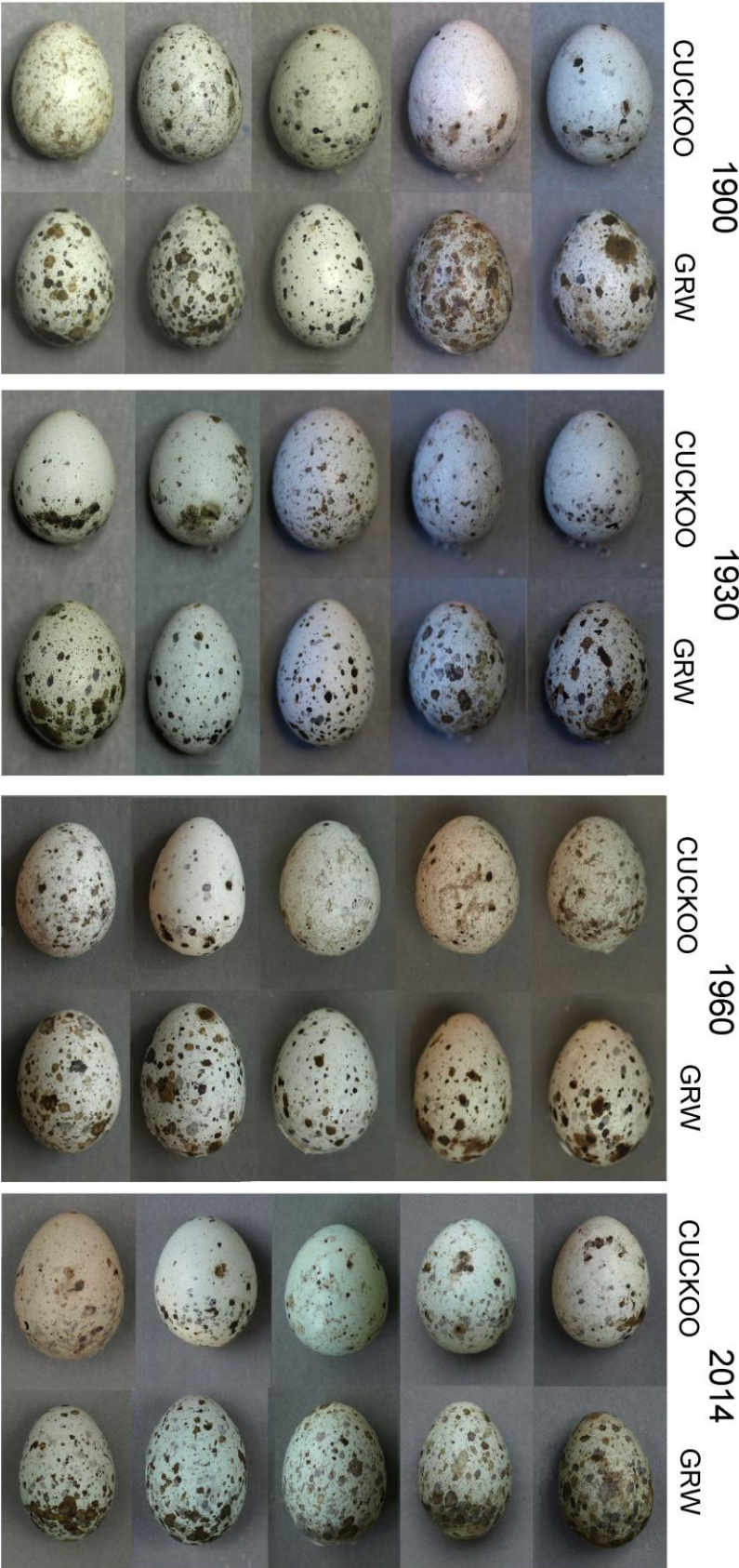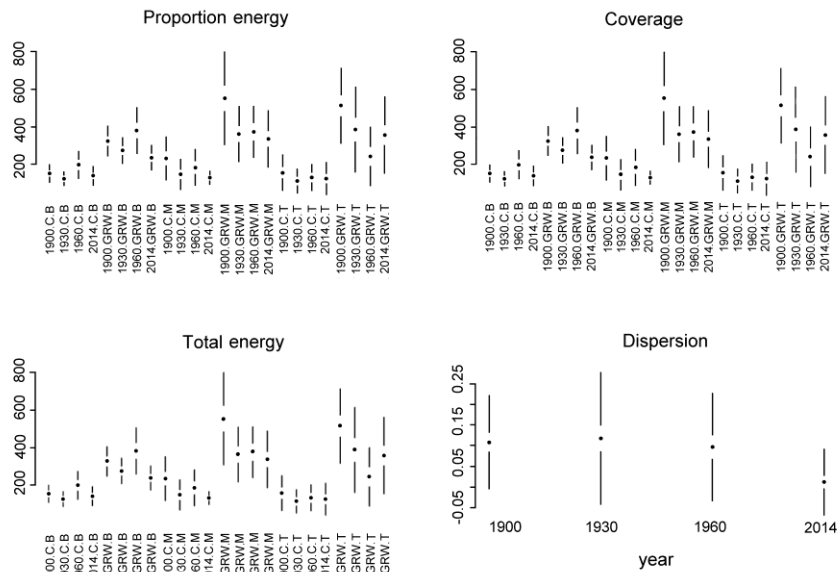
699   unexplained variances.
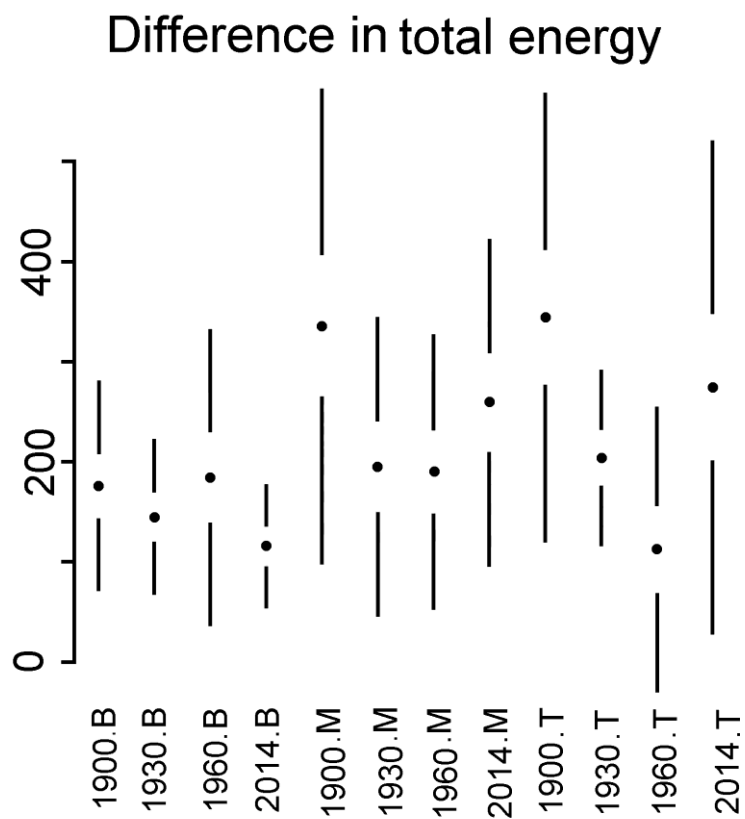
700

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704   Fig. 1

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707   Fig. 2

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710   Fig. 3

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Difference in total energy

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713

714   Fig. 4

715

Total energy

716

717

718