The global distribution of avian eggshell colours suggest a thermoregulatory benefit of darker pigmentation. — Source link ↗

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1 **Temperature drives the evolution and global distribution of avian eggshell colour**

2

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9 **The survival of a bird's egg depends upon its ability to stay within strict thermal limits.**

10 **Avian eggshell colours have long been considered a phenotype that can help them stay**

11 **within these thermal limits[1,2], with dark eggs absorbing heat more rapidly than bright eggs.**

12 **Although disputed[3,4], evidence suggests that darker eggs do increase in temperature more**

13 **rapidly than lighter eggs, explaining why dark eggs are often considered as a cost to trade-**

14 **off against crypsis[5–7]. Although studies have considered whether eggshell colours *can* confer**

15 **an adaptive benefit[4,6], no study has demonstrated evidence that eggshell colours *have***

16 **actually adapted for this function. This would require data spanning a wide phylogenetic**

17 **diversity of birds and a global spatial scale. Here we show evidence that darker and**

18 **browner eggs have indeed evolved in cold climes, and that the thermoregulatory advantage**

19 **for avian eggs is a stronger selective pressure in cold climates. Temperature alone**

20 **predicted more than 80% of the global variation in eggshell colour and luminance. These**

21 **patterns were directly related to avian nesting strategy, such that all relationships were**

22 **stronger when eggs were exposed to incident solar radiation. Our data provide strong**

23 **evidence that sunlight and nesting strategies are important selection pressures driving egg**

24 **pigment evolution through their role in thermoregulation. Moreover, our study advances**

25 **understanding of how traits have adapted to local temperatures, which is essential if we are**

26 **to understand how organisms will be impacted by global climate change.**

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28 The impact of global climate patterns on the evolution and distribution of traits is an area of

29 increasing importance as global temperatures continue to rise. Birds' eggs are an ideal system for

1

30   exploring the intersection between climate and trait diversity, because a tight thermal range is

31   necessary for the survival of the developing embryo[8], as eggs are unable to regulate their own

32   temperature[9]. As a result, many birds have adapted incubation behaviours and nest characteristics

33   in response to local conditions[10,11]. In addition to these behavioural adaptations, the adaptive

34   value of eggshell coloration for thermoregulation has been of longstanding interest[1,2,5]. These

35   eggshell colours are generated by just two pigments[12] and eggshell coloration is known to reflect

36   local environmental conditions[13].

37   The white colour found on many eggs (e.g., ostrich eggs) reflects incident solar radiation

38   from their surfaces, but can draw the attention of predators[14]. By contrast, dark brown or heavily

39   speckled eggs (e.g., artic loon eggs) may escape the visual detection of predators, particularly in

40   ground nesting birds[15], but these darker eggs should heat more rapidly when left in the sun[1,16].

41   Therefore, in hot climes the thermal costs must be balanced against the adaptive benefits of

42   cryptic pigmentation, while in cold climes thermoregulation and crypsis provide synergistic

43   benefits to birds laying dark brown eggs. Thus, the potential trade-off between thermal

44   constraints and crypsis are not equivalent across the globe; eggs found near the poles should be

45   darker, while those found near the equator should have higher luminance (appear brighter) and

46   more variable colours. The strength of these relationships should covary with nest types, such

47   that they are stronger in nests exposed to more light.

48   To examine these ecogeographic patterns, we quantified egg colours across their known

49   geographic ranges. To accomplish this we generated coordinates of avian eggshell coloration

50   within an opponent colour space spanning 634 species, representing 32 of the extant 36 orders of

51   birds[17] (Fig. 1). Coordinates within this space correspond with avian perceived colour and

52   luminance (brightness), and they directly relate to physical metrics of colour (see Methods).

53   Then, we simulated random nests (n = 3,577,243) within each species' breeding range and

54   assigned each species' eggshell colour and luminance. Next, we calculated the phylogenetic

55   mean colour and luminance (Fig. 1) for species found within each sampling area of an equal area

56   hexagonal grid, and associated annual temperature, and other climatic variables, with these

57   eggshell phenotypes. We then used a spatial Durbin error model to predict eggshell colours and

58   luminance values by climate variables to account for spatial autocorrelation. Lastly, to explore

59   the direct effects of solar heating on eggshell colours, we tested the heating and cooling rates of

60   white, blue-green, and brown *Gallus gallus domesticus* eggs under natural sunlight conditions.

61

62   **Fig. 1**. **An opponent colour space**
63   **illustrating the avian perceivable**
64   **variation in eggshell coloration.**
65   These data are based on the
66   difference between two opponent
67   channels (colour) and avian
68   perceivable luminance. Inset eggs
69   represent where three distinct
70   eggshell colour morphs fall within
71   this space. We illustrate the locations
72   for eggshell colours for the black
73   tinamou *Tinamus osgoodi*, peregrine
74   falcon *Falco peregrinus*, and Olive
75   sparrow *Arremonops rufivirgatus*,
76   representing blue-green, brown, and
77   white egg colours, respectively. Each
78   egg is depicted alongside a 1 cm scale
79   bar.

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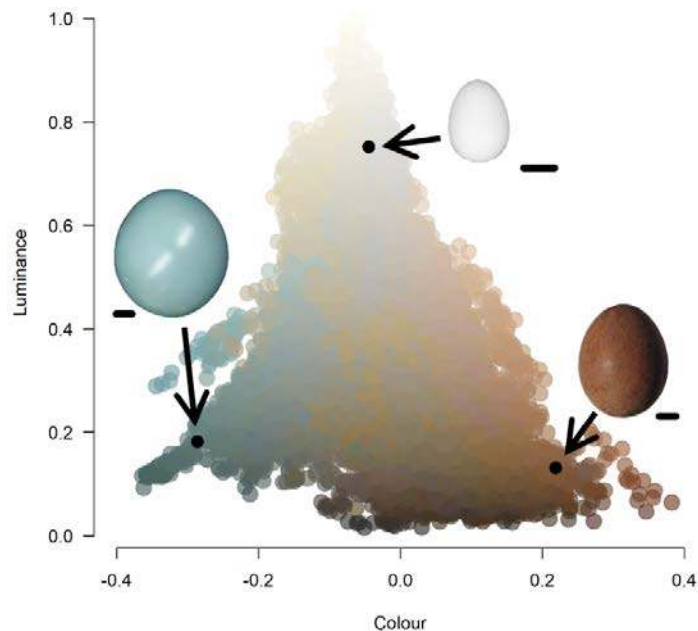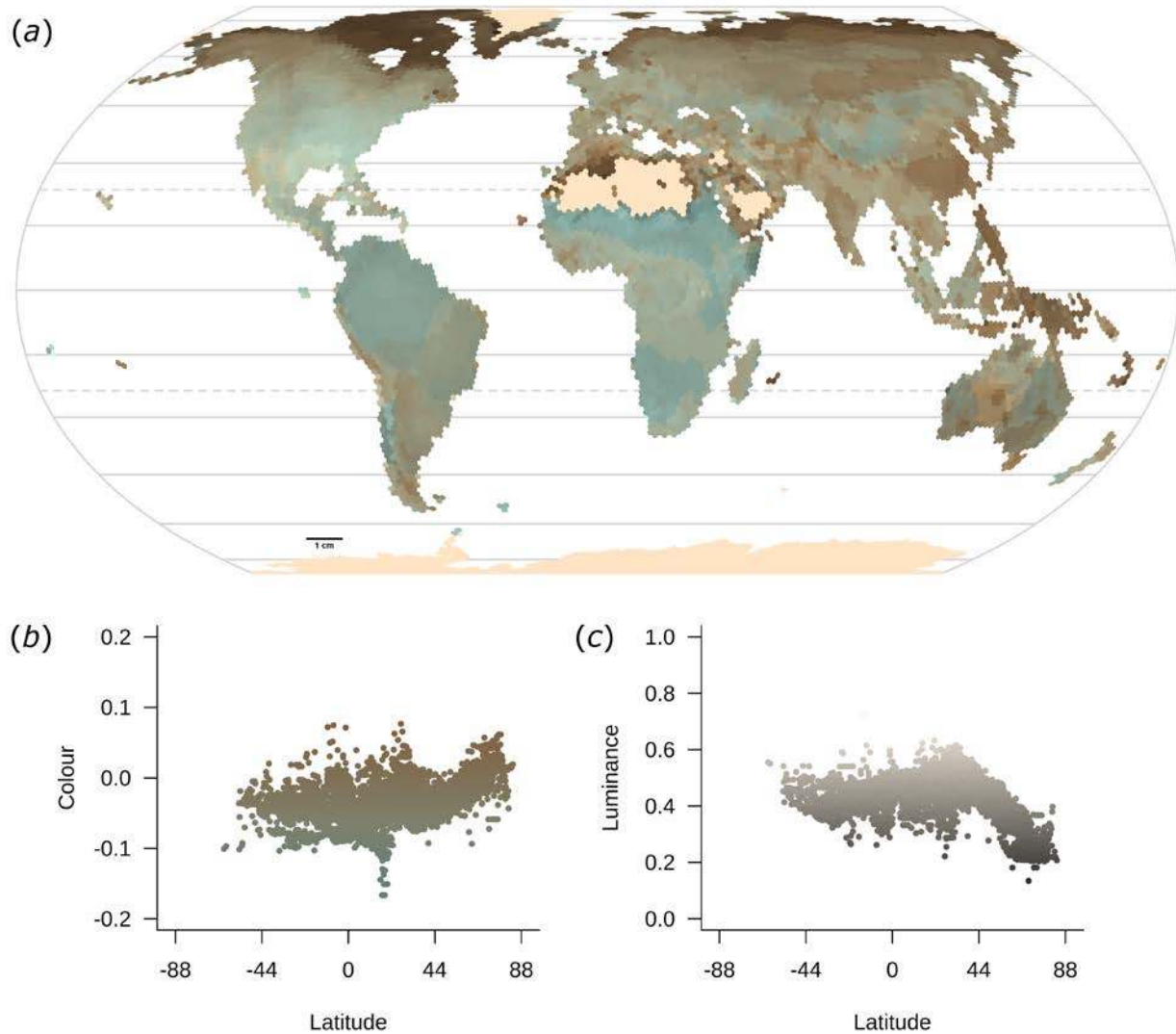
Fig. 2. **An equal Earth projection of the global distribution of avian eggshell colour**. Depicted in a **a**, bivariate plot illustrating continuous variation in blue-green to brown eggshell and dark to light eggshell colours, in units of standard deviation from their means. Both avian perceived **b**, *colour* ($R^2 = 0.83$, *latitude*: $z = -0.06$, $p = 0.95$; *latitude²*: $z = 4.67$, $p < 0.0001$) and **c**, *luminance* ($R^2 = 0.88$, *latitude*: $z = 2.08$, $p = 0.04$; *latitude²*: $z = -9.26$, $p < 0.0001$) vary non-linearly across latitude, such that dark brown eggs are more likely at northerly latitudes.

We found that avian eggshell colours are darker and browner near the Arctic, and have greater luminance and more variable colours near the equator (Fig. 2). Temperature accounted for 83.3% and 88.0% of the variance in avian egg colour and luminance, respectively, with higher latitudes having significantly browner (*temperature*: $z = -5.29$, $p < 0.0001$; *temperature²*: $z = 2.04$, $p =$

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94   0.04) and darker eggs (*temperature*: z = 13.32, p < 0.0001; *temperature²*: z = −8.50, p < 0.0001).

95   These striking, nonlinear relationships with latitude and temperature suggest that avian eggshell

96   colours are adaptive for thermal regulation in cold climes, but not in other environments. In

97   support of this, we found direct linear associations between annual temperature and eggshell

98   colour and brightness within two distinct climate regions that are associated with cold climates

99   (*colour*: R^2 = 0.82, z = −9.30, p < 0.0001; *luminance*: R^2 = 0.94, z = 14.79, p < 0.0001; Fig.

100   3a,b), while those patterns are weaker in other climate regions (*colour*: R^2 = 0.79, z = −3.03, p =

101   0.002; *luminance*: R^2 = 0.72, z = 0.59, p = 0.56; Fig. 3c,d). Although the role of thermoregulation

102   in driving egg colour evolution has long been proposed as an important selective pressure[1,2], dark

103   brown colours are often considered costly because egg temperatures are maintained close to their

104   upper thermal limit[4]; thus, brown colours would be counterproductive to shedding incident heat.

105   Instead, our results illustrate that this classic trade-off is dependent upon geography, where

106   brown colours are adaptive for thermoregulation in only some places on Earth.
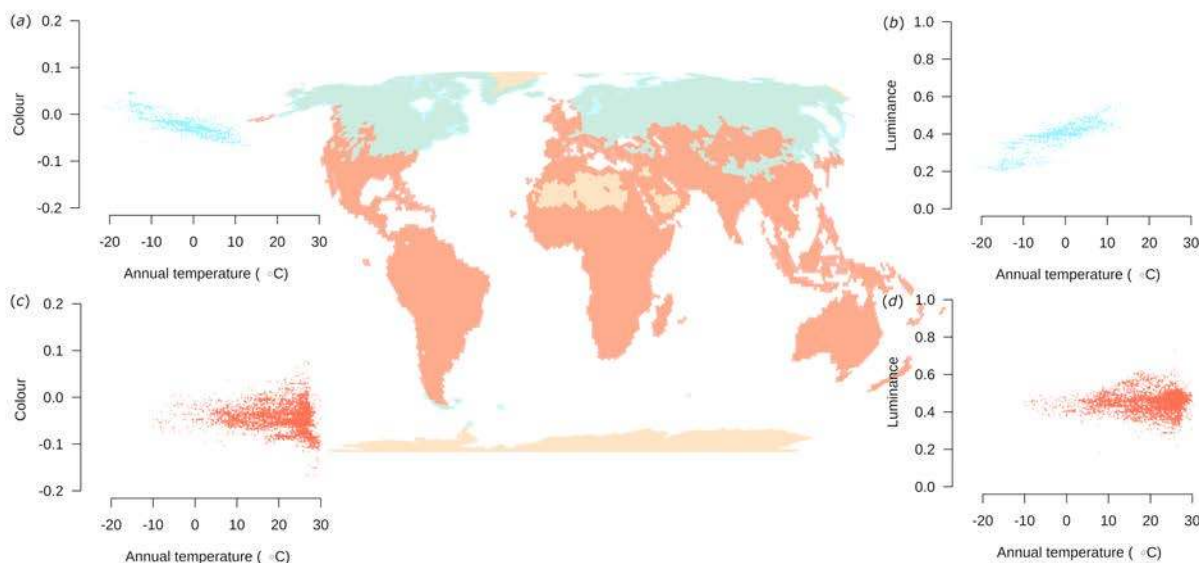
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108   **Fig. 3**. **Global relationship between temperature and eggshell colour and luminance.**
109   Variation in avian perceived **a,c**, colour and **b,d**, luminance in **a-b**, cold Köppen climate regions
110   (blue dots, *n* = 2,159) compared to **c-d**, other ecoregions (pink dots, *n* = 4,523). The central
111   inset depicts those climate regions on the Earth. See Fig. 1 and methods for details.

112   Using a linear model, and under natural solar radiation, luminance and egg mass

113   explained 91% of the variance in surface heat gain ($F_{2,45}$ = 232.70, R^2_{adj} = 0.91, p < 0.0001;

114   *luminance*: z = −1.70, p < 0.0001; *mass*: z = 0.45, p = 0.003), while colour and mass only

115   predicted 71% of the variance ($F_{2,45}$ = 58.85, R^2_{adj} = 0.71, p < 0.0001; *colour*: z = 1.15, p <

116   0.0001; *mass*: z = 0.53, p = 0.0005; ΔAICc = 55; Fig. 4). Specifically, dark brown eggs heated

117   faster (7.28 °C h^{-1}; $F_{3,44}$ = 127.3, R^2_{adj} = 0.89, p < 0.0001) than light brown (6.78 °C h^{-1}; p =

118   0.006), blue-green (5.71 °C h^{-1}; p < 0.0001), or white eggs (4.17 °C h^{-1}; p < 0.0001) and retained

119   heat longer (Extended Data Fig. 1a). This evidence suggests that pigmentation is an important

120   factor in egg thermoregulation, such that darker eggs are more adaptive than lighter eggs in

121   colder climes, especially in exposed nests. Because darker brown colours are more common in

122   the coldest places, our findings suggest that the dark brown eggshell pigment, protoporphyrin,

123   provides a greater thermal adaptive benefit than the blue-green eggshell pigment, biliverdin.

124   Thus, birds may adapt an optimal colour to their locale.

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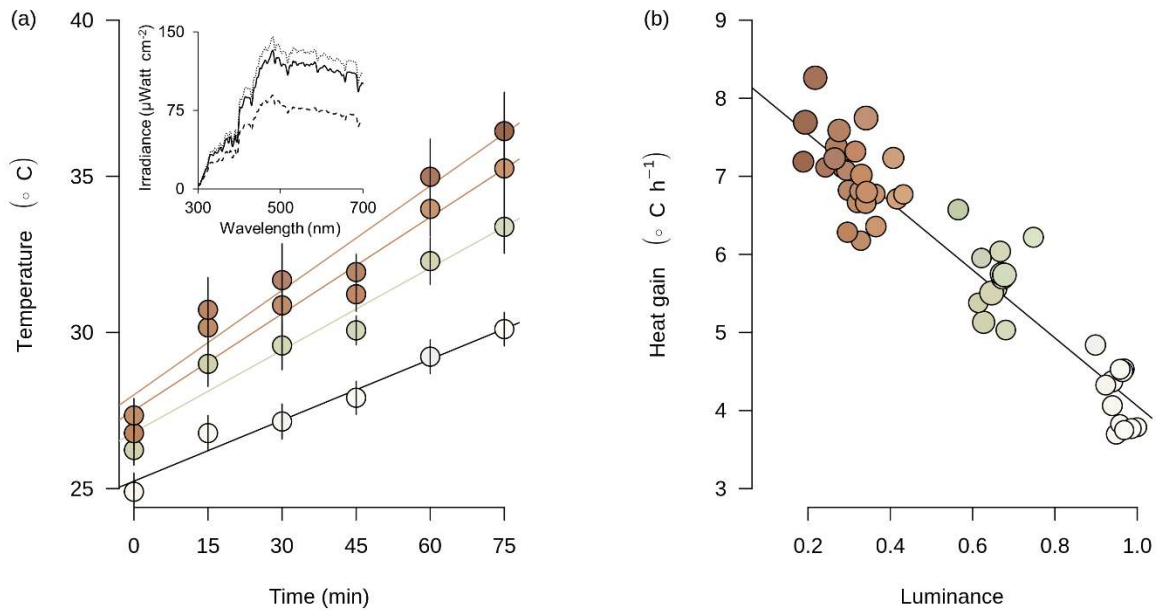
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Fig. 4. Eggshell heat gain. The heat gain ± s.e. for **a**, white, blue, light brown, and dark brown (bottom to top) chicken *Gallus gallus domesticus* eggs left outside at 27°C. The eggs were exposed to direct sunlight, except for cloud cover between 30-45 minutes after the start of the experiment (note the negative residuals at 45 minutes for each line). Inset includes solar irradiance measures at the start (solid black line), end (dotted grey line), and during cloudy conditions (dashed line) of the experiment. We also plot the **b**, eggshell surface heat gain in °C h^{-1} over this 75 min period, for each egg-based luminance and mass (dot size) illustrates relative egg sizes).

In cold climates, the ability to maintain temperature for longer periods of time afforded by darker coloration is particularly important[18]. This is not to say that species laying exposed eggs will leave their eggs unattended for longer, but instead, when unattended, dark eggs would have greater heat retention over comparable time periods. Eggshell pigmentation thus can confer an additional advantage over the chill tolerance found in some species[8]. By contrast, in warmer climates dark eggs might be more costly because they heat relatively quickly (e.g., nearly twice as fast as white eggs). In these environments, species are subjected to competing selection pressures and while eggs may have greater luminance (less pigmentation) in these warmer climes the colour is likely impacted by a range of other selective pressures: solar filtration[5,19], anti-

7

146   microbial defence[20], signalling of mate quality[21], and egg recognition[22]. Additionally, crypsis[7]

147   and eggshell strength[23] are known to influence egg coloration, and are likely important selective

148   pressures globally. This interpretation is supported by our data. Egg colour was increasingly

149   variable nearer the equator, indicating other selective pressures (e.g., ecological or behavioural)

150   are acting on eggshell coloration.

151   As predicted, the strength of these relationships varied with nest types that experience

152   differing levels of solar irradiance, such that the ability for temperature to predict colour and

153   brightness was strongest in ground nesting birds which are exposed to the most light (*colour*: R^2

154   = 0.77, z = −7.82, p < 0.0001; *brightness*: R^2 = 0.77, z = 9.63, p < 0.0001), was weaker in cup

155   nesting birds that often nest in dense foliage (*colour*: R^2 = 0.62, z = 8.68, p < 0.0001; *brightness*:

156   R^2 = 0.78, z = 10.93, p < 0.0001), and was weakest in cavity nests entirely enclosed from light

157   (*colour*: R^2 = 0.68, z = −1.02, p = 0.31, *brightness*: R^2 = 0.75; z = 2.46, p = 0.01). Interestingly,

158   recent research has documented that in non-avian dinosaurs these two eggshell pigments

159   emerged in species employing exposed nesting strategies[24]. This evidence suggests that nesting

160   ecology was a pervasive and important selective pressure driving the evolution and distribution

161   of eggshell colours.

162   The chemical properties of eggshell pigments underlying eggshell colours may have

163   adapted in response to other environmental forces. For example, eggshell pigments may protect

164   the developing embryo's DNA from ionizing radiation, due to their absorption peaks in the UV

165   range[5]. The blue-green pigment, biliverdin, absorbs more UV light than protoporphyrin (the

166   brown pigment)[5]; therefore, we would expect more intense blue-green coloration in locales with

167   high UVB radiation. Cold regions had on average 3.4 times less average monthly UVB radiation

168   (mean UVB ± s.e.; *cold*: 276.54 ± 4.24 kJ·m^{-2}; *other*: 943.82 ± 3.68 kJ·m^{-2}) than other regions[25],

169   yet in both regions blue-green eggs were associated with locales with higher UVB and the

170   relationship was strongest in cold climes which have lower UVB levels (*cold*: $R^2 = 0.82$, $z =$

171   -7.54, $p < 0.0001$; *other*: $R^2 = 0.79$, $z = -4.28$, $p < 0.0001$). Similarly, luminance was only

172   related to UVB levels in cold climes (*cold*: $R^2 = 0.94$, $z = 11.24$, $p < 0.0001$; *other*: $R^2 = 0.72$, $z =$

173   0.44, $p = 0.66$), suggesting that these relationships are driven by the strong correlation between

174   UVB and temperature ($r = 0.91$, $CI_{0.95} = 0.91$ to 0.92, $p < 0.0001$). Differentiating the

175   independent selection pressures exerted by either is an important area of future research.

176   It is also possible that protoporphyrin protects the egg from microbial invasion because

177   protoporphyrin has photo-dependent anti-microbial activity[20,26]. Because microbial loads are

178   associated with humidity[27], we expect to find browner eggs in more humid places. However,

179   although we found that in cold regions humidity significantly predicted eggshell colour ($R^2 =$

180   0.79, $z = 3.80$, $p < 0.0002$) and luminance ($R^2 = 0.93$, $z = -2.57$, $p = 0.01$), these patterns were

181   not as strong in other climate regions where the risk of microbial infection would be expected to

182   be higher (*colour*: $R^2 = 0.79$, $z = 2.20$, $p = 0.03$; *luminance*: $R^2 = 0.72$, $z = 0.56$, $p = 0.58$).

183   Overall, our results indicate that temperature is the main selective pressure driving avian eggshell

184   colour in colder northern climates, and that other selective pressures may be more important at

185   warmer climates.

186   Here we provide a robust analysis across the full phylogenetic diversity of birds and at a

187   global scale to consider how abiotic factors have shaped the evolution and distribution of a trait.

188   We illustrate why such scale, scope, and depth is necessary to understand a classic example of an

189   ecological trade-off. Thus, while our findings provide a framework for understanding the

190   selective pressures shaping eggshell colours, they also provide insight into the forces driving

191   pigmentation generally. We show that abiotic pressures such as temperature constrain the

192 expression of phenotypes, and may limit the role of alternative selective pressures in some places

193 while those same traits may be less constrained in other places on Earth. Such explorations of the

194 impact of climate on phenotypes[28], particularly those inherently linked with survival[29], are

195 necessary if we wish to quantify climate change impacts. As temperatures rise in the Arctic[30], the

196 egg colours found in that region could be shifting from adaptive to maladaptive, which could

197 result in a loss of biodiversity. Therefore, our findings provide a roadmap for identifying regions

198 at greater risk due to increasing global temperatures and prioritizing conservation efforts. Thus,

199 in addition to illustrating how abiotic factors have shaped trait diversity, our research outlines

200 novel and unexplored consequences of anthropogenic climate change.

201

202 **Online content**

203 Any methods, additional references, Nature Research reporting summaries, source data, statements

204 of data availability and associated accession codes are available at __. Birdlife Range data can be

205 requested at http://datazone.birdlife.org/species/requestdis. Digital Chart of the World basemap

206 can be found at: https://worldmap.harvard.edu/data/geonode:Digital_Chart_of_the_World. Natural

207 Earth Data maps can be found at: https://www.naturalearthdata.com/. Worldclim temperature data

208 is available at http://worldclim.org/version2. National Center for Atmospheric Research UV data

209 can be found at: https://www2.acom.ucar.edu/modeling/tuv-download. Atlas of the Biosphere

210 humidity data can be found at: https://nelson.wisc.edu/sage/data-and-models/atlas/maps.php.

211 Köppen climate data can be found at http://www.gloh2o.org/koppen/.

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284

293   **Author contributions** The concept was developed by D.H., data was collected by D.H. and
294   P.C., colour analyses were conducted by D.H and I.R., biogeographic models were developed
295   and implemented by P.W., P.K, and D.H., the initial draft was prepared by P.W. and D.H., and
296   all authors edited the paper.
297

298   **Competing interests** The authors declare no competing interests
299

300   **Additional information**
301   **Extended data** is available for this paper at _.
302   **Correspondence and requests for materials** should be addressed to D.H.
303

304   **METHODS**

305   *Colour estimation:* We used spectral reflectance data of avian eggshells (n = 634) spanning all

306   avian orders excluding Eurypygiformes (two species: Kagu and Sunbittern), Leptosomiformes

307   (one species: Cuckoo Roller), Mesitornithiformes (three Mesites species), and Pteroclidiformes

308   (16 Sandgrouse species)[1]. Of those orders represented in our data, 87±4% of the families within

309   them were sampled. These reflectance data were smoothed using a locally weighted polynomial

310   function. These data had previously been used to determine the extant variation in avian eggshell

311   coloration[17] within the avian tetrahedral colour[32,33]. We then modelled avian perceived colour of

312   each eggshell using a noise limited neural model to estimate quantum catch for each

313   photoreceptor. We calculated relative photoreceptor and double cone quantum catch[34] assuming

314   the average photoreceptor sensitivity of an ultraviolet sensitive bird and the double cones of the

315   blue tit *Cyanistes caeruleus*. To provide a comparable objective measurement of perceived

316   eggshell colours we used an ideal illuminant with equal irradiance across all wavelengths for

317   these analyses. Then we constructed an opponent colour space, using these quantum catches to

318   calculate responses to opponent channels corresponding with relatively short and long

319   wavelength light[35]. Specifically, we calculated the coordinates of eggshell colours within an

320   opponent space defined by perceived eggshell luminance (y axis), and by opponent channels

321   corresponding with the perception of relatively short and long wavelength light[35] defined by

322   eggshell quantum catches to calculate responses (x axis). Specifically,

323     $SU = (q_s - q_u) / (q_s + q_u)$

324     $LMS = (0.5 (q_l + q_m) - q_s) / (0.5(q_l + q_m) + q_s)$

325   where, q_u, q_s, q_m, q_l represent the quantile catch of the ultraviolet, short, medium, and long

326   wavelength-sensitive photoreceptors, respectively[35]. The difference between LMS and SU

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327   (divided by 2) standardized all colour values to a minimum of −1 and a maximum of +1,

328   corresponding with blue-green and brown respectively (Fig. 1). The eggshell luminance was

329   standardized to the brightest value. Unlike previous analyses that quantified avian eggshell

330   coloration[17,36,37], this approach uses quantum catch from all four receptors, while providing a

331   quantification of variation in coloration from blue-green to brown, along with a second

332   dimension of capturing variation in perceived eggshell luminance (Fig. 1). Although species

333   differ in their photoreceptor sensitivity, the coordinates within opponent colour spaces were

334   calculated for the average ultraviolet- and violet-sensitive avian viewer, two broadly divergent

335   types of avian vision[38], were highly correlated (*colour*: $\beta = 0.93$, $CI_{0.95} = 0.88$ to 0.92, $p <$

336   0.0001, Pagel's $\lambda_{max}=0.85$; *luminance*: $\beta = 1.00$, $CI_{0.95} = 1.00$ to 1.00, $p < 0.0001$, Pagel's $\lambda_{max}=$

337   0.85; Extended Data Fig. 2). Our use of avian perceived colour and luminance is important for

338   future studies involving conspecific signalling[21], brood parasitism[39], and avian predation[40]. Our

339   calculated avian perceived colour and luminance relate to a measure of blue-green chroma (the

340   sum of the reflectance within 450 and 550nm divided by the sum total reflectance within 300 and

341   700nm) and brightness (the average reflectance within 300 to 700nm)[41] (*colour*: $\beta = -0.97$, $CI_{0.95}$

342    $= -0.99$ to -0.95, $p < 0.0001$; *luminance*: $\beta = 0.98$, $CI_{0.95} = 0.98$ to 0.99, $p < 0.0001$),

343   respectively. We chose to quantify colour using an avian perceptual system because birds

344   actively select eggs, and therefore these data have value for meta-replication when testing

345   hypotheses related to avian signalling.

346

347   *Biogeographical sampling:* We downloaded bird distribution maps from Birdlife[42], and buffered

348   entirely oceanic ranges by 10 km using the 'Digital Chart of the World' base map[43] to restrict all

349   ranges to land. We chose the 10 km buffer because it was well within one sampling area (see

350 below). Then, we removed all lakes from all sampled species bird ranges using a 1:10,000,000

351 map of worldwide lakes from Natural Earth Data version 3.0.0[44]. We obtained environmental

352 data from WorldClim[45], National Center for Atmospheric Research[25] and the Atlas of the

353 Biosphere[46] datasets. Next we randomly generated one nest every 10,000 km^2 within each

354 species' breeding or resident range[42]. Each point was then assigned its species' colour and

355 luminance value. We overlaid an equal area hexagonal discrete global grid (ISEA aperture 3,

356 resolution 7, $n = 7,158$), and within each hexagon (hereafter locales) we averaged both egg and

357 environmental data. Köppen climate regions[47] were summarized to each locales' modal primary

358 category (tropical, arid, temperate, cold, and polar). We pooled cold and polar regions to

359 represent 'cool' regions, and pooled the remaining regions as 'other'. All biogeographic

360 sampling was conducted using ArcGIS ArcMap version 10.5 (Esri, Redlands, CA).

361

362 *Phylogenetic and geospatial analysis:* We accounted for the phylogenetic relatedness among

363 birds by constructing a phylogenetic hypothesis using a sample of 9,999 fully resolved

364 phylogenetic trees from a recent complete avian phylogeny[48,49]. Using these data, we calculated

365 the Bayesian maximum credibility tree (Extended Data Fig. 3), using the mean branch lengths of

366 the candidate set using DendroPy[50], dropping 10% of trees as burn in. We then assigned all

367 nodes an ancestral nest type, assuming equal rates using a maximum likelihood estimation[51]. For

368 each locale, we calculated the phylogenetic mean colour and luminance for all birds, as well as

369 birds nesting exclusively on the ground, in open nests, dome nests, cavities, or in mounds using

370 the 'phyloMean' function in the 'motmot 2.0' package. We removed any locale that could not be

371 phylogenetically controlled for (e.g., fewer than 3 species), which reduced our final sample size

372 to 6,692 locales. Mean species richness across each climate region in our final dataset ($n =$

373   6,692) were all greater than 15 (*tropical*: 30.50 ± 0.37, *arid*: 24.75 ± 0.42, *temperate*: 36.22 ±

374   0.65, *cold*: 40.28 ± 0.47, *polar*: 15.32 ± 0.41). Although phylogenetic signal[52] varied across

375   geography (*colour*: Moran's I = 0.84, p < 0.0001, *luminance*: Moran's I = 0.89, p < 0.0001),

376   phylogenetic means and non-phylogenetic means were highly correlated (*colour*: R^2 = 0.90, z =

377   70.43, p < 0.0001; *luminance*: R^2 = 0.91, z = 80.43, p < 0.0001; Extended Data Fig. 2a)

378   suggesting that any bias introduced by controlling for full phylogenetic signal (Brownian

379   motion) was minimal. Significance testing for Moran's I was two tailed.

380

381   *Thermoregulation and colour:* We tested whether the thermoregulatory properties of the egg

382   could be predicted by eggshell colour and luminance using domestic chicken *Gallus gallus*

383   *domesticus* eggs (Carrol's, Pete and Jerry's, and Stop and Shop) under natural illumination

384   conditions. These eggs (*n* = 48) were either dark brown, light brown, blue-green, or white. Each

385   egg's mass was recorded using a microbalance (Ohaus Adventure Pro, model AV114C, ±

386   0.0001g) to the nearest centigram, their surface reflectance was recorded using an Ocean Optics

387   Jaz spectrophotometer (Ocean Optics, Jaz, Dunedin, Florida, USA), and then each egg's

388   coordinates were calculated within the opponent space. The eggs were then sorted into 12 groups

389   of four, each containing an egg of each colour in a random order. These eggs were acclimated to

390   room temperature ~24.1 °C overnight, and then placed in direct sunlight on 24 August 2018 at

391   27°C. We measured temperature using a thermal imaging camera (FLIR Infra-Cam) at 15-minute

392   intervals for 75 minutes spanning solar noon (30 min prior to 45 minutes after solar noon). Mean

393   egg temperature for each egg was calculated using calibrated thermal images in ImageJ[53]. To

394   verify that estimates of surface temperature measured by the FLIR Infra-Cam correspond with

395   internal temperatures, we inserted a Omega type T thermocouple (Omega SSRTC-TT-T-24-36)

16

396   2.5 cm into a new set of dark brown (n = 3) and white eggs (n = 3) and recorded internal

397   temperature using a thermocouple logger (Omega HH506RA); internal and external temperature

398   were highly related (r = 0.92, $CI_{0.95}$ = 0.83 to 0.95, p < 0.0001; Extended Data Fig. 1*b*).

399

400   *Statistical analysis:* We accounted for spatial autocorrelation in both dependent and independent

401   variables using a spatial Durbin error model, using first and second order Queen's contiguity[54]

402   weighting and lower-upper matrix decomposition. A first order Queen's contiguity considers all

403   neighbours for each locale, while in this case a second contiguity considers all neighbouring

404   locales as well as all their neighbours (weights were roughly equivalent to ~350 km). We use

405    $AICc$[55,56] to determine whether the model weighted by the first or second order contiguity better

406   explained our data, and we report the model with the lowest AICc. We calculate and report total

407   impact statistics (e.g., z scores and two tailed p values) and Nagelkerke pseudo-R^2 for each

408   spatial Durbin error model. All isolated locales and locales with missing information were

409   removed from weight files, which is a requirement of the spatial Durbin error model. This

410   resulted in datasets of eggshell coloration and luminance of the eggs from locales containing all

411   birds (n = 6,692), ground nesting birds (n = 6,539), open nesting birds (n = 6,475), and cavity

412   nesting birds (n = 6,557). Phylogenetic mean colour and luminance for each nest type was

413   considered in the presence of all possible nest types at each locale, rather than truncating the

414   dataset. Dome nesting birds and mound builders were retained for these calculations (Extended

415   Data Fig. 3), but we do not have predictions for these groups so we do not explore their

416   independent relationships. We predicted the rate of heating of chicken eggs under natural

417   incident solar radiation based on colour, luminance, and mass using a general linear model.

418

Data availability

The data and codes that support the findings of this study are available from the corresponding author upon request.

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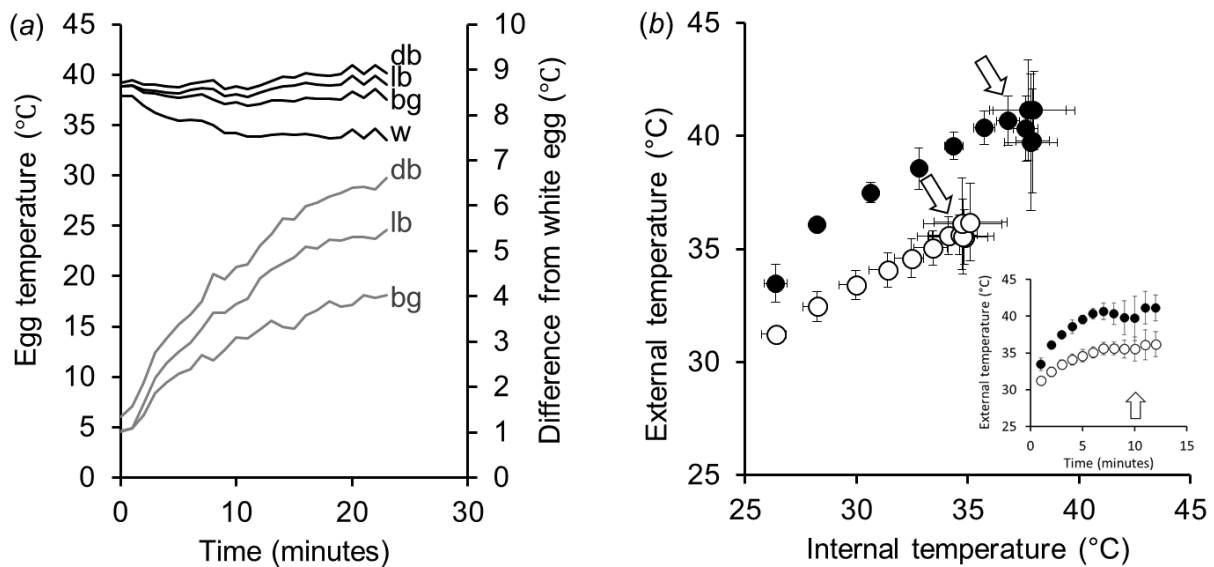
485
486   **Extended Data Fig. 1**. | **Egg temperatures**. Eggshell surface temperatures under **a**, natural
487   ambient light conditions (24.1 °C) for dark brown (db), light brown (lb), blue-green (bg), and white
488   (w) domestic chicken eggs ($n = 12$). All eggs were heated together overnight to 37°C in a Powers
489   Scientific Inc. (model DROS33SD) incubator on locally sourced topsoil, sand, and fallen leaves to
490   approximate a scratch nest. Over this trial, brown eggs retained their intial temperature, while blue-
491   green eggs lost temperature slowly, and white eggs lost temperature rapidly. We measured egg
492   temperatures every minute for 24 minutes using a FLIR Infra-Cam. After 24 minutes, dark brown
493   eggs were 7°C warmer than white eggs (grey lines, second y axis). A new set of eggs' external surface
494   tmperatures were **b**, related to their internal temperatures. Dark brown (filled dots, $n = 3$) and white
495   (open dots, $n = 3$) eggs were left under natural ambient light conditions (33.9 °C; see Methods for
496   details). The external temperatures change more rapidly than internal temperatures when wind
497   increases convective cooling (arrows), as experienced 10 minutes into this trial (inset).
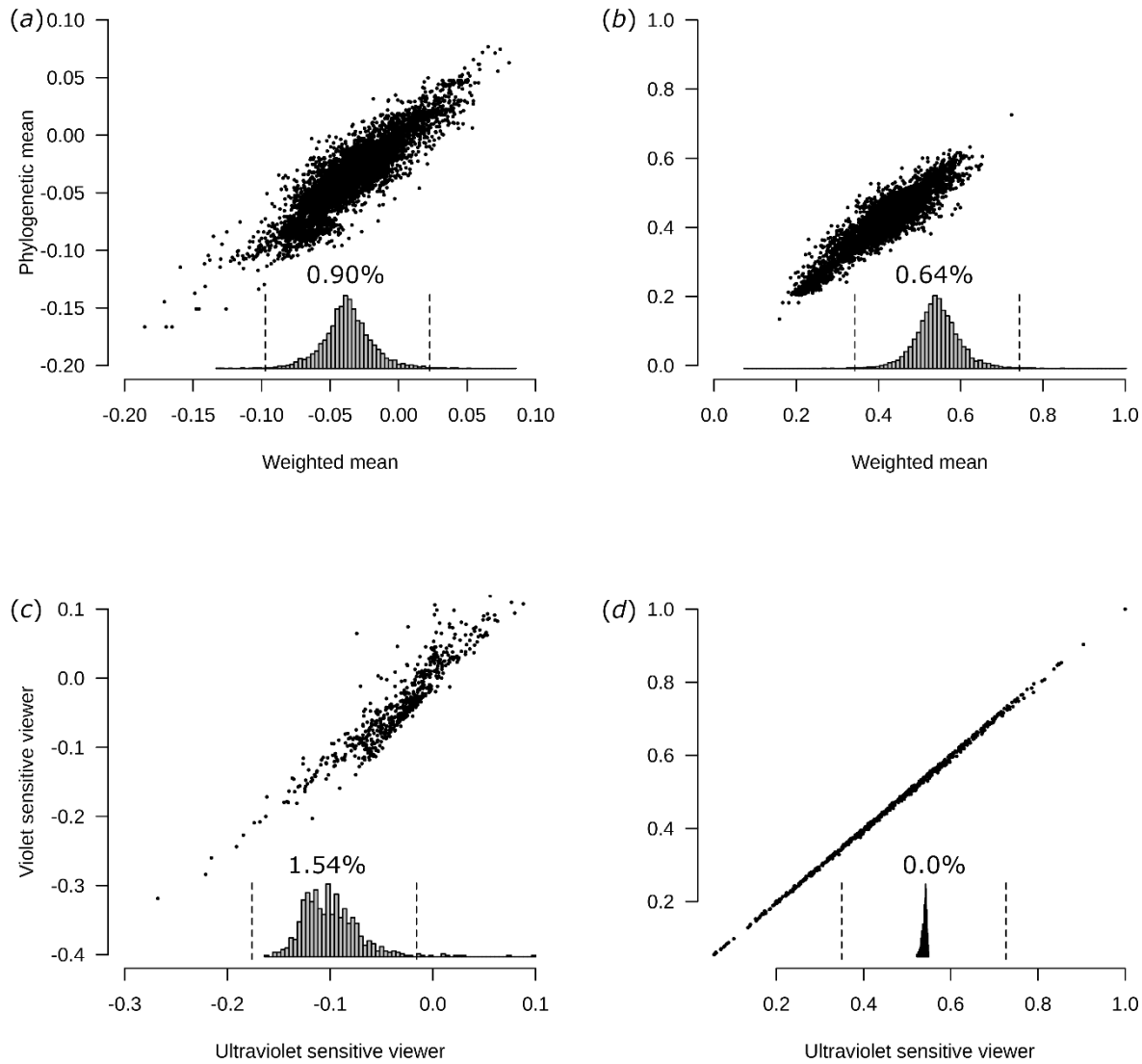
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500 **Extended Data Fig. 2**. | **Comparison of other methods**. Relationships between the phylogenetic
501 and weighted means for **a**, colour and **b**, luminance, as well as the relationship between the **c**, colour
502 and **d**, luminance components of the opponent colour space calculated for the average violet- and
503 ultraviolet-sensitive avian viewer. Here the weighted means were calculated using an intercept-only
504 phylogenetic generalized least squares, where estimates correspond with weighted means[57] but the
505 maximum likelihood value for Pagel's lambda[52] is calculated for each locale. These figures illustrate
506 the maximum degree of error introduced into our analyses (residuals) by our application of
507 phylogenetic means and ultraviolet-sensitive visual systems. In both cases, more fine-tuned variation
508 likely exists in our data, but these illustrate extremes (e.g., no phylogenetic correction versus full
509 Brownian motion, and two common but broadly divergent visual systems). The inset histograms
510 represent the residuals from each's respective tests (see Results). Units are scaled, and presented on a
511 comparable scale (−2.5 to 2.5 standard deviations) and dashed lines represent −1 and 1 standard
512 deviation, respectively. The percentages above each histogram represent deviations more extreme
513 than 1 standard deviation.

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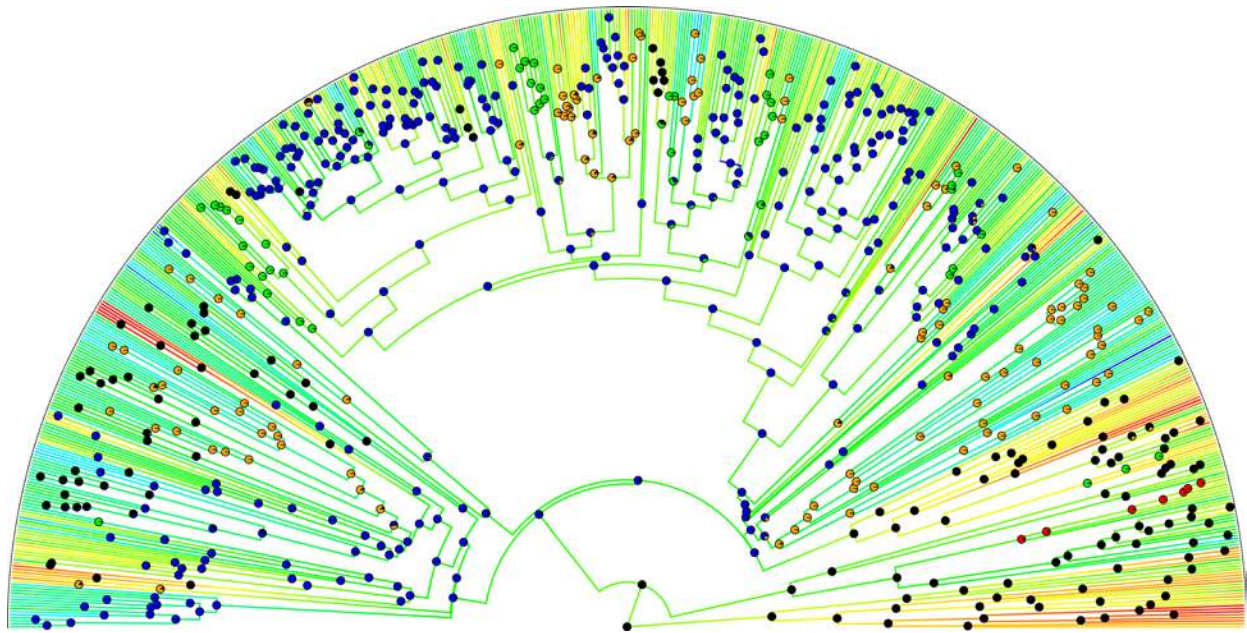
517
518 **Extended Data Fig. 3. | Phylogenetic relationships**. The maximum clade credibility tree used in
519 this study. Here we plot branch lengths in continuous coloration representing avian perceived
520 eggshell luminance from dark (red) to bright (blue). At each node, we illustrate pie charts
521 representing the most likely ancestral state for nest types: ground nesting (black), cup nesting (blue),
522 dome nesting (green), cavity nesting (orange), and mound nesting (red).
523