Surface texture heterogeneity in maculated bird eggshells

Marie R. G. Attard[1,2,3], James Bowen[2] and Steven J. Portugal[1,4,*]

[1]Department of Biological Sciences, School of Life and Environmental Sciences,

Royal Holloway University of London, Egham, Surrey, TW20 0EX, United Kingdom

[2]School of Engineering & Innovation, Open University, Milton Keynes, MK7 6AA,

United Kingdom

[3]Mapping and Geographic Information Centre, British Antarctic Survey, Cambridge

CB3 0ET, UK

[4]The Natural History Museum, Tring, Herts, HP23 6AP, United Kingdom

*Corresponding Author: Steven Portugal; email: steve.portugal@rhul.ac.uk

26 **Abstract**

27 Many of the world's 10,000 bird species lay coloured or patterned eggs. The large

28 diversity of eggshell patterning among birds, achieved through pigment, has been

29 attributed to a few selective agents such as crypsis, thermoregulation, egg

30 recognition, mate signalling, egg strength and protecting the embryo from UV.

31 Pigmentation may influence the texture of eggshells, which in turn may be important

32 for dealing with water and microbes. We measured surface roughness (S_a, nm)

33 surface skewness (S_{sk}) and surface kurtosis (S_{ku}), which describe different aspects

34 of surface texture, across 204 bird species with maculated (patterned) eggs and 166

35 species with immaculate (non-patterned) eggs. Using phylogenetically controlled

36 analyses, we tested whether maculated eggshells have different surface topography

37 between the foreground colour and background colour, and between the background

38 colour of maculated eggshells and the surface of immaculate eggshells. Secondly,

39 we determined to what extent variation in eggshell pigmentation of the foreground

40 and background colour are determined by phylogenetic relatedness, and whether

41 certain life-history traits are important predictors of eggshell surface structure. We

42 show that the surface of maculated eggs consists of a rougher foreground pigment

43 compared to the background pigment across 71% of the 204 bird species (54

44 families) investigated. Species that lay immaculate eggs showed no difference in

45 surface roughness, kurtosis or skewness compared to background pigment of

46 maculated eggs. The difference in eggshell surface roughness between foreground

47 and background pigmentation was greater among species that occupied dense

48 habitats, such as forests with closed canopies, compared to those that nest in open

49 and semi-open habitats (e.g., cities, deserts, grasslands, open shrubland and

50 seashores). Among maculated eggs, foreground texture was correlated with habitat,

51   parental care, diet, nest location, avian group and nest type, while background

52   texture was correlated with clutch size, annual temperature, development mode and

53   annual precipitation. Surface roughness among immaculate eggs was greatest for

54   herbivores, and species that have larger clutch sizes. Together, this suggests that

55   multiple life-history traits have influenced the evolution of eggshell surface textures in

56   modern birds.

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Introduction

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The colour of bird eggs and the patterns that adorn them vary widely among species. Most non-passerines – including owls (Strigiformes), some ducks (Anatidae), pigeons (Columbidae), hummingbirds (Trochilidae) and woodpeckers (Picidae) - lay pure white eggs, without obvious maculation [1]. Unmaculated eggs are believed to be the ancestral state for Aves [2], although the earliest birds may have been capable of colouring their eggs, as has been reported in some dinosaurs [3]. In contrast, most Passeriformes – perching songbirds – lay maculated eggs, typically with reddish markings concentrated as a ring on the blunt end [4,5]. Nearly all colour of bird eggshells is derived from only two pigments; protoporphyrin IX, responsible for the red-brown colouration, and biliverdin, for the blue-green colouration [6]. Eggshell maculation is thought to serve numerous functions, including crypsis, thermoregulation and microbial defence. In passerines, it has been proposed that maculation may additionally aid as a defence mechanism against possible attack from avian brood parasites. For example, the eggs of tawny-flanked prinias (*Prinia subflava*) have a uniform background colour with coloured blotches and fine squiggles scrawled over the entire shell, which are difficult to replicate by parasitic cuckoo finches (*Anomalospiza imberbis*), allowing hosts to identify and reject foreign eggs [4]. In some species, the markings reinforce structural integrity in regions where the eggshell has thinned [1], while in others patterning may serve as a sexually selected signal through which a female can advertise the quality of both herself and her imminent nestlings [7,8, and references therein]. Most cavity-nesters lay white eggs as they are already well hidden from predators and hard to see, so colour

101 would be very little use as signals, while ground-nesting shorebirds that lay their

102 eggs in exposed locations tend to have strongly patterned eggs that blend in well

103 with their nest environment, demonstrably increasing offspring survival [9]. The

104 extent of eggshell variation across birds is remarkable, but a function of egg

105 patterning that has received comparably less attention is the surface texture it

106 creates.

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108 Surface texture, or surface topography, refers to the three-dimensional features of a

109 surface, defined by surface orientation and roughness [10]. Surface roughness

110 relates to its waviness (height) and asperity; the deviation of a surface from its mean

111 plane is characterised by variance of the height to form peaks and valleys [11].

112 Roughness influences surface functionality – not only surface properties such as

113 hydrophobicity, optical and plasmonic behaviour, adhesion and friction – but also

114 bulk properties, such as fracture toughness and fatigue resistance [12, and

115 references within]. There are numerous examples of smooth and rough surfaces in

116 nature, each fulfilling a specific function. Surface roughness impacts the ability of

117 water droplets to attach to the surface, and thus impacts bacteria adhesion and

118 removal. For example, fruits or vegetables with rough surfaces (e.g., oranges,

119 avocados and cantaloupes) are better protected from bacterial infection compared to

120 fruits with smooth surfaces (e.g., apples) [13]. Sun beetles (Pachnoda *spp.*) can

121 more easily grasp rougher substrate particles with their claw tip compared to smooth

122 substrate particles, as rough surfaces generate a greater friction force [14]. Similarly,

123 the glossy appearance of tinamou eggshells is produced by an extremely smooth

124 cuticle causing light to reflect in the specular direction, whereas the matt appearance

125 of chicken (*Gallus gallus*) eggs is produced by having a rough cuticle, causing light

126 to be scatted in multiple directions [15]. Thus, it is apparent that the texture of the

127 surface has implications for bacterial removal, visual signalling and light responses.

128 How these patterns pan out across a broader spectrum of the avian phylogeny has

129 yet to be determined.

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131 Mroz *et al*. [16] noted that the maculated eggs of turkey (*Meleagris gallopavo*) have a

132 different surface structure on the foreground colour versus background colour, and in

133 turn, this base colour was similar to immaculate eggs laid by the same species [16].

134 The difference in roughness between foreground and background pigment has never

135 been properly quantified in birds, and it is unknown whether differences in surface

136 textures of immaculate and maculate eggs is universal, or what may be determining

137 this potential difference. Based on Mroz's findings, Attard *et al*. [17] proposed that

138 eggshell roughness will be greater on the foreground – the colour maculated parts –

139 than the background base pigmentation of species with maculated eggs. In the

140 present study we set out to test this. We hypothesised that (*1*) foreground

141 pigmentation would be rougher and more peaked than background pigmentation, (*2*)

142 there would be no distinction between the background pigmentation of maculated

143 eggs and the pigmentation of immaculate eggs, and (*3*) variation in the surface

144 topography of the foreground and background pigmentation would be influenced by

145 life-history traits associated with the nest environment (see table 1 for full definitions

146 and hypotheses). To test these hypotheses, we compiled high-resolution three-

147 dimensional scans of avian eggshells from 486 species to characterise their surface

148 structure.

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2. Materials and Methods

2.1 Egg sampling

Empty eggshells from 486 species (1,838 eggs) were sampled at the Western Foundation of Vertebrate Zoology (WFVZ) at Camarillo, United States of America and Natural History Museum (NHM) at Tring, United Kingdom. Fragments (surface area ~1.5 cm^2) were cut from the equatorial region of each egg using a micro-tool rotary saw with diamond-coated thin cutting wheel (Dremel 4000, Bosch Leinfelden, GER). Eggshell fragments were gently cleaned with a cotton bud dipped in distilled water, then allowed to dry for at least 24 h. Eggshells coated with a thin, chalky layer of vaterite were not cleaned as vaterite crystals dissolve upon contact with water [35].

2.2 Interferometer

The surface topography of eggshell surfaces was obtained using a three-dimensional non-contacting optical profilometer (LeicaSCAN DCM3D, Leica Microsystems, Germany) connected to a white light interferometric microscope. For each eggshell fragment, a section along the surface was scanned at three non-overlapping locations at a focal depth of 100 µm (100 focal planes at 1 µm resolution) using the 20 x objective magnification to give a measurement area of 636.61 x 477.25 µm^2 (pixel resolution = 768 x 576).

During scanning, maculation was recorded for each eggshell fragment as 0 – if the fragment was immaculate, 1 – for maculation present but with a clear, dominant background colour that was able to be separated from the foreground, and 2 – for

176 widespread maculation that covered most of the fragment that could not be

177 separated from background [36]. For maculated (spotted, squiggly or blotched)

178 eggshells, the foreground and background pigment was scanned separately, each in

179 three different locations (total 6 scans per fragment). Scans were manually cropped

180 in Scanning Probe Image Processor, SPIP version 4.4.3.0 (Image Metrology,

181 Hørsholm, Denmark) if both foreground and background pigments were in the image

182 so only the region of interest remained. Eggs that were too difficult to divide into

183 foreground and background pigments (e.g., densely speckled eggshells) were

184 excluded from the study. Cropped scans, and scans that did not require cropping

185 (total 7,013 scans), were then transferred to SPIP for processing and analysis. We

186 used the plane correction tool to automatically correct plane distortions in the data

187 using polynomial functions. In this case, a second-order polynomial was used as the

188 slope on the data was linear. The mean z-value was then adjusted to 0. Scans which

189 had elevation data for less than 40% of pixels were manually inspected in SPIP to

190 determine if the scan quality was sufficient for analysis. Some scans were cropped to

191 remove low quality regions of the scan for analysis. Scans were taken under a

192 narrow focal plane, so the centre of the field of view usually captured the topography

193 well and were suitable for inclusion in the analysis, even where pixel information was

194 absent around the scan edges. Scans of brood parasite eggshells (total 40 scans

195 from 5 brood parasite species) were excluded from the study, as they represent a

196 unique example of an unusual breeding strategy, which benefits from specific

197 eggshell adaptations [37]. However, we have provided eggshell surface texture

198 values for brood parasites that were scanned within the Figshare repository.

199

200 After removing brood parasites, surface texture values from multiple locations on the

201 same egg were averaged for immaculate eggs, and maculated eggs with a score of

202 1 to obtain a specimen mean value. Cook's distance was applied to specimen S_a, S_{sk}

203 and S_{ku} values (see below for definitions) using R statistical software (R v4.1.2) [38]

204 to identify outliers and/or influential values, which were subsequently removed from

205 analysis [39]. We retained surface texture measurements from 1,873 specimens

206 across 345 species after removing low-quality scans and influential values. S_a, S_{sk}

207 and S_{ku} values were averaged per species from specimen-specific values for

208 phylogenetic comparative analyses.

209

210 Scans were processed using SPIP to quantify surface roughness (S_a, nm), surface

211 skewness (S_{sk}) and surface kurtosis (S_{ku}) (figure 1). S_a expresses, as an absolute

212 value, the difference in height of each point compared to the arithmetical mean of the

213 surface [40]. Surface skewness describes the asymmetry of the height distribution

214 histogram. If $S_{sk} = 0$, a symmetric height distribution is indicated. If $S_{sk} < 0$, it can be

215 a bearing surface with holes and if $S_{sk} > 0$ it can be a flat surface with peaks. S_{sk}

216 values numerically greater than 1.0 may indicate extreme holes or peaks on the

217 surface [40]. The surface kurtosis (S_{ku}) describes the "peakedness" of the surface

218 topography. Smaller values indicate broader height distributions and vice versa for

219 values greater than 3.0. S_a, S_{sk} and S_{ku} are based on surface height distribution [41]

220 and are scale-dependent (figure 1). Therefore, these measures depend on the

221 available measurement scale and the sampling interval of the measurement

222 technique. As the distribution of S_a and S_{ku} values across species were skewed, we

223 log-10 transformed these response variables to achieve a normal distribution for

224 statistical analysis. S_{sk} values were not transformed as they had a normal distribution

225 across species.

226

2.3 Life-history and ecological data

228 We collected literature data on 13 life-history traits (table 1) that represent different

229 ecological and mechanistic causes of eggshell surface texture heterogeneity across

230 modern birds. Ecological explanations address evolutionary function, such as why

231 eggshell heterogeneity in maculated eggs exists, while mechanistic explanations

232 address how interspecific differences in eggshell texture are achieved [39]. Table 1

233 lists the hypotheses, rationale and definitions of each predictor. The life-history traits

234 investigated include different aspects of parent behaviour, embryo development,

235 nest environment and climate conditions that could potentially influence the evolution

236 of avian eggshell texture, and surface heterogeneity in maculated eggs.

237

2.4 Phylogenetic comparative analysis

239 Phylogenetic comparative analyses and plots were processed in R. To analyse the

240 evolution of surface texture among maculate and immaculate bird eggshells within a

241 comparative context, we used the most complete molecular phylogeny of extant bird

242 species (available from http://www.birdtree.org) to generate 10,000 trees using the

243 primary backbone of Hackell *et al*. [42] for all species in our study. Pagel's lambda

244 (λ) was used to determine the extent of phylogenetic signal for S_a, S_{sk} and S_{ku} for the

245 foreground and background pigment of maculated eggs and eggshell surface of

246 immaculate eggs. Phylogenetic signal was measured using the *phylosig* function in

247 the package 'phytools' [43]. At $\lambda = 0$ the trait of interest may vary randomly across a

248   phylogeny while at $\lambda = 1$, closely related species tend to exhibit more similarity in trait

249   expression.

250

251   The association between eggshell texture and life-history traits was assessed using

252   Phylogenetic Generalised Least Squares (*pgls*) regression models to account for

253   phylogenetic uncertainty in comparative analyses across species. Each *pgls* model

254   tested one or more comparative hypotheses using an entire set of equally likely

255   trees. We ran two simple *pgls* models: firstly, to test if the foreground to background

256   pigmentation of maculated (patterned) eggs is significantly different, and secondly, to

257   test if the base colour of species with maculated eggs is significantly different to

258   species with immaculate (non-patterned) eggs.

259

260   For each maculated egg, we calculated the difference between foreground and

261   background pigment S_a, S_{sk} and S_{ku} values to assess the degree of contrast in

262   surface texture, referred to herein as ΔS_a, ΔS_{sk} and ΔS_{ku}, respectively. More positive

263   values indicated the foreground pigment has greater roughness or texture than the

264   background pigment. This ratio was averaged across all eggs from the same species

265   to use as the response variable for phylogenetic generalised least squares (*pgls*)

266   modelling to investigate drivers of species-specific differences in the contrast ratio

267   between foreground and background surface textures across species that lay

268   maculated eggs. We ran a separate series of *pgls* models using actual values of

269   foreground and background pigmentation for maculated egg as the response

270   variable and included climate and life-history traits as predictors. Likewise, we ran a

271   separate series of *pgls* models to assess the influence of life-history traits on the

272   evolution of eggshell surface topography among species that lay immaculate eggs.

273 Sample sizes and categorical predictors included in each *pgls* analysis are provided

274 in table S1. Pagel's lambda (λ) [44] was measured for foreground, background and

275 delta values using the package 'phytools' [45] to determine to what extent related

276 species were more likely to share similar roughness values for maculated eggs.

277

278 We tested for collinearity amongst pertinent life-history traits, and only selected

279 uncorrelated variables (with paired-correlation less than <0.75) and VIF under 10 as

280 predictor variables (see Attard *et al*. [17,39] for details). We included up to 5

281 predictors per *pgls* model. For *pgls* using multiple predictors, we performed

282 conditional model-averaging for *pgls* models with a delta Akaike Information Criterion

283 (AICc) score of ≤ 2 relative to the top-ranked model. Model averaging allows us to

284 average the parameter estimates across multiple models that best explain the data

285 and avoids the issue of model uncertainty [46].

286

287 **3. Results**

288

289 In this study, we measured and compared the surface roughness (S_a), surface

290 skewness (S_{sk}) and surface kurtosis (S_{ku}) among maculated and immaculate avian

291 eggshells. These parameters describe aspects of surface texture. S_a is defined as

292 the difference in height of each point compared to the surface average, S_{sk} is the

293 degree of asymmetry of the height distribution histogram (i.e., if the surface is flat

294 with peaks or is a surface with holes), and S_{ku} describes the broadness of the

295 surface peaks.

296

3.1 Differences in pigment surface topography in patterned and non-patterned avian eggshells

The correlations between S_a, S_{ku} and S_{sk} for foreground pigment, background pigment and pigmentation on immaculate eggs were weak, confirming that these surface structure variables provide different quantitative information about eggshell surface characteristics (table S2). As such, each of these values were used to represent different aspects of eggshell surface texture in our analysis.

Among the 187 species analysed with maculated eggs, 71% had higher S_a values for the foreground than background regions (figure 2). Digital elevation models for several species with the most extreme difference in foreground and background roughness are shown in figure 3. S_{sk} and S_{ku} values were higher in the foreground versus background pigmentation for 55% and 47% of species, respectively. There was a significant positive correlation in the foreground region relative to the background region for S_a (*pgls*: Estimate=0.43, R^2=0.10, *p*<0.001) and S_{sk} (*pgls*: Estimate=0.24, R^2=0.05, *p*<0.001), meaning that when foreground pigment has higher eggshell roughness and symmetry of surface heights above the mean plane, so too will the background pigment. However, there was no association between foreground and background S_{sk} (*pgls*: Estimate=0.02, R^2<0.01, *p*=0.70) values. Together, this suggests that overall, among maculated eggs, the foreground pigment tends to be rougher and slightly more symmetrical for any peaks present compared to background pigment, but differences in surface peakedness is variable between species. Species that lay immaculate eggs (*n*=174) showed no difference in S_a (R^2=-0.00, *p*=0.34), S_{ku} (R^2=-0.05, *p*=0.99) and S_{sk} (R^2=-0.02, *p*=0.43) values compared to the background pigment of species with maculated eggs (*n*=192).

322

3.2 Phylogenetic signal for eggshell surface topography

There was a weak phylogenetic signal for surface texture difference between foreground and background pigment ($\lambda=0.06$ for ΔS_a, and $\lambda=0.00$ for ΔS_{sk} and ΔS_{ku}), which were significantly different from 1 ($p<0.001$), but not significantly different from 0 ($p=0.22-1.00$) (table 2). This means that heterogeneity in eggshell surface texture among species with maculated eggs has evolved mainly independently of phylogeny and close relatives are no more similar than distant relatives [47]. The phylogenetic signal for most eggshell topographical measures was low ($\lambda = 0.00-0.39$) and significantly different from 1 ($p<0.001$) for maculated eggs when pigment types were analysed separately. For foreground and background colour, the phylogenetic signal for S_{sk} was significantly different from 0 and 1 ($p<0.001$ and $p<0.001$, respectively), whereas S_a and S_{ku} were not significantly different from 0 (see table 2). Species with immaculate eggs had no phylogenetic signal for S_{sk} ($\lambda=0.00$, $p=1.00$ for $\lambda=0$, $p<0.001$ for $\lambda=1$), and intermediate phylogenetic signal for S_a ($\lambda=0.30$, $p=0.03$ for $\lambda=0$, $p<0.001$ for $\lambda=1$) and S_{ku} ($\lambda=0.68$, $p<0.001$ for $\lambda=0$ and $\lambda=1$). Among species that lay immaculate eggs, S_{sk} has evolved independently of phylogeny, whereas both phylogeny and life-history contribute to the evolution of S_a and S_{ku}.

340

3.2 Association between topographic heterogeneity and life-history among maculate eggshells

Species that occupy dense habitats had higher ΔS_a than those in semi-open ($z=2.01$, $p=0.04$) and open habitats ($z=2.29$, $p=0.01$) (figure 4, table S3). Dense habitats included forests with a closed canopy, or species that live in lower vegetation strata of dense thickets, shrubland, mangroves or marshland, whereas open habitats

347   included deserts, open water, grassland, seashores and cities, and semi-open

348   habitats included open shrubland, parkland and forest edges [34]. None of the life-

349   history traits were significantly associated with ΔS_{sk} or ΔS_{ku} (table S4-S5).

350

351   **3.3 Influence of life-history on foreground and background eggshell**

352   **pigmentation**

353   When running *pgls* models for S_a, S_{sk} and S_{ku} against body mass, background S_a

354   and foreground S_{ku} values were significantly (positively) associated with body mass

355   (Estimate=0.10, R^2=0.02, *p*=0.03 and Estimate=0.03, R^2=0.06, *p*<0.001,

356   respectively). Background S_{ku} significantly increased with higher annual precipitation

357   (*z*=2.72, *p*<0.01; figure 5*a*) and clutch size (*z*=0.05, *p*=0.02; figure 5*b*) (table S6).

358   Background S_{sk} was negatively associated with larger clutches (*z*=3.70, *p*<0.001;

359   Figure 5c) and higher annual temperature (*z*=2.23, *p*=0.03; figure 5*d*) (table S7).

360   Species with altricial young had lower S_{sk} background values than species with

361   precocial young (*z*=4.84, *p*<0.001; figure 5*e*). None of the life-history traits were

362   significantly associated with S_a for the background pigment (table S8-S9).

363

364   S_a of the foreground pigment was lower among species occupying open habitats

365   compared to dense habitats (*z*=3.20, *p*=0.001; figure 6*a*), species that return to the

366   nest with wet plumage (*z*=1.99, *p*=0.05; figure 6*b*), and omnivores compared to

367   herbivores (*z*=2.02, *p*=0.04; figure 6*c*) and insectivores (*z*=2.93, *p*=0.001; figure 6*c*)

368   (table S09). Species that nest close to (or on) water had higher foreground S_{ku} than

369   species that nest above (*z*=2.98, p<0.01) or on the ground (*z*=3.02, *p*<0.01) (figure

370   6*d*, table S11). Foreground S_{sk} was significantly higher in species that nest on the

371   ground than above ground (*z*=2.59, *p*=0.01; figure 6*e*), and in Passeriformes

372 compared to non-Passeriformes (z=2.59, p=0.01, figure 6f). Species that lay their

373 eggs in enclosed nests also had higher foreground S_{sk} compared to species that use

374 exposed (z=2.77, p=0.01) or semi-enclosed (z=2.02, p=0.04) nests (figure 6g, table

375 S11).

376

377 **3.4 Association between eggshell surface texture and life-history among**

378 **immaculate eggshells**

379 Among the 174 species analysed with immaculate eggs, S_a was found to be

380 significantly higher in herbivores than omnivores (z=3.68, p<0.001), invertivores

381 (z=3.12, p<0.001) and carnivores (z=1.98, p=0.05) (figure 7a), and was negatively

382 associated with clutch size (z=2.60, p=0.01; figure 7b) (table S12). None of the life-

383 history traits were significantly associated with S_{ku} or S_{sk} among species with

384 immaculate eggs, based on conditionally averaged models (table S13-S14).

385

386 **4. Discussion**

387 We found that the surface of maculated eggs consists of a rougher foreground

388 pigment compared to the background pigment across 71% of the 204 bird species

389 (54 families) investigated. For maculated eggs, the texture of the foreground was

390 primarily correlated with parental care, habitat, diet, nest location and nest type,

391 while background texture was correlated with annual temperature, clutch size,

392 development mode and annual precipitation. For those species which lay

393 immaculate eggs, there were no differences in surface roughness (S_a), kurtosis (S_{sk})

394 or skewness (S_{ku}) compared to the background regions of maculated eggs. Surface

395 roughness among immaculate eggs was greatest for herbivores, and species that

396 have larger clutch sizes. The phylogenetic signal was low for our measured traits,

397 suggesting that multiple life-history traits are likely governing the evolution of

398 eggshell surface textures, and the eggshell surface properties measured have

399 evolved independently of phylogeny; close relatives are no more similar than distant

400 relatives.

401

402 **4.1 Surface roughness (S_a)**

403

404 The phylogenetic signal for S_a was low, suggesting life-history traits are contributing

405 more to the relationships between background and foreground S_a. Despite this, none

406 of our measured life-history traits were highlighted as significantly influencing S_a,

407 potentially meaning an alternate trait not included in our analyses may be

408 determining S_a. One such factor may be dominant nest material; we did not include

409 nest materials in our analyses due to the high variability of such materials used

410 within a species. Another element it was not possible to determine for heavily

411 maculated eggs was the percentage of surface area covered with foreground versus

412 background pigment. Thus, it is feasible that an eggshell could exhibit a high

413 foreground-to-background ratio, but the functionality of that ratio may be unclear if

414 the foreground pigment consists of only a few spots in total.

415

416 Among the non-Passeriformes, foreground S_a was highest for Kentish plovers

417 (*Charadrius alexandrinus*), razorbills (*Alca torda*), black vultures (*Coragyps atratus*)

418 and killdeers (*Charadrius vociferus*). Killdeer and Kentish plovers are ground-nesting

419 shorebirds, and their nests are a shallow depression or scape lined with shells,

420 pebbles, grass and leaves [48,49]. Patterning on plover eggs have been proposed to

421 help strengthen the eggshells along with providing crypsis [50,51]. Killdeer prefer

422   lighter-coloured nesting materials, either to help keep the nest cool or conceal it [48].

423   In Kentish plover eggshells, the relative quantities of protoporphyrin to biliverdin are

424   positively correlated with the fractional dimension (FD; a higher FD correlates with

425   greater surface complexity) of spottiness, meaning that a combination of eggshell

426   patterning characteristics (spot size and distribution, degree of spottiness and

427   convolution of the spot outlines) influence the relative quantities of each pigment.

428   Eggshell patterning is proposed to partly determine the propagation of cracks [52],

429   as eggshells with a higher fractional dimension of spottiness may require more

430   fracture energy to crack than maculated eggs with a lower fractional dimension, and

431   therefore should be more resistant to breakage [50]. In our study, we found that most

432   species with maculated eggs had a rougher foreground pigment compared to the

433   background pigment. As crack length in heterogeneous materials is smaller than in

434   more homogenous materials [50], we propose that eggs of species with a greater

435   difference in surface texture among foreground and background pigments are more

436   resistant to crack propagation.

437

438   **4.2 Surface skewness (S_{sk})**

439

440   We found that species which nest on the ground, have enclosed nests, or are

441   Passerines, have a higher foreground S_{sk} compared to the background part of the

442   shell. A high skewness reflects more 'pointed' peaks with a flatter surface between

443   peaks, while low skewness describes more 'rounded' peaks and no flat surface

444   between peaks. Thus, such holes in the background component of the eggshell may

445   indicate the presence of pores, suggesting pores on the eggshell surface of ground-

446   nesting species, those in enclosed nests and those in the Passerines group, are

447 present more in the background of the shell, rather than the foreground pigmented

448 regions. In certain bird species studied (e.g., Gavidae) it has been observed that

449 pigment can run down the pore channel and into the mammillary cones at the bottom

450 [53], suggesting that pores are not exclusively found only in the background part of

451 the shell, but also found in what would be considered the foreground pigmented

452 areas. In many instances, pore mouths are not visible on the surface at all, often

453 instead being hidden from view by the cuticle [53]. However, there can be indications

454 of pore openings present in the deeper valley-like regions of the shell, and in those

455 species where a cuticle is entirely absent [54]. The distribution and difference in pore

456 openings between species, and whether these coincide with foreground pigment

457 spotting, could potentially be linked to the presence or absence of a cuticle, or to

458 specific structures that are found on eggs that potentially deal with heavy dirt or

459 detritus. For example, eggshell cuticles – made of organic matter – are prevalent in

460 precocial species (e.g., common quails, common ostriches (*Struthio camelus*),

461 greylag geese), but absent in many altricial species studied (e.g., feral pigeons

462 (*Columba livia*), canaries (*Serinus canaria*), budgerigars (*Melopsittacus undulatus*))

463 [54]. A lack of cuticle may explain why we found that S_{sk} in the background colour of

464 the eggshell of altricial species was lower when compared to those which are

465 precocial. A total lack of a cuticle will have implications for the overall eggshell

466 surface heterogeneity and topography, particularly influencing how pores appear on

467 the outer surface of the shell, and thus influencing S_{sk}.

468

469 Pore structure itself may further influence the degree of peakedness of the surface

470 (S_{ku}) and overall surface topography. Although pores are typically – but not always –

471 covered in amorphous organic matter, different pore types may influence the S_{ku} of

472   the egg surface. For example, pore types that are characterised by extensive

473   branched pores venting into grooves present in the external shell surface (seen in,

474   e.g., American rheas (*Rhea americana*)) [55] are likely to have a greater influence on

475   S_{ku} and S_{sk} than those pore types such as a simple, unbranched, funnel-shaped

476   pore, seen in many bird species [55]. For certain species, particularly those typically

477   associated with aquatic environments, unique eggshell structures are present, which

478   in turn are likely to influence S_a, S_{sk} and S_{ku}. For example, spheres on the outer

479   surface of the shell of grebes (Podicipediformes) contain calcium phosphate [56],

480   while the shells of cormorants (Phalacrocoracidae), flamingos (Phoenicopteridae)

481   and anis (Crotophaga) contain the calcium polymorph vaterite [35,57,58]. The

482   implications of these structures on overall surface heterogeneity is currently poorly

483   understood.

484

485   For a number of species, further examination of the brown (likely protoporphyrin)

486   pigment spots showed the presence of needle-shaped calcite crystals which were

487   morphologically markedly different from the main calcite crystals [50]. This

488   morphological difference in pigment calcite crystals has not been quantified across a

489   variety of species, but may be a contributing factor to both the difference in our

490   measured parameters (S_a, S_{sk}, S_{ku}) between species, and also between the

491   background and foreground measurements. Although a focus of our study was the

492   comparison of background and foreground parts of the eggshell, immaculate

493   eggshells are not themselves devoid of pigment. The eggshells of scops owls (*Otus*

494   *scops*), Barbary doves (*Streptopelia risoria*) and white storks (*Ciconia ciconia*) all

495   contain detectable quantities of protoporphyrin despite being immaculate and white

496   to the human eye, while the immaculate eggshells of black-footed penguins

497 (*Spheniscus demersus*) and common woodpigeons (*Columba palumbus*) contain

498 both protoporphyrin and biliverdin [59]. The presence of these pigments in

499 immaculate eggshells further brings into their question the true function of these

500 pigments, while confirming that one single hypothesis for the role of pigmentation is

501 unlikely to explain its diversity, presence and absence in eggshells.

502

503 **4.3 Surface kurtosis (S_{ku})**

504

505 Tyler [60] noted that in the eggshells of divers (Gaviidae), the surface resembled

506 something akin to flat hillocks with valleys in-between, with the brown pigment spots

507 giving a rough overall appearance to the shell where pigments spots were present

508 on the outer surface. Tyler also observed, however, that these pigment spots were

509 not just on the outer surface of the shell, but rather were found throughout the shell

510 itself, at depths that would be invisible from just looking at the outer surface. The

511 presence of brown – assumingly protoporphyrin – spots within the shell itself raises

512 questions about their function. For example, we found that species which nest close

513 to water have higher foreground (pigment) S_{ku} compared to background. It could be

514 hypothesised that this higher S_{ku} may contribute to preventing water from clogging up

515 pores and generally making the surface dirty, i.e., increase hydrophobicity. However,

516 if the pigment spots are occurring throughout the entirety of the shell – not just the

517 outer surface – this suggests that the function goes beyond just contact with water or

518 detritus. A fruitful further investigation (see Future Directions, below) would be to

519 measure the properties of those pigment spots contained within the shell itself, and

520 determine if their properties match that of the outer surface pigment spots. If the

521 surface properties of those pigment spots within the shell match that of the outer

522 surface, it suggests they perform a similar function, but that this function is unlikely to

523 be related to water or detritus on the outer shell surface. Such functions for internal

524 pigment spots are more likely to be linked to thermoregulation [2,61]. For example,

525 Maurer *et al*. [61] demonstrated that darker pigment spots acted as a form of

526 'sunblock', reducing the transmission of potentially harmful UV rays through the

527 shell, protecting the developing embryo. Pigment spots on both the outer surface

528 and those contained within the shell can both act to reduce UV transmission. If,

529 however, upon further investigation, the properties of the outer surface pigment and

530 inner pigments spots are different, this suggests that (*i*) while appearing visibly

531 similar, pigments spots can have differing structural properties, and (*ii*) the function

532 of pigment spots are, as has been previously suggested [e.g., 2], not mutually

533 exclusive. In this instance, pigment spotting contained within the shell could act as

534 UV shielding and increasing structural integrity, while pigment spots on the outer

535 shell surface, with higher S_{ku} than the background base colour, may act to reduce

536 adhesion of detritus, water and, in turn, potential harmful microbes.

537

538 Prior work on eggshell nanostructures and cuticle functionality highlighted the role

539 that risk of microbial infection and caking of the eggshell in detritus plays in

540 determining eggshell surface properties. For example, eggs of species that typically

541 nest in damp and humid environments exhibit higher rates of gas exchange under

542 standard conditions than those of species which nest in drier environments [32], and

543 have cuticular nanospheres present on the outer surface of the eggshell [62]. These

544 cuticular nanospheres prevent the accumulation of water on the eggshell surface,

545 helping protect the egg from bacterial and microbial infection. Interestingly, in the

546 present study, we found that the eggs of birds who were found in regions which

547   experienced relatively high levels of precipitation showed the opposite trend in S_{ku} to

548   those birds which nest close to or near water. Birds inhabiting areas with high

549   precipitation showed a higher background base-colour S_{ku} to the pigmented

550   foreground regions. For methodological reasons, we were unable to measure

551   relative foreground-background S_a, S_{ku} and S_{sk} in species whose eggs were heavily

552   maculated, as it was not possible to differentiate between background base colour

553   and pigment spotting. Therefore, for those species we did measure that exhibit

554   maculation, the background base-colour was the dominant surface. Thus, it is

555   possible that a higher S_{ku} for the background has positive advantages for dealing

556   with heavy precipitation; the same pattern was observed in S_{sk}. As S_{ku} describes the

557   "peakedness" of the surface topography, it is possible that a background eggshell

558   surface structure where the S_{ku} is higher assists with dealing with the higher

559   precipitation.

560

561   **4.4 Future directions**

562   While our study measured 1,838 eggs from 486 species, understanding how S_a, S_{sk}

563   and S_{ku} vary between background and foreground eggshell surfaces across a wider

564   selection of species would be beneficial. Moreover, studying the eggshells of species

565   with maculated eggs that have a large-scale global distribution across multiple

566   environmental gradients would help decode the functional reasons behind eggshell

567   surface properties, and the relationship between foreground and background S_a, S_{sk}

568   and S_{ku}. Such species could include peregrine falcons (*Falco peregrinus*), ospreys

569   (*Pandion haliaetus*), house sparrows (*Passer domesticus*) and barn swallows

570   (*Hirundo rustica*) which have extensive distributions, spanning multiple

571   environmental gradients. Establishing whether the relationships identified between

572　foreground and background S_a, S_{sk} and S_{ku} in the present study are consistent – or

573　not – across differing environments but within species would help determine the

574　likely function of these background/foreground differences.

575

576　Eggshell properties are known to be a labile trait [63]. For example, studies focusing

577　on museum collections demonstrated that the eggs of reed warblers (*Acrocephalus*

578　*scirpaceus*) were brighter in years with higher rainfall, and were bluer and greener in

579　colder years [64]. Such studies suggest that changes in colour have a structural

580　function, and may aid in thermoregulation and dissipation of water. In the present

581　study we used eggs with limited temporal data associated with them, particularly

582　those eggshells from the destructive collection at the Natural History Museum in

583　Tring [e.g., 61]. Moving forward, eggshells in museum collections with date

584　information could be used to determine how the intra-specific relationships between

585　background and foreground S_a, S_{sk} and S_{ku} change over time in response to different

586　wet seasons and broader scale climate changes. Similarly, determining the

587　consistency of the relationships between background and foreground S_a, S_{sk} and S_{ku}

588　within clutches would be valuable. For example, the last egg to be laid within a clutch

589　is typically distinct from the others due to different patterning of the maculation [2].

590　Thus, how these differences in the degree of maculation manifest in structural

591　properties would be important, and suggests the last egg may be different in its

592　properties.

593

594　Comparatively little is currently known about how pigment deposition varies between

595　species [8], and the potential implications this may have on textural properties.

596　Whether protoporphyrin or biliverdin is deposited deeper within the shell could likely

597　　influence the difference in S_a, for example, between the foreground and background.

598　　Moreover, while many studies work on the assumption that eggshell colour is derived

599　　from just these two pigments, recent studies on the eggshells of tinamous

600　　(Tinamidae) discovered the presence of two additional pigments; uroerythrin and

601　　bilirubin [65]. These two pigments likely offer different potential structural functions

602　　beyond the currently proposed functions of protoporphyrin and biliverdin. These

603　　pigments may contribute to the glossy nature of the eggs of tinamous, for example,

604　　in conjunction with nanostructured surface calcite and calcium phosphate crystals

605　　[15]. Hamchand *et al.* [65] noted that uroerythrin and bilirubin were photodegradable,

606　　suggesting these pigments have additional functions beyond what has been

607　　proposed for protoporphyrin. Thus, moving forward, a greater number of species

608　　require their pigment type to be determined, and their respective quantities

609　　extracted, rather than assuming only protoporphyrin and biliverdin are present.

610

611　　Lastly, studies showing that the location of pigment spots can, for some species,

612　　mark areas of thinner eggshell due to lower calcium availability [1,8] warrants further

613　　investigation with respect to eggshell surface properties. While this phenomenon has

614　　only been documented in a few species, experimental manipulation of calcium

615　　availability would provide the opportunity to ascertain if pigment spots present on

616　　regions of thinner eggshell due to low calcium availability differ in their S_a, S_{sk} and

617　　S_{ku} properties in comparison to pigment spots present on eggshells produced in

618　　areas with plentiful calcium availability.

619

Data accessibility

The data and code used for statistical analysis are available at:

https://figshare.com/s/ccb5e8588944af1ea529. Statistical outputs are provided in

the electronic supplementary material.

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Conflict of interest

The authors declare no conflict of interest.

Author's contributions

S.J.P., J.B. and M.R.G.A. developed the project. M.R.G.A. collected and analysed
the data and prepared figures. J.B. provided access and training on the
interferometer. S.J.P., J.B. and M.R.G.A. and contributed to the interpretation of the
findings. M.R.G.A. wrote the first draft of the manuscript, which all authors then
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648

References

650 1.   Gosler AG, Higham JP, Reynolds SJ. 2005 Why are birds' eggs speckled?

651   *Ecol. Lett.* **8**, 1105–1113. (doi:10.1111/j.1461-0248.2005.00816.x)

652 2.   Kilner RM. 2006 The evolution of egg colour and patterning in birds. *Biol. Rev.*

653   **81**, 383–406. (doi:10.1017/S1464793106007044)

654 3.   Wiemann J, Yang T-R, Norell MA. 2018 Dinosaur egg colour had a single

655   evolutionary origin. *Nature* **563**, 555–558.

656 4.   Lack DL. 1968 *Ecological adaptations for breeding in birds*. London: Methuen.

657 5.   Davies NB, Brooke M de L. 1989 An experimental study of co-evolution

658   between the cuckoo, Cuculus canorus, and its hosts. I. Host egg

659   discrimination. *J. Anim. Ecol.* **58**, 207–224.

660 6.   Hargitai R, Boross N, Hámori S, Neuberger E, Nyiri Z. 2017 Eggshell biliverdin

661   and protoporphyrin pigments in a songbird: are they derived from erythrocytes,

662   blood plasma, or the shell gland? *Physiol. Biochem. Zool.* **90**, 613–626.

663 7.   Moreno, J, Osorno, JL. 2003 Avian egg colour and sexual selection: does

664   eggshell pigmentation reflect female condition and genetic quality? **6**, 803–

665   806.

666 8.   Cherry MI, Gosler AG. 2010 Avian eggshell coloration: new perspectives on

667   adaptive explanations. *Biol. J. Linn. Soc.* **100**, 753–762.

668 9.   Skrade PDB, Dinsmore SJ. 2013 Egg crypsis in a ground-nesting shorebird

669   influences nest survival. *Ecosphere* **4**, 1–9.

670　10. Leach R. 2013 Introduction to Surface Topography. In *Characterisation of*

671　　　*Areal Surface Texture*, Berlin, Heidelberg: Springer. (doi:10.1007/978-3-642-

672　　　36458-7_1)

673　11. Davim JP. 2011 *Tribology for engineers: A practical guide*. Elsevier.

674　12. Jacobs TDB, Junge T, Pastewka L. 2017 Quantitative characterization of

675　　　surface topography using spectral analysis. *Surf. Topogr. Metrol. Prop.* **5**,

676　　　13001.

677　13. Wang H, Feng H, Liang W, Luo Y, Malyarchuk V. 2009 Effect of surface

678　　　roughness on retention and removal of Escherichia coli O157: H7 on surfaces

679　　　of selected fruits. *J. Food Sci.* **74**, E8–E15.

680　14. Dai Z, Gorb SN, Schwarz U. 2002 Roughness-dependent friction force of the

681　　　tarsal claw system in the beetle Pachnoda marginata (Coleoptera,

682　　　Scarabaeidae). *J. Exp. Biol.* **205**, 2479–2488. (doi:10.1242/jeb.205.16.2479)

683　15. Igic B *et al.* 2015 A nanostructural basis for gloss of avian eggshells. *J. R. Soc.*

684　　　*Interface* **12**, 20141210. (doi:10.1098/rsif.2014.1210)

685　16. Mróz E, Michalak K, Faruga A, Horbanczuk JO, Orłowska O. 2008 Shell

686　　　microstructure and hatchability of turkey eggs. *Anim. Sci. Pap. Reports* **26**,

687　　　129–140.

688　17. Attard MRG, Portugal SJ. 2021 Climate variability and parent nesting

689　　　strategies influence gas exchange across avian eggshells. *Proc. R. Soc. B*

690　　　**288**, 20210823. (doi:10.1098/rspb.2021.0823)

691　18. Dunning JB. 2008 *CRC handbook of avian body masses*. Florida: Boca Raton.

692　19. Wilman H, Belmaker J, Simpson J, de la Rosa C, Rivadeneira MM, Jetz W.

693　　　2014 EltonTraits 1.0: Species-level foraging attributes of the world's birds and

694　　　mammals. *Ecology* **95**, 2027–2027. (doi:10.1890/13-1917.1)

695   20.   Pigot AL *et al.* 2020 Macroevolutionary convergence connects morphological

696   form to ecological function in birds. *Nat. Ecol. Evol.* **4**, 230–239.

697   (doi:10.1038/s41559-019-1070-4)

698   21.   Sheard C, Neate-Clegg MHC, Alioravainen N, Jones SEI, Vincent C,

699   MacGregor HEA, Bregman TP, Claramunt S, Tobias JA. 2020 Ecological

700   drivers of global gradients in avian dispersal inferred from wing morphology.

701   *Nat. Commun.* **11**, 2463. (doi:10.1038/s41467-020-16313-6)

702   22.   Jetz W, Sekercioglu CH, Böhning-Gaese K. 2008 The worldwide variation in

703   avian clutch size across species and space. *PLOS Biol.* **6**, e303.

704   (doi:10.1371/journal.pbio.0060303)

705   23.   Lislevand T, Figuerola J, Szekely T. 2007 Avian body sizes in relation to

706   fecundity, mating system, display behavior, and resource sharing. *Ecology* **88**,

707   1605. (doi:10.1111/sifp.12091)

708   24.   Myhrvold NP, Baldridge E, Chan B, Sivam D, Freeman DL, Ernest SKM. 2015

709   An amniote life-history database to perform comparative analyses with birds,

710   mammals, and reptiles. *Ecology* **96**, 3109. (doi:10.1890/15-0846R.1)

711   25.   Billerman SM, Keeney BK, Rodewald PG, Schulenberg TS, editors. 2020 *Birds*

712   *of the World*. Ithaca; NY; USA.: Cornell Laboratory of Ornithology. See

713   https://birdsoftheworld.org/bow/home.

714   26.   Augustine S, Lika K, Kooijman SALM. 2019 Altricial-precocial spectra in animal

715   kingdom. *J. Sea Res.* **143**, 27–34. (doi:10.1016/j.seares.2018.03.006)

716   27.   Starck JM. 1993 Evolution of Avian Ontogenies. In *Current Ornithology* (ed DM

717   Power), pp. 275–366. Boston, MA: Springer US. (doi:10.1007/978-1-4615-

718   9582-3_6)

719   28.   Starck JM, Ricklefs RE. 1998 *Patterns of development: the altricial–precocial*

720   *spectrum*. Oxford: Oxford University Press.

721   29.   Kern MD, Cowie RJ. 1995 Humidity levels in Pied flycatcher nests measured

722   using capsule hygrometers. *Auk* **112**, 564–570. (doi:10.1093/auk/112.3.564)

723   30.   Ke D, Lu X. 2009 Burrow use by Tibetan ground tits *Pseudopodoces humilis*:

724   coping with life at high altitudes. *Ibis (Lond. 1859).* **151**, 321–331.

725   (doi:10.1111/j.1474-919X.2009.00913.x)

726   31.   Messens W, Grijspeerdt K, Herman L. 2005 Eggshell penetration by

727   Salmonella: a review. *Worlds. Poult. Sci. J.* **61**, 71–86.

728   (doi:10.1079/WPS200443)

729   32.   Portugal SJ, Maurer G, Thomas GH, Hauber ME, Grim T, Cassey P. 2014

730   Nesting behaviour influences species-specific gas exchange across avian

731   eggshells. *J. Exp. Biol.* **217**, 3326–3332. (doi:10.1242/jeb.103291)

732   33.   Heenan CB, Seymour RS. 2012 The effect of wind on the rate of heat loss

733   from avian cup-shaped nests. *PLoS One* **7**, e32252.

734   (doi:10.1371/journal.pone.0032252)

735   34.   Tobias JA, Sheard C, Seddon N, Meade A, Cotton AJ, Nakagawa S. 2016

736   Territoriality, social bonds, and the evolution of communal signaling in birds.

737   *Front. Ecol. Evol.* **4**, 74. (doi:10.3389/fevo.2016.00074)

738   35.   Portugal SJ, Bowen J, Riehl C. 2018 A rare mineral, vaterite, acts as a shock

739   absorber in the eggshell of a communally nesting bird. *Ibis (Lond. 1859).* **160**,

740   173–178. (doi:10.1111/ibi.12527)

741   36.   Brulez K, Choudhary PK, Maurer G, Portugal SJ, Boulton RL, Webber SL,

742   Cassey P. 2014 Visual scoring of eggshell patterns has poor repeatability. *J.*

743   *Ornithol.* **155**, 701–706.

744   37.   López A V., Fiorini VD, Ellison K, Peer BD. 2018 Thick eggshells of brood

745   parasitic cowbirds protect their eggs and damage host eggs during laying.

746   *Behav. Ecol.* **29**, 965–973. (doi:10.1093/beheco/ary045)

747   38.   R Core Team. 2020 R: A language and environment for statistical computing.

748   R Foundation for Statistical Computing. URL https://www.r-project.org/.

749   39.   Attard MRG, Bowen J, Corado R, Hall LS, Dorey RA, Portugal SJ. 2021

750   Ecological drivers of eggshell wettability in birds. *J. R. Soc. Interface* **18**,

751   20210488. (doi:10.1098/rsif.2021.0488)

752   40.   Jørgensen JF. 2021 SPIP^{TM} User's and Reference Guide.

753   41.   Järnström J, Ihalainen P, Backfolk K, Peltonen J. 2008 Roughness of pigment

754   coatings and its influence on gloss. *Appl. Surf. Sci.* **254**, 5741–5749.

755   (doi:https://doi.org/10.1016/j.apsusc.2008.03.043)

756   42.   Hackett SJ *et al.* 2008 A phylogenomic study of birds reveals their evolutionary

757   history. *Science (80-.).* **320**, 1763–1768. (doi:10.1126/science.1157704)

758   43.   Anderson D, Burnham K. 2002 *Model Selection and Multi-model Inference: A*

759   *Practical Information-Theoretic Approach*. 2nd edn. New York, USA: Springer-

760   Verlag.

761   44.   Pagel M. 1999 Inferring the historical patterns of biological evolution. *Nature*

762   **401**, 877–884. (doi:10.1038/44766)

763   45.   Revell LJ. 2012 phytools: An R package for phylogenetic comparative biology

764   (and other things). *Methods Ecol. Evol.* **3**, 217–223. (doi:10.1111/j.2041-

765   210X.2011.00169.x)

766   46.   Hinne M, Gronau QF, van den Bergh D, Wagenmakers E-J. 2020 A

767   conceptual introduction to bayesian model averaging. *Adv. Methods Pract.*

768   *Psychol. Sci.* **3**, 200–215. (doi:10.1177/2515245919898657)

769   47.   Molina-Venegas R, Rodríguez MÁ. 2017 Revisiting phylogenetic signal; strong

770   or negligible impacts of polytomies and branch length information? *BMC Evol.*

771   *Biol.* **17**, 53. (doi:10.1186/s12862-017-0898-y)

772 48. Kull Jr RC. 1977 Color selection of nesting material by killdeer. *Auk* , 602–604.

773 49. Gómez-Serrano MÁ, López-López P. 2014 Nest site selection by Kentish

774   Plover suggests a trade-off between nest-crypsis and predator detection

775   strategies. *PLoS One* **9**, e107121.

776 50. Gómez J, Liñán-Cembrano G, Castro M, Pérez-Hurtado A, Ramo C, Amat JA.

777   2019 The protoporphyrin content of Kentish Plover *Charadrius alexandrinus*

778   eggshells is better predicted by the fractal dimension of spottiness than by

779   colour. *J. Ornithol.* **160**, 409–415. (doi:10.1007/s10336-018-1616-2)

780 51. Nguyen LP, Nol E, Abraham KF. 2007 Using digital photographs to evaluate

781   the effectiveness of plover egg crypsis. *J. Wildl. Manage.* **71**, 2084–2089.

782   (doi:10.2193/2006-471)

783 52. Carpinteri A, Chiaia B. 1996 Crack-resistance behavior as a consequence of

784   self-similar fracture topologies. *Int. J. Fract.* **76**, 327–340.

785   (doi:10.1007/BF00039781)

786 53. Tyler C. 1969 A study of the egg shells of the Gaviiformes, Procellariiformes,

787   Podicipitiformes and Pelecaniformes. *J. Zool.* **158**, 395–412.

788   (doi:https://doi.org/10.1111/j.1469-7998.1969.tb02157.x)

789 54. KE. M, Mikhailov KE. 1997 *Avian eggshells: an atlas of scanning electron*

790   *micrographs*. Hertfordshire: British Ornithologists' Club Occasional

791   Publications.

792 55. Tullett SG. 1978 Pore Size Versus Pore Number in Avian Eggshells. In

793   *Respiratory Function in Birds, Adult and Embryonic* (ed J Piiper), pp. 219–226.

794   Berlin, Heidelberg: Springer Berlin Heidelberg.

795   56.   Board RG. 1981 The microstructure of avian eggshells, adaptive significance

796   and practical implications in aviculture. *Wildfowl* **32**, 132–136.

797   57.   Tullett SG, Board RG. 1976 Oxygen flux across the integument of the avian

798   egg during incubation. *Br. Poult. Sci.* **17**, 441–450.

799   58.   R.G. B, Perrott HR. 1979 Vaterite, a constituent of the eggshells of the

800   nonparasitic cuckoos, *Guira guira* and *Crotophagi ani*. *Calcif. Tissue Int.* **29**,

801   63–69. (doi:10.1007/BF02408056)

802   59.   Kennedy GY, Vevers HG. 1976 A survey of avian eggshell pigments. *Comp.*

803   *Biochem. Physiol. -- Part B Biochem.* **55**, 117–123. (doi:10.1016/0305-

804   0491(76)90183-8)

805   60.   Tyler C. 1969 Avian egg shells: their structure and characteristics. *Int. Rev.*

806   *Gen. Exp. Zool.* **4**, 81–130. (doi:10.1016/B978-0-12-368104-1.50007-9)

807   61.   Maurer G, Portugal SJ, Hauber ME, Mikšík I, Russell DGD, Cassey P,

808   Tschirren B. 2015 First light for avian embryos: Eggshell thickness and

809   pigmentation mediate variation in development and UV exposure in wild bird

810   eggs. *Funct. Ecol.* **29**, 209–218. (doi:10.1111/1365-2435.12314)

811   62.   D'Alba L, Maia R, Hauber ME, Shawkey MD. 2016 The evolution of eggshell

812   cuticle in relation to nesting ecology. *Proc. R. Soc. B Biol. Sci.* **283**, 20160687.

813   (doi:10.1098/rspb.2016.0687)

814   63.   Stein LR, Badyaev A V. 2011 Evolution of eggshell structure during rapid

815   range expansion in a passerine bird. *Funct. Ecol.* **25**, 1215–1222.

816   (doi:10.1111/j.1365-2435.2011.01887.x)

817   64.   Avilés JM, Stokke BG, Moksnes A, Røskaft E, Møller AP. 2007 Environmental

818   conditions influence egg color of reed warblers *Acrocephalus scirpaceus* and

819   their parasite, the common cuckoo *Cuculus canorus*. *Behav. Ecol. Sociobiol.*

61, 475–485. (doi:10.1007/s00265-006-0275-0)

65. Hamchand R, Hanley D, Prum RO, Brückner C. 2020 Expanding the eggshell colour gamut: uroerythrin and bilirubin from tinamou (Tinamidae) eggshells. *Sci. Rep.* **10**, 11264. (doi:10.1038/s41598-020-68070-7)

826 **Table 1.** Predictions and definitions for possible explanations for variation in eggshell topography in birds. Source lists references

827 for definitions, and primary databases used to compile bird life-history traits. Hypotheses are numbered 1 to 13.

ID	Predictor	Logic	Definition	Source
1	Body mass	As adult body mass is correlated with egg mass, eggshells of heavier birds will have rougher foreground and background pigment compared to eggshells of smaller birds.	Mean body mass (g) of adult birds.	Data from Dunning *et al*. [18], with updates from Wilman *et al*. [19] and Pigot et al. [20]. Database compiled by Sheard *et al*. [21].
2	Clutch size	Species with larger clutches will be more likely to experience greater friction from rubbing against adjacent eggs. Therefore, larger clutches will have rougher surfaces, particularly the foreground pigmentation.	Number of eggs per brood, measured as geometric mean of the typical minimum and maximum clutch size.	Databases from Jetz, Sekercioglu and Böhning-Gaese [22], Lislevand *et al*. [23] and Myhrvold *et al*. [24]. Gaps filled in using HBW Alive [25] and other sources.
3	Diet	The composition of the eggshell is correlated with diet. Herbivores have thicker eggshells invertivores and omnivores (Attard and Portugal, unpublished). As microbes can more easily enter the egg of thinner eggshells, species that consume insects or are omnivores will develop smoother, more hydrophobic eggshell surfaces to minimise microbial adhesion.	**(1) Plant:** Diet primarily consists of fruit, buds, seeds or plants **(2) Insectivore**: Diet primarily consists of insects **(3) Omnivore/Carnivore**: Diet is omnivorous, carnivorous or a scavenger	Category based on Wilman *et al*. [19], updated from HBW Alive [25] and other sources. Database from Sheard *et al*. [21].

4	Mode of development	Longer incubation duration will promote the accumulation of microbes on the eggshell surface. Precocial species require more incubation time than altricial species, thus are expected to possess smoother eggshell surfaces.	**(1) Altricial:** Newly born young are relatively immobile, naked, and usually require care and feeding by the parents. **(2) Precocial:** Newly born young are relatively mobile, covered in feathers, and independent.	Category based on Augustine *et al.* [26], Stark [27] and Stark and Ricklefs [28]. Data from HBW Alive [25] and other sources.
5	Nest type	Nests in cavities or burrows have a higher relative humidity than open-top nests [29] and are more insulated [30]. As the level of bacterial penetration through the shell increases with higher temperature and relative humidity [31], the shell surfaces of eggs laid in enclosed nests will be rougher and more hydrophobic than eggs laid in semi-enclosed and exposed nests.	**(1) Exposed:** Nest is open above and has no side walls (no nest, scrape, saucer, platform, heap). **(2) Semi-enclosed:** Nest is partially open and has side walls (cup, bowl, pendant, sphere, dome, pouch). **(3) Enclosed:** Nest is entirely enclosed (cavity, burrow, crevice).	Category from this paper. Data from HBW Alive [25] and other sources.
6	Nest location	Elevated nests have lower risk of flooding, water accumulation or exposure to dirt and animal faeces, therefore will have smoother surfaces compared to burrows and ground-nesting species, due to reduced risk of infections.	**(1) Ground:** Nest location in or on the ground. **(2) Water:** Floating on water. **(3) Elevated:** Nest located in tree, bush, shrub, wall, cave roof, cliff or attached to reed.	Category based on Portugal *et al.* [32]. Data from HBW Alive [25] and other sources.
7	Habitat	Eggs of species breeding in open habitats are more vulnerable to heat loss due to exposure to wind [33], therefore their eggshells are expected to have smoother surfaces to reduce heat loss compared to eggs of species breeding in semi-open and dense habitats.	**(1) Open:** Species primarily occurs in desert, grassland, open water, open moorland, low shrubs, rocky habitats, seashores and cities. **(2) Semi-open:** Species primarily occurs in open shrubland and bushland, scattered bushes, parkland, forest edge.	Habitat scores from Tobias *et al.* [34]. Database compiled by Sheard *et al.* [21].

			(3) **Dense:** Species primarily occurs in forest with a closed canopy, or in the lower vegetation strata of dense thickets, shrubland, mangroves or marshland.	
8	Nest lining	Incorporation of nest lining will trap moisture, resulting in smoother surfaces.	(1) **Lined:** Nest lining is always or sometimes present. (2) **Not lined:** Nest lining is absent.	Category from this paper. Data from HBW Alive [25] and other sources.
9	Incubating parent	Eggs are more prone to microbial penetration when the parent leaves the nest uncovered. This is more likely to occur if incubation is not shared between parents, hence these eggs are more likely to have smoother eggshells.	(1) **Not shared:** Contact incubation of eggs by single adult. (2) **Shared:** Contact incubation of eggs by two adults.	Category from Portugal *et al*. [32]. Data from HBW Alive [25] and other sources.
10	Parental contact	The wet incubating parent returning to the nest will increase the nest's humidity, thus eggshells of these species are expected to have rougher eggshells.	(1) **Wet plumage:** Adults return habitually to the nest with wet plumage. This included species that feed on freshwater or marine prey, or use nests built on water. (2) **Dry plumage**: Adults did not return habitually to the nest with wet plumage.	Category from Portugal *et al*. [32]. Data from HBW Alive [25] and other sources.
11	Parental care	The eggshells of species that provide biparental care are expected to have rougher surfaces, as nest humidity and temperature can be better maintained when both parents assist.	(1) **Uniparental:** The brood is provisioned and/or defended by one adult (2) **Biparental:** The brood is provisioned and/or defended by at least two adults	Category from Portugal *et al*. [32]. Data from HBW Alive [25] and other sources.
12	Annual temperature	As the level of bacterial penetration through the shell increases with higher temperature [31], eggshells of eggs	Average annual mean temperature (BIO1) of breeding/resident range.	From Sheard *et al*. [21], based on WorldClim v1 data

		incubated in warmer climates will have smoother surfaces to avoid microbial colonisation.		[21].
13	Annual precipitation	Eggshells incubated in environments with higher annual precipitation will be rougher, to combat temporary periods of excessive rain.	Average annual mean precipitation (BIO12) of breeding/resident range.	From Sheard *et al.* [21], based on WorldClim v1 data [21].

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Table 2. Estimates of phylogenetic signal in surface roughness (S_a), surface

skewness (S_{sk}) and surface kurtosis (S_{ku}) in all birds, separated based on

pigmentation as follows: (*1*) difference in surface texture between foreground to

background pigmentation of maculated eggs, (*2*) surface texture of foreground and

background of maculate eggs analysed separately, and (*3*) surface texture of

immaculate eggs. S_a and S_{ku} were log-10 transformed prior to analysis. The *p*-value

tests the null hypothesis for both no phylogenetic signal ($\lambda = 0$) and a Brownian

motion model ($\lambda = 1$) of evolution, and are shown in brackets under the log-likelihood

ratios.

Egg type	Pigment	Response variable	Pagel's λ	Log likelihood	Log likelihood for model λ = 0	Log likelihood for model λ = 1
Maculated	Difference between foreground and background	ΔS_a	0.06	-2036.95	1.47 (0.22)	-224.97 **(<0.001)**
		ΔS_{sk}	0.00	-53.78	-0.003 (1.00)	-104.99 **(<0.001)**
		ΔS_{ku}	0.00	-373.84	-0.01 (1.00)	-434.58 **(<0.001)**
	Foreground	S_a	0.39	-150.98	3.49 (0.06)	-228.57 **(<0.001)**
		S_{sk}	0.10	-14.92	8.59 **(<0.01)**	-98.60 **(<0.001)**
		S_{ku}	0.13	190.04	-6.77 (0.01)	-91.92 **(<0.001)**
	Background	S_a	0.07	-116.50	1.44 (0.23)	-196.84 **(<0.001)**
		S_{sk}	0.20	-19.46	18.77 **(<0.001)**	-101.10 **(<0.001)**
		S_{ku}	0.06	149.46	1.84 (0.17)	38.63 **(<0.001)**
Immaculate	Immaculate eggs	S_a	0.30	-121.78	4.73 **(0.03)**	-196.01 **(<0.001)**
		S_{sk}	0.00	-70.70	-0.00 (1.00)	-117.33 **(<0.001)**
		S_{ku}	0.68	74.30	16.45 **(<0.001)**	-23.08 **(<0.001)**

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(a) Sine wave; S_{sk} = 0.00; S_{ku} = 1.50   (b) Spikes; S_{sk} = 0.61; S_{ku} = 2.11   (c) Inverted spikes; S_{sk} = -0.61; S_{ku} = 2.11

(d) Tall humps; S_{sk} = -1.04; S_{ku} = 2.85   (b) Medium humps; S_{sk} = -1.23; S_{ku} = 3.38   (c) Short humps; S_{sk} = -1.32; S_{ku} = 3.63

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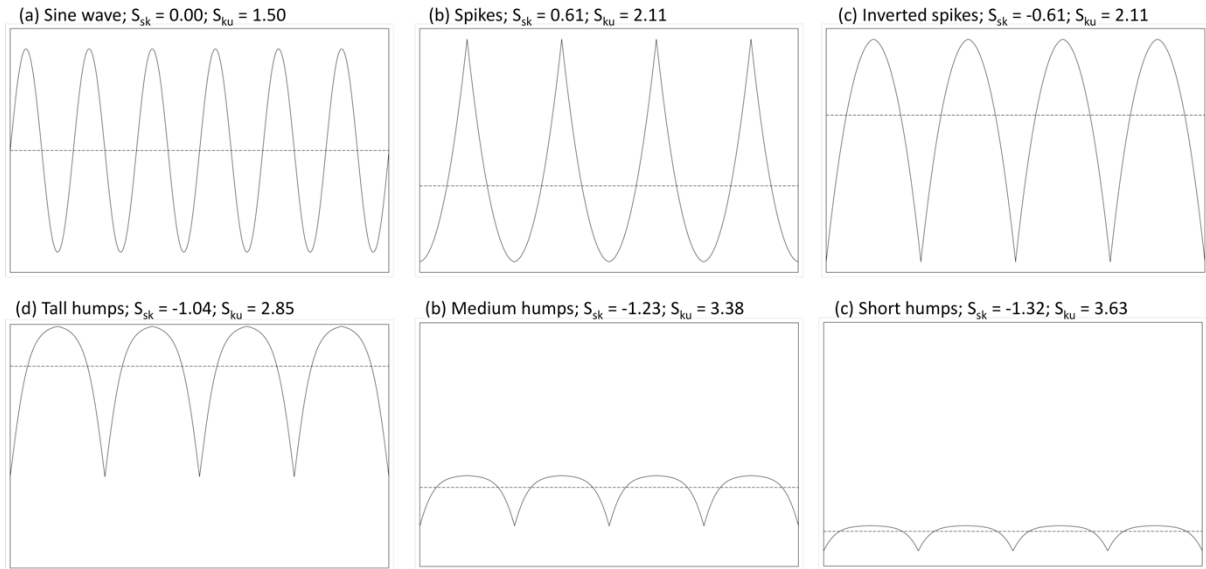
Figure 1. Schematic visualising relative values of S_{sk} and S_{ku}. (*a*) Sine wave; S_{sk} = 0.00; S_{ku} = 1.50; immaculate example: ruby-throated hummingbirds, *Archilochus colubris* (S_{sk} = -0.02, S_{ku} = 2.80). (*b*) Spikes; S_{sk} = 0.61; S_{ku} = 2.11; immaculate examples: wood storks, *Mycteria americana* (S_{sk} = 0.56, S_{ku} = 3.59) or black-necked stilts, *Himantopus mexicanus* (S_{sk} = 0.62, S_{ku} = 3.92). (*c*) Inverted spikes; S_{sk} = -0.61; S_{ku} = 2.11; immaculate example: barred cuckoo-doves, *Macropygia unchall* (S_{Sk} = -0.61, S_{ku}= 3.27) or red-shouldered hawks, *Buteo lineatus* (S_{sk} = -0.60, S_{ku} = 3.62). (*d*) Tall humps; S_{sk} = -1.04; S_{ku} = 2.85; immaculate example: brown pelicans, *Pelecanus occidentalis* (S_{sk} = -1.02, S_{ku} = 4.87). (*e*) Medium humps; Ssk = -1.23; Sku = 3.38; immaculate example: firewood-gatherers, *Anumbius annumbi* (S_{sk} = -1.08, S_{ku} = 6.16). (f) Short humps; Ssk = -1.32; Sku = 3.63; immaculate example: Manx shearwaters, *Puffinus puffinus* (S_{sk} = -1.48, S_{ku} = 7.09). All of the values are dimensionless. The dashed line indicates the average height of the line section. All figures are generated from equations and are not the actual species examples mentioned.
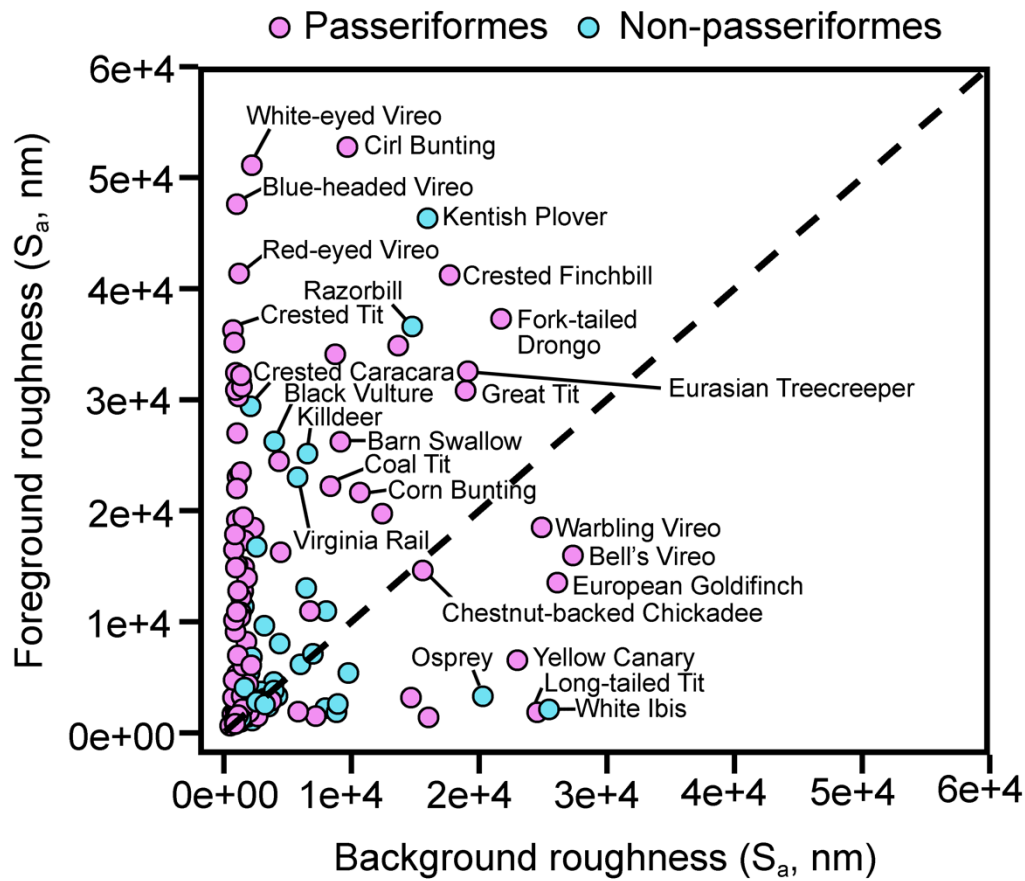
Figure 2. Eggshell surface roughness (S_a, nm) of foreground and background pigment of 204 bird species with maculated eggs. Dotted line has a slope of 1, with mean species S_a values above the line having rougher foreground versus background pigment surface. Species are colour coded based on avian clade (pink = Passeriformes; blue = Non-Passeriformes) and a subset of species are labelled. The data in the figure is not corrected for phylogenetic relatedness.

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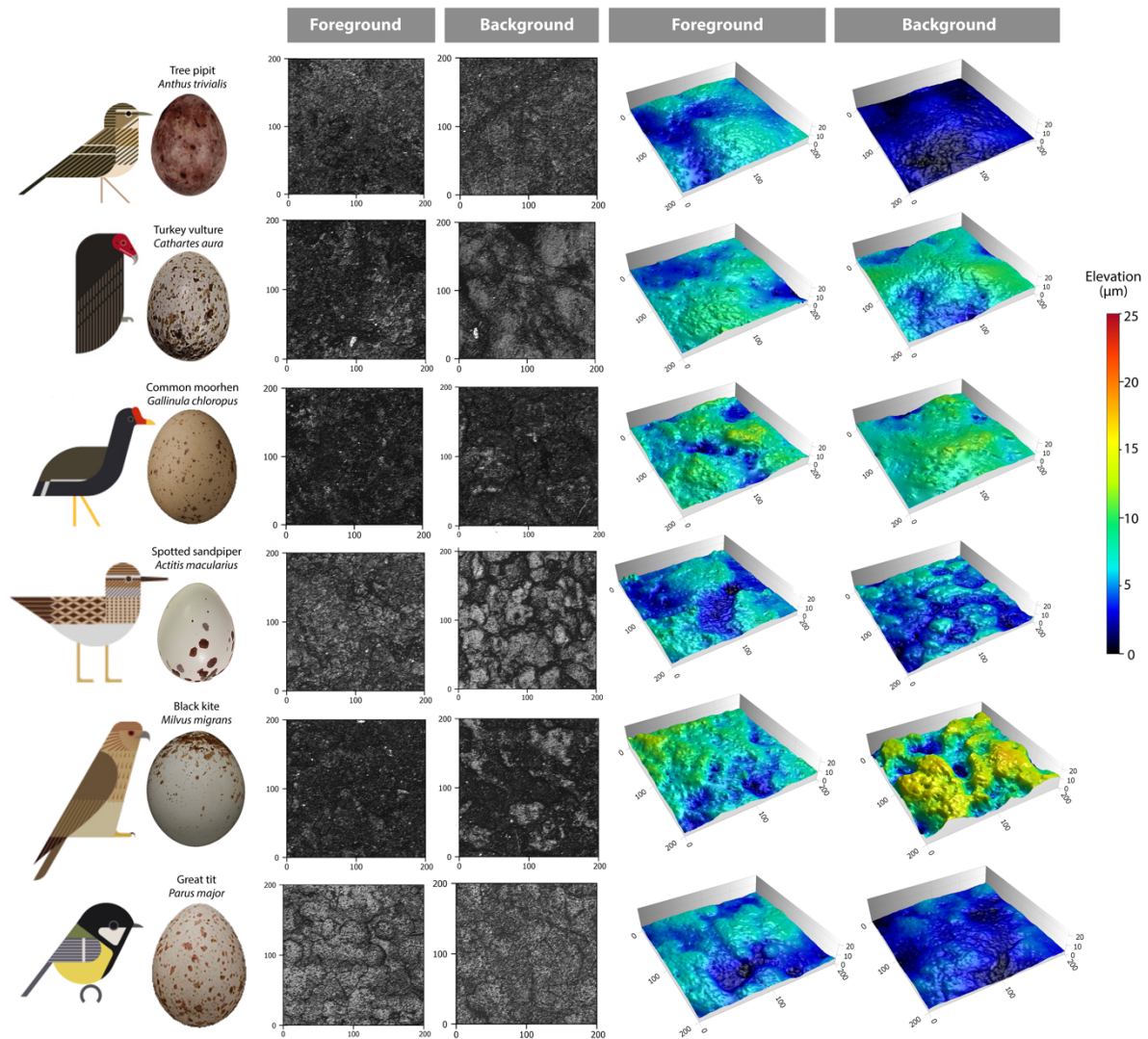
Figure 3. Surface topography of maculated eggshells from a selection of species included in this study. Greyscale two-dimensional images of the surface topography are shown for the foreground and background pigment. Digital elevation models of the foreground and background pigment for one specimen per species (1 μm resolution, dimensions 200 um x 200 μm). Bird art by Scott Partridge and egg photos by the Western Foundation of Vertebrate Zoology. The egg photos are not to scale. More information can be found in the supplementary information.
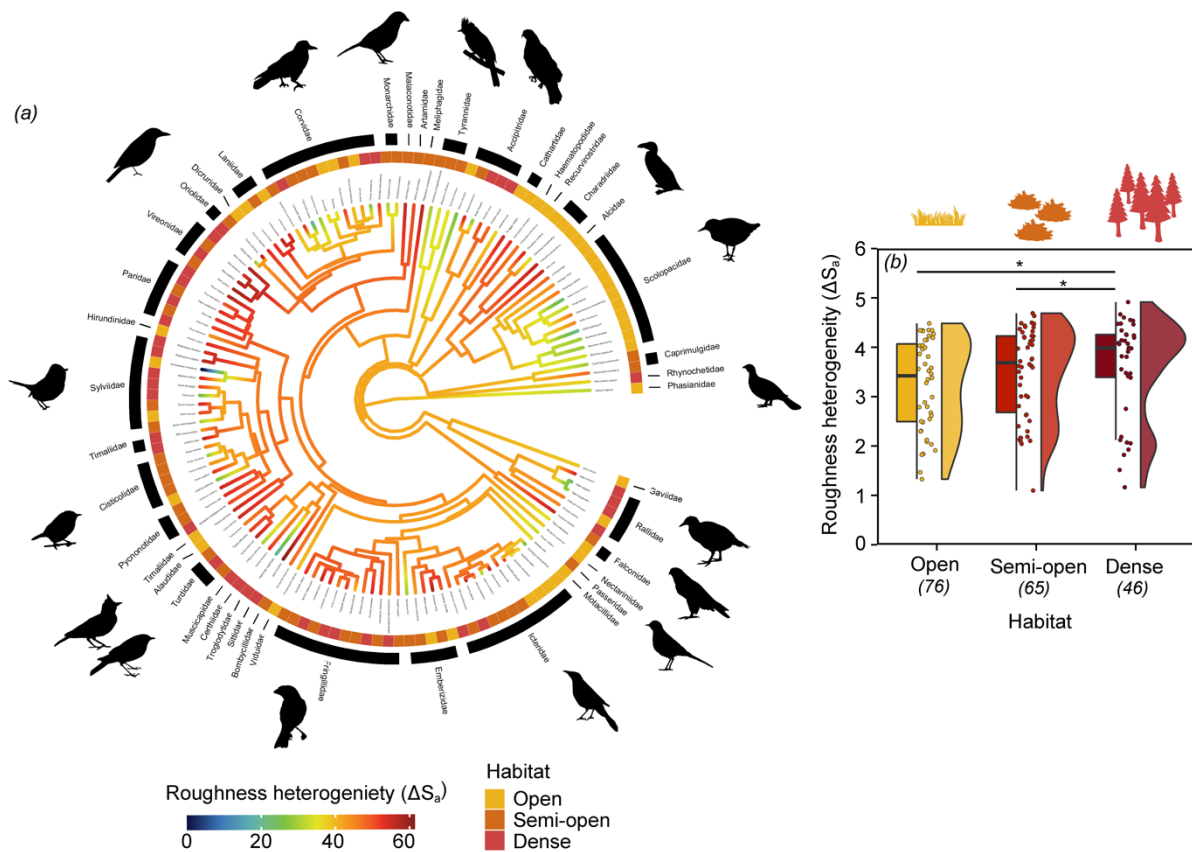
Figure 4. (*a*) Phylogenetic tree showing significant predictors of surface heterogeneity (ΔS_a) among maculated eggs. Branch colours depicts ΔS_a (nm) for each species. Scientific names are shown as tip labels and avian family is separated using black bars. (*b*) Boxplot and violin plot showing the difference in foreground to background roughness as a function of habitat. Species that occupy dense habitats had higher ΔS_a than those in semi-open and open habitats. A single asterisk (*) signifies significant ($p < 0.05$) differences for pairwise comparisons. (*b*) is not corrected for phylogenetic relatedness. Silhouette illustrations came from PhyloPic (http://phylopic.org) and SVG Silh (https://svgsilh.com/), contributed by various authors under public domain licence (see electronic supplementary material).

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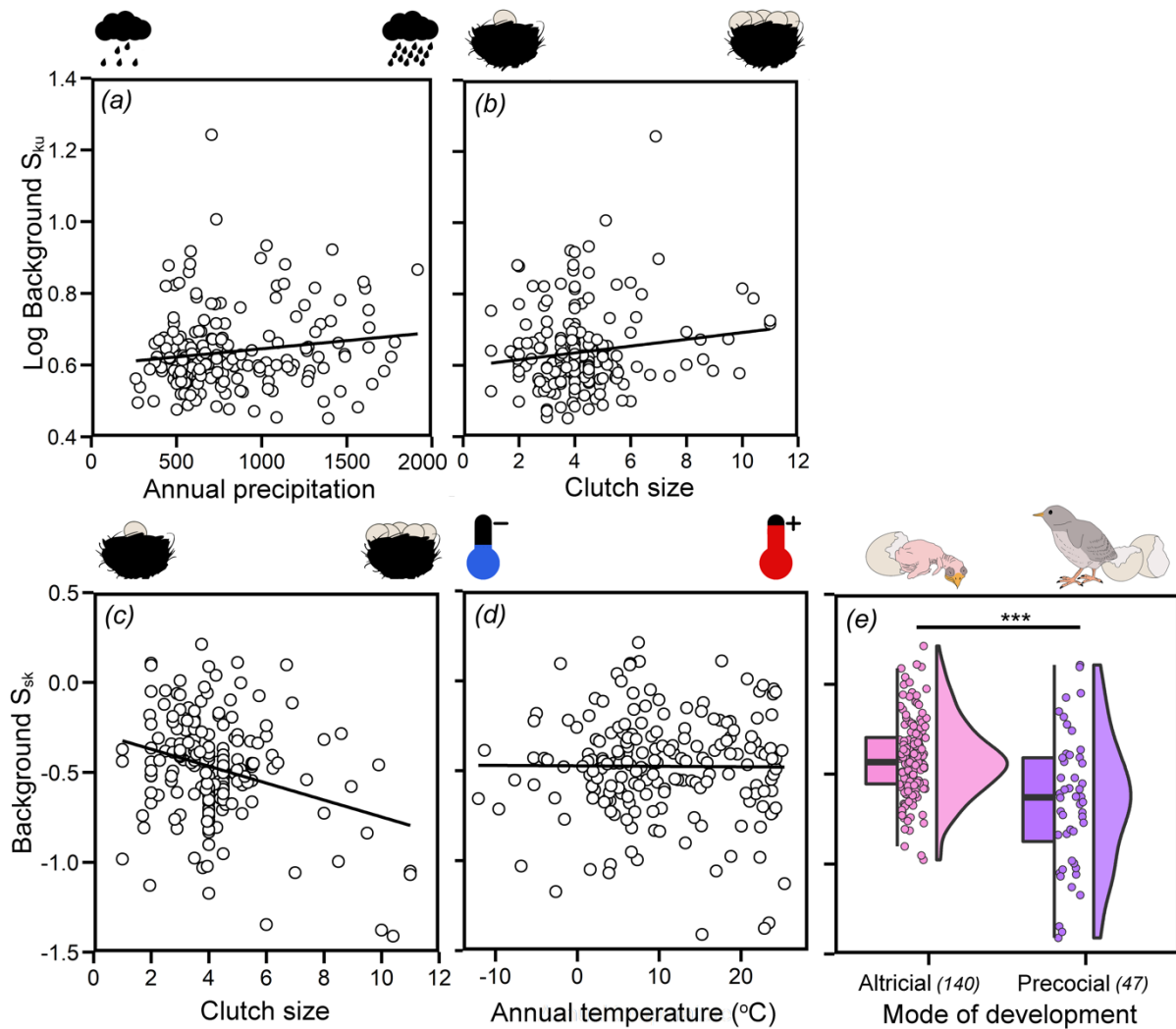
Figure 5. Significant predictors of eggshell surface kurtosis (S_{ku}) and skewness (S_{sk}) for background pigment among species that lay maculated eggs. Background S_{ku} is plotted as a function of (*a*) annual precipitation and (*c*) clutch size. Background S_{sk} is plotted as a function of (*b*) annual temperature), (d) clutch size and (e) mode of development. Three asterisks (***) signifies $p < 0.001$ for pairwise comparisons. The data in the figures are not corrected for phylogenetic relatedness. None of the life-history traits were significant for background surface roughness (S_{a}) in conditionally averaged models. Silhouette illustrations came from SVG Silh (https://svgsilh.com) under public domain licence (see electronic supplementary material).

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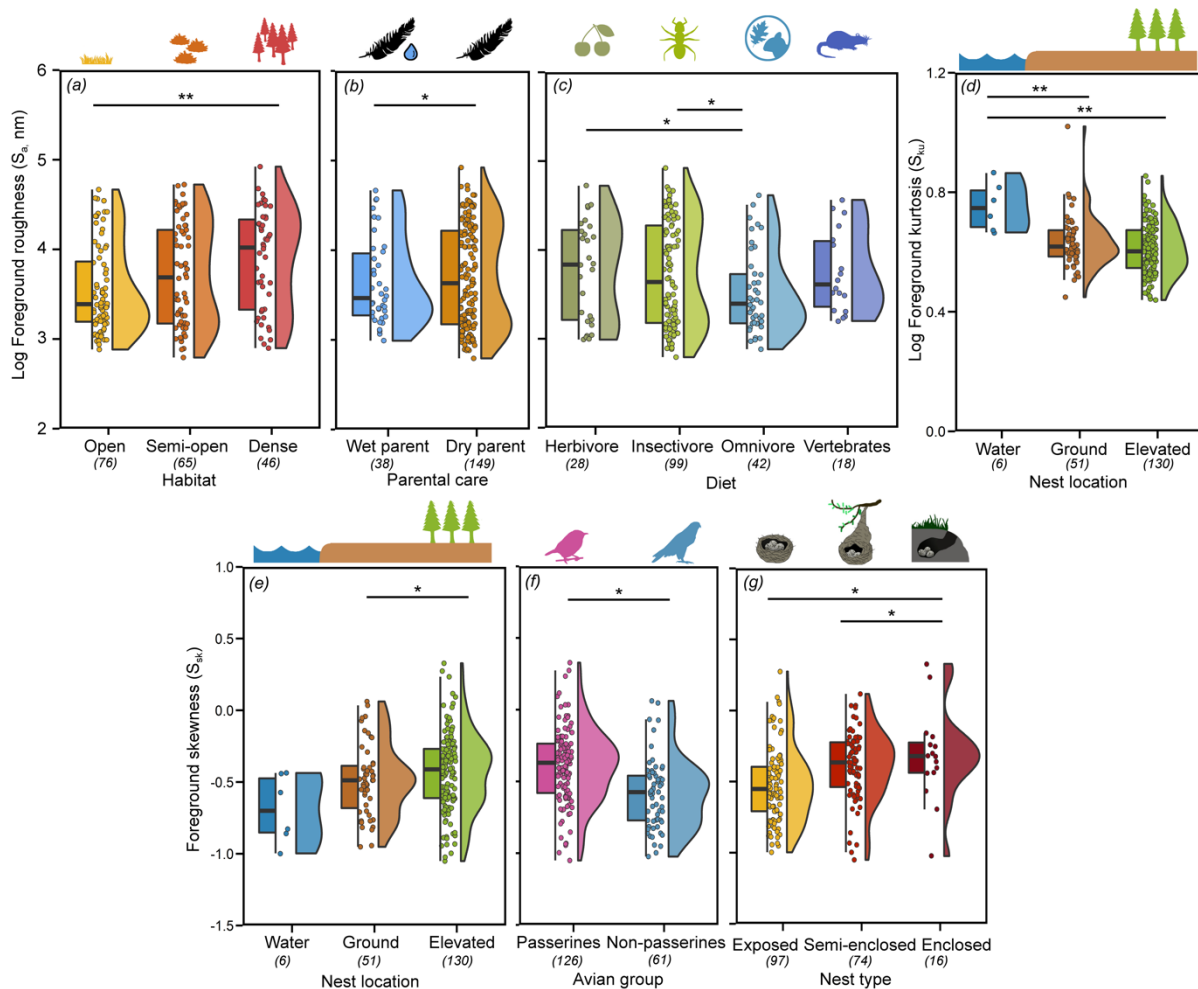
Figure 6. Significant predictors of foreground surface roughness (S_a, nm), kurtosis

(S_{ku}) and skewness (S_{sk}) among species that lay maculated eggs. Foreground S_a is

plotted as a function of (*a*) habitat, (*b*) parental contact, and (*c*) diet. Foreground S_{ku}

is plotted as a function of (*d*) nest location, while foreground S_{sk} is plotted as a

function of (*e*) nest location, (*f*) avian group and (*g*) nest type. The data in the figures

are not corrected for phylogenetic relatedness. Significant differences between

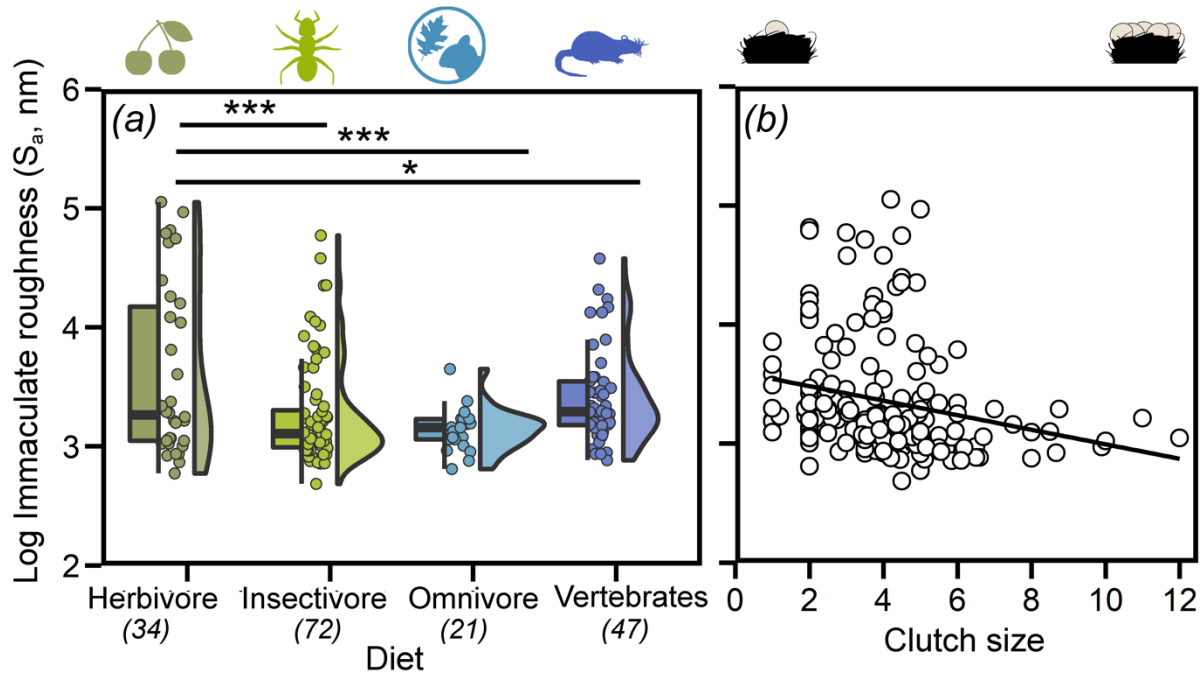categorical variables based on conditionally averaged models are given in asterisks

with ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$. Silhouette illustrations came from

PhyloPic (http://phylopic.org) and SVG Silh (https://svgsilh.com/), contributed by

various authors under public domain licence (see electronic supplementary material).

Figure 7. Significant predictors of surface texture among species that lay immaculate eggs. Surface roughness (S_a) is plotted as a function of (*a*) diet, and (*b*) clutch size. Significant differences between categorical variables based on conditionally averaged models are given in asterisks with ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$. Silhouette illustrations came from the authors or Silh (https://svgsilh.com) under public domain licence (see electronic supplementary material).