

The global distribution of avian eggshell colours suggest a thermoregulatory benefit of darker pigmentation

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The survival of a bird's developing embryo depends on the egg's ability to stay within strict thermal limits. How eggshell colours help maintain thermal balance is a long-standing and contested question. Using data spanning a wide phylogenetic diversity of birds on a global spatial scale, we find evidence that eggshell pigmentation may have been shaped by thermoregulatory needs. Birds living in cold habitats, particularly those with nests exposed to incident solar radiation, have darker eggs. We show evidence that darker eggs heat more rapidly than lighter ones when exposed to solar radiation. This evidence suggests that egg pigmentation could play an important role in thermoregulation in cold climates, while a range of competing selective pressures further influence eggshell colours in warmer climates. These findings advance our understanding of thermoregulation in the distribution of natural colours.

Dark colours play a crucial role in thermoregulation for ectotherms, from fungi to lizards^{1,2}. These colours often absorb more solar radiation and ectotherms with these colours are usually more common in colder climates^{2,3}. This is a broad ecogeographical pattern known as Bogert's rule⁴. Eggs are a useful system for exploring the role of colour in thermoregulation and life history trade-offs^{5–7}. Birds' eggs are particularly well-suited for addressing these questions because the survival of the developing embryo depends on a narrow thermal range⁸ and eggs cannot regulate their own temperature⁹.

Birds' eggs vary in colour from the underlying white calcium carbonate to an array of blue-green and brown colours generated by the pigments biliverdin and protoporphyrin, respectively^{10,11}. When exposed to incident solar radiation, eggs absorb solar energy and risk either heating or cooling beyond their thermal limits¹². In addition, an exposed egg may also attract the attention of visual predators⁷; thus, brighter eggs bear a greater risk than brown or spotted eggs^{13–17}. Wherever ambient temperatures approach an egg's upper thermal limit, any adaptive benefit of a dark eggshell colour must be balanced against a thermal cost (opposing pressures). By contrast, when ambient temperatures are below an egg's thermal limit, dark brown eggs should provide both a thermoregulatory and cryptic benefit (synergistic pressures). Although studies have found that darker eggs heat more rapidly when illuminated^{12,18}, this finding is not universally supported¹⁹ and a variety of methodological issues complicate the interpretation of earlier findings²⁰. However, if eggshell colours play a role in thermoregulation, we expect that, on a global scale, birds living in colder environments will have darker and browner eggs.

Predation is probably an important selection pressure on egg colours and patterns^{7,21} and rates of nest predation are generally lower in the far north and higher towards the tropics^{22–24}. In addition, predation rates tend to be lower on eggs that are a uniform brown colour or spotted^{14,21}, both generated by protoporphyrin.

In fact, when disturbed some parents deliberately soil their eggs to reduce predation risk²⁵. Therefore, in contrast to our expectations with thermoregulation, we expect browner and darker eggs in areas of higher predation (that is, hotter regions). This expectation is similar to what would be predicted if eggshell pigments are selected for antimicrobial defence²⁶. Specifically, protoporphyrin has photo-dependent (that is, light-activated) antimicrobial properties and microbial load is highest in warm humid environments^{27,28}; thus, for both hypotheses, we expect eggs to vary from dark brown to lighter blue-green along an environmental gradient from humid to arid. Likewise, if eggshell pigments have adapted to filter out harmful ultraviolet (UV) radiation to protect the developing embryo^{18,29}, we would expect darker eggshell colours in areas with high levels of UVB radiation. However, in such places, we expect darker blue-green eggs because biliverdin absorbs more UV light than protoporphyrin^{18,30}.

To examine these global patterns (Supplementary Table 1), we quantified eggshell brightness and colour for 634 species, representing 36 of the extant 40 orders of birds¹⁰ (Fig. 1). We mapped these within each species' breeding range³¹ and calculated the mean blue-green chroma (hereafter, colour) and brightness for the unique assemblage of birds found within each sampling area (hereafter, locale; Fig. 2), accounting for phylogenetic non-independence within each locale (see Methods). Next, we summarized environmental variation into two principal components representing solar intensity and aridity (Extended Data Fig. 1). We then predicted phylogenetically corrected eggshell colour and brightness at each locale by these environmental variables using spatial error models (assemblage-level analysis³²). This level of analysis examines spatial trends by species co-occurring at each locale. Examining similar patterns across species adapted to different ranges is another way to study ecogeographical patterns (interspecific-level analysis). Using both methods provides a more robust analysis of ecogeographical patterns. Therefore, we ran a complementary analysis predicting colour

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and brightness across species using phylogenetic generalized least squares³³. For both sets of analyses, we considered whether these relationships covary with three nest types (ground, cup and cavity nests) that we expect vary in exposure to solar radiation. Finally, to explore the direct effects of solar heating on eggshell colours, we tested whether eggshell colour and brightness predicted the rates of heating from ambient temperature and the rates of cooling from incubation temperature of variably coloured chicken *Gallus gallus domesticus*, duck *Anas platyrhynchos domesticus* and Japanese quail *Coturnix japonica* eggs under natural sunlight conditions.

Results

We found that our assemblage-level data of avian eggshell brightness and colour (Fig. 2) were predicted by environmental conditions ($n=6,692$), which relate to solar intensity (principal component 1 (PC1)) and aridity (principal component 2 (PC2)). Solar intensity had a clear non-linear relationship with brightness and colour (brightness: $\chi^2=16,140$, $P<0.0001$; colour: $\chi^2=13,877$, $P<0.0001$; Fig. 3). Specifically, eggs found at locales with the lowest solar intensities were significantly darker (PC1: $\beta=0.39$, 95% confidence interval (CI)=0.29 to 0.49, $P<0.0001$; PC1²: $\beta=-0.27$, 95% CI=-0.37 to -0.18, $P<0.0001$) and browner (PC1: $\beta=0.40$, 95% CI=0.29 to 0.51, $P<0.0001$; PC1²: $\beta=-0.19$, 95% CI=-0.31 to -0.07, $P=0.001$); however, this analysis detected that eggs in hot humid locales with the highest solar intensity also tended to have slightly darker and browner eggs (Fig. 3). Because solar intensity relates to both temperature and UVB radiation³⁴ (Extended Data Fig. 1), these patterns suggest that thermoregulation or solar filtration influences avian eggshell colours. However, if eggshell pigments protect the developing embryo's DNA from ionizing radiation¹⁸, we expected darker blue-green colouration in locales with high UVB radiation¹⁸. Instead, we found eggs were significantly lighter in these locales and darker in colder locales, supporting the predictions of thermoregulation and at best suggesting a trade-off with solar filtration¹⁸. It is unsurprising that solar filtration was not robustly supported on this scale because eggshells generally transmit very little (<0.10%) UV light³⁵.

In addition to these findings, we found that aridity was linearly related to eggshell brightness, so that eggshells were significantly darker in drier habitats (PC2: $\beta=-0.08$, 95% CI=-0.14 to -0.01, $P=0.02$). However, this pattern was notably weaker than the relationship between eggshell brightness and solar intensity and is more clearly illustrated within specific nest types (see further on). By contrast, eggshell colours did not correlate with aridity (PC2: $\beta=-0.03$, 95% CI=-0.11 to 0.05, $P=0.44$). These patterns contrast with the predictions of Gloger's rule¹, but provide strong support for Bogert's rule⁴. Interestingly, both colour and brightness were most variable at higher solar intensities (Fig. 3), supporting our prediction that synergistic forces operate in locales with lower solar intensity (that is, where ambient temperature is closer to the egg's lower thermal limit), while opposing forces operate in locales with greater solar intensity (that is, where ambient temperature is above the egg's thermal limit). This is consistent with the hypothesis that birds may be impacted by a range of competing selective pressures, such as solar filtration^{18,36}, antimicrobial defence²⁶, signalling of mate quality³⁷, eggshell strength^{38,39} or egg recognition^{40–42} in these locales.

As predicted, we found significant interactions between these environmental variables and nest types (ground, cup, and cavity nests) that experience differing levels of solar irradiance^{43–45}. Specifically, ground-nesting birds that should face stronger selection for thermoregulation⁴⁶, crypsis²⁴ and microbial defence⁴⁷, had significantly darker and browner eggs than cup- and cavity-nesting birds (Table 1 and Extended Data Fig. 2), as may be expected by these hypotheses (Supplementary Table 1). We also found a significant non-linear increase in brightness as solar intensity increased for all nests (Table 1). Both ground- and cup-nesting birds had

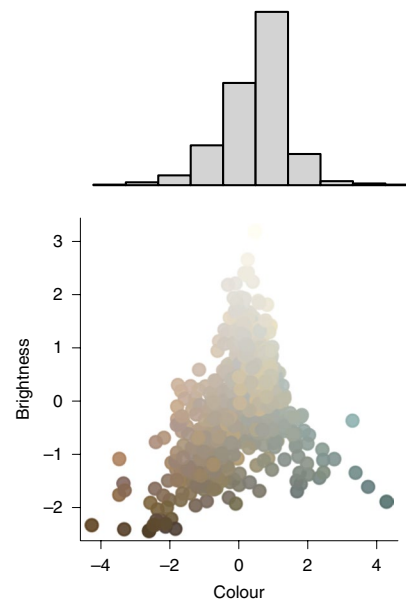


Fig. 1 | Variation in avian eggshell brightness and colouration. Brightness and colour of the eggshells for 634 species of extant birds. Eggshell brightness represents the mean reflectance, while colour represents the blue-green chroma. Both values were centred and scaled for all analyses (see Methods). We focused on blue-green chroma because all birds' eggs vary from blue-green to brown¹⁰ and this colour metric corresponds with avian perceived variation in colours (see Extended Data Fig. 7). The colours of the dots represent the colours of each species' eggshell. Marginal histograms are presented along each axis to assess the variability of these data.

relatively darker eggs in cold places (note the positive slopes in Extended Data Fig. 2a), which is also broadly consistent with the thermoregulation hypothesis.

Although the eggs of cavity-nesting birds were generally bright, we found that they were relatively darker in the coldest and hottest locales (Extended Data Fig. 2). The relatively darker (and less blue-green) eggs of cavity nesters in hot humid locations probably explains the decrease in eggshell brightness in locales with high solar intensities (see Fig. 3). We encourage future research to study the evolution of eggshell brightness in cavity-nesting birds in different habitats. However, because the eggs of cavity nesters are not exposed to as much light⁴⁴, the thermoregulatory and cryptic benefits of eggshell pigmentation would not be expected. Instead there may be a selective advantage for brighter eggs that parents can see in these low-light conditions^{48–50}. Relative to the eggs of ground-nesting birds, both cup- and cavity-nesting birds had bluer eggs in cool and humid habitats (Table 1), which is not a specific prediction of thermoregulation, solar filtration, crypsis or antimicrobial defence. For birds nesting in exposed locations, it is possible that crypsis in ectotherms may be under stronger selection by humidity that darkens the soil, rather than temperature^{4,51}.

An interspecific-level analysis found that eggshell brightness was predicted by environmental variables (Fig. 4). We found that eggshell brightness was positively related to solar intensity, such that species living in colder habitats have darker eggs (Fig. 4). This was true for all nest types (Extended Data Fig. 3) and supports the findings of our assemblage-level analyses for these patterns. The relationships between eggshell brightness and aridity varied by nest type, such that cup-nesting species tended to have darker eggs in wet environments and brighter eggs in arid environments (Fig. 4 and Supplementary Table 1); however, contrary to our findings with eggshell brightness, eggshell colour did not vary with solar intensity or aridity when examined at the interspecific level. Therefore,

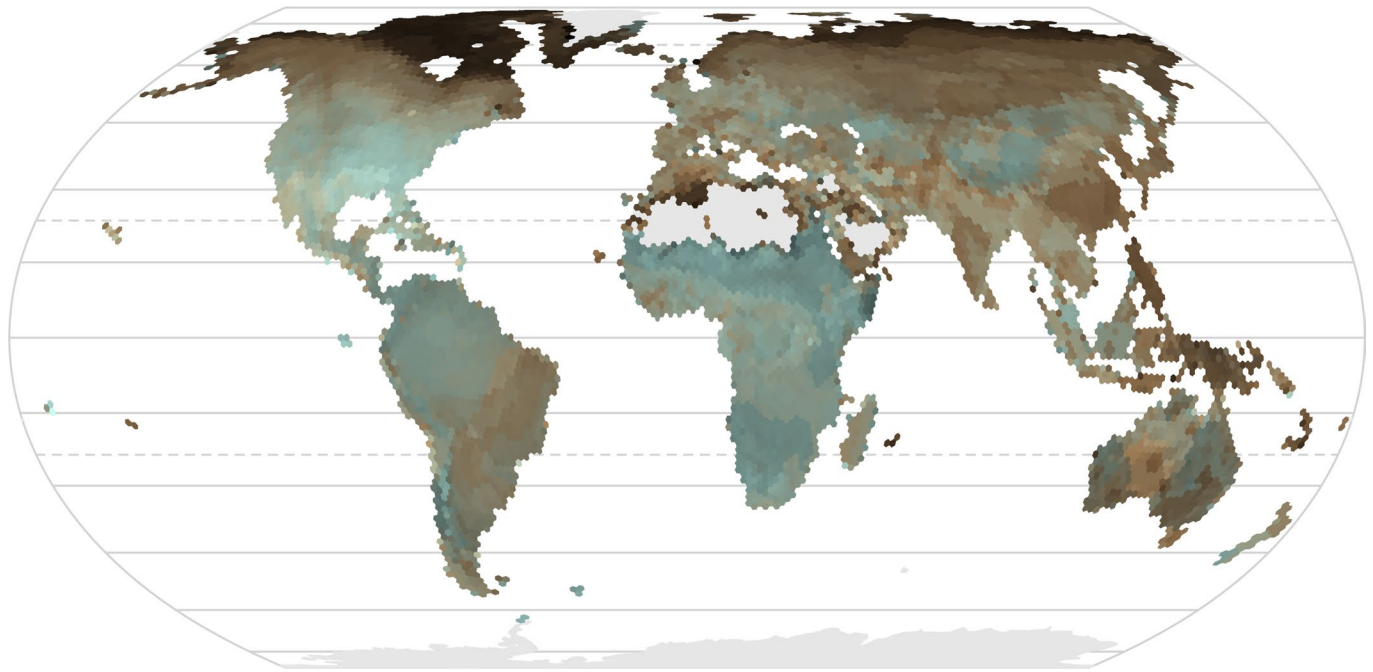


Fig. 2 | An equal Earth projection of the global distribution of avian eggshell colour. Global variation in avian eggshell colour and brightness using a bivariate plot. Each locale ($n = 6,692$) varies in relative eggshell colour and brightness. Plotted colours range from the blue-green of the eggshell of the grey catbird *Dumetella carolinensis* to the brown of the eggshell of the peregrine falcon *Falco peregrinus*. Locales where data were lacking or removed due to low sample size are not shown. An alternative colour scheme is available for colour-blind readers (Supplementary Fig. 1).

unlike the assemblage-level results, interspecific colour variation probably does not support antimicrobial defence²⁶ and crypsis⁵². We found these tests robust to the exclusion of influential species (Extended Data 4).

These results all suggest that darker surface pigmentation, regardless of the specific eggshell pigment, is found in places where solar radiation, and therefore temperature, is lower. Thus, darker colours may be adaptive for maintaining thermal balance, since these darker eggshells absorb more of the limited solar irradiance in these colder climates (see further on) when the egg is uncovered and most vulnerable to heat loss. Because avian eggshells, both pigmented and unpigmented, reflect a large amount of near-infrared solar radiation^{53,54}, they should heat less rapidly than structures pigmented with melanin⁵³, which absorb relatively more near-infrared radiation⁵³. However, roughly half the solar radiation that reaches Earth's surface is within the UV and visible spectrum (300–700 nm)⁵ and eggshell reflectances vary greatly within this region^{10,55}. This interpretation is consistent with our empirical measurements of variably coloured chicken eggshell surface temperatures over 25 min periods (Fig. 5a), which represents the estimated length of a natural foraging bout⁵⁶. In the absence of solar radiation, all eggs shed heat at the same rate, regardless of their brightness or colour (Fig. 5b,c); however, when those same eggs were illuminated under natural sunlight, darker eggs maintained their incubation temperatures for longer periods than white eggshells (Fig. 5b). Using a different set of eggs, we determined that darker eggs heated from ambient temperature more rapidly than white eggs (Fig. 5c). Importantly, a comparison of less variable duck and Japanese quail eggs found a congruous relationships between heat loss and eggshell brightness (Fig. 5b,c and Extended Data Fig. 5). Together, these tests suggest that darker pigmentation provides a valuable reduction in heat loss.

Discussion

Previous investigations of the impact of environmental variables on eggshell colour and brightness, which also spanned large regional

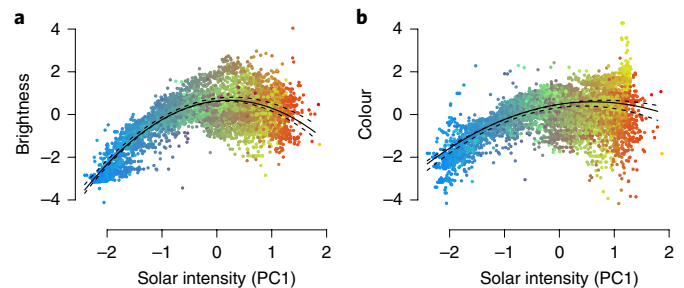


Fig. 3 | Relationship between solar intensity and eggshell colour. a,b, Solar intensity ($n = 6,692$) predicted both eggshell brightness (a) and colour (b) (solid lines). We show the 95% CIs for these relationships (dashed lines). In these plots, we depict each locale with colours representing their relative values of solar intensity (PC1). Dark blue, cold and relatively humid locales; red, hot and humid locales; light blue, cold and dry locales; yellow, hot and dry locales (see Extended Data Fig. 1 for further details).

scales, found limited⁵⁷ or no evidence⁵⁸ that avian eggshell colour or brightness vary with temperature. However, in this study we used a larger array of colours, a broader spatial range and wider phylogenetic diversity. Dark brown eggshell colouration is generally considered costly^{12,18} because egg temperatures are maintained close to their upper thermal limit¹⁹, or selectively neutral^{20,53}; however, our findings support the claim that these darker eggs may be adaptive for thermoregulation in colder locales¹. For the majority of birds, parents actively regulate incubation temperature and attempt to reduce predation risk^{59,60}. In cold environments, where predation pressure is lower^{22,23} and temperatures risk chilling eggs below their lower limit⁸, foraging bouts are shorter and trips to and from the nest are more frequent to maintain incubation temperature⁶¹. The daily energy expenditure for these adults can be up to 50% greater than birds living in temperate regions⁶²; thus, traits that can reduce

Table 1 | Parameter estimates from a spatial error panel model

Parameter	Brightness ^a				Colour ^b			
	β	s.e.m.	<i>t</i>	<i>P</i>	β	s.e.m.	<i>t</i>	<i>P</i>
Intercept	−0.61	0.02	−28.00	<0.0001	−0.72	0.03	−27.64	<0.0001
Solar intensity	0.23	0.01	16.64	0.0001	0.30	0.02	17.53	<0.0001
Solar intensity ²	−0.16	0.01	−13.84	0.0001	−0.08	0.02	−5.56	0.0001
Nest type (cup)	0.55	0.03	17.73	<0.0001	0.90	0.04	24.48	<0.0001
Nest type (cavity)	1.82	0.03	58.59	<0.0001	0.99	0.04	27.09	<0.0001
Aridity	0.004	0.01	0.40	0.69	0.27	0.01	21.52	<0.0001
Solar intensity × nest type (cup)	−0.0006	0.02	−0.03	0.98	−0.43	0.02	−17.69	<0.0001
Solar intensity × nest type (cavity)	−0.21	0.02	−10.79	<0.0001	−0.26	0.02	−10.70	<0.0001
Solar intensity ² × nest type (cup)	−0.001	0.02	−0.08	0.94	0.24	0.02	11.34	<0.0001
Solar intensity ² × nest type (cavity)	−0.04	0.02	−2.56	0.01	0.26	0.02	12.40	<0.0001
Aridity × nest type (cup)	−0.07	0.01	−4.80	<0.0001	−0.13	0.02	−7.53	<0.0001
Aridity × nest type (cavity)	0.21	0.01	15.42	<0.0001	−0.19	0.02	−10.59	<0.0001

^a $\rho = 0.69 \pm 0.006$, $AIC_c = 25,481.24$, $P < 0.0001$. ^b $\rho = 0.63 \pm 0.006$, $AIC_c = 36,229.42$, $P < 0.0001$. The models predict brightness and colour. Here ρ is a coefficient parameter that demonstrates the level of spatial dependence in each model ($\pm$ its associated s.e.m.). We show AICc and a significance test for each model. All parameter estimates are made with reference to the eggs of ground-nesting birds. We include solar intensity (PC1) and aridity (PC2), which are two separate principal components describing environmental conditions, a quadratic term for solar intensity and an interaction with both environmental scores. See Methods for further details.

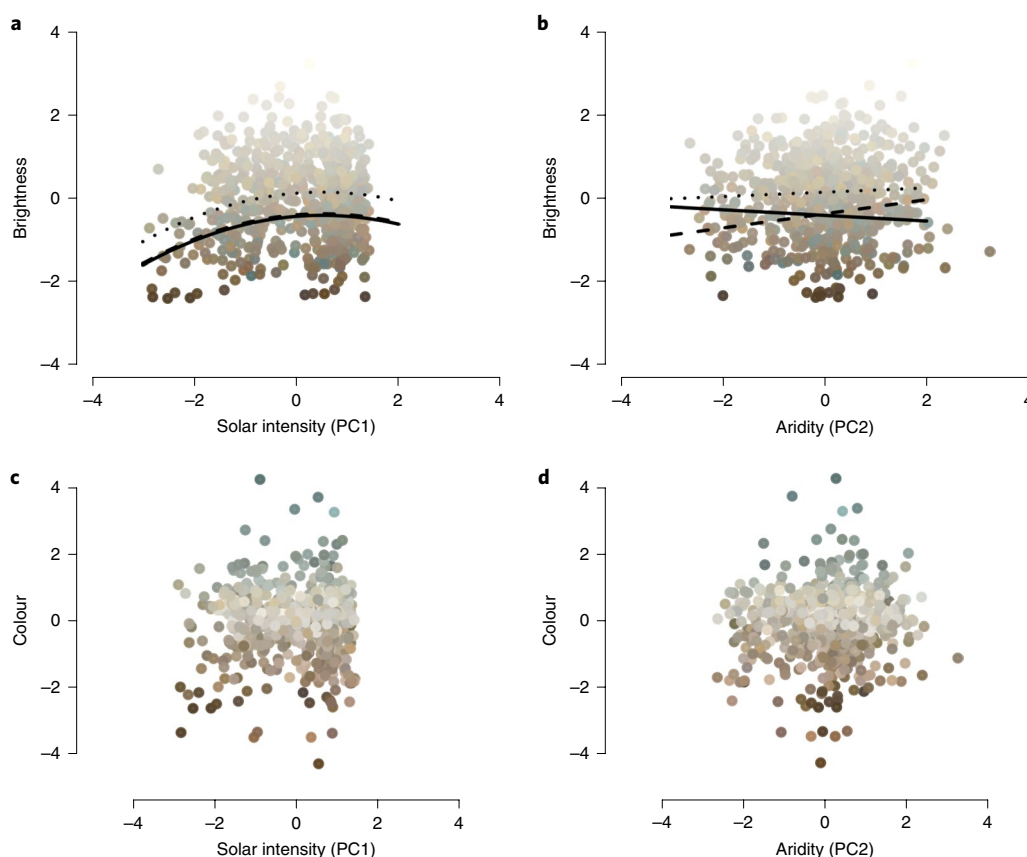


Fig. 4 | Interspecific patterns. a–d, Relationship between eggshell brightness and solar intensity (PC1) (**a**) and eggshell brightness and aridity (PC2) (**b**) across 618 species (brightness: $\chi^2 = 74.62$, Pagel's lambda $\lambda_{ML} = 0.85$, $P < 0.0001$). In addition, we show the relationship between colour and solar intensity (**c**) and colour and aridity (**d**) (colour: $\chi^2 = 20.98$, $\lambda_{ML} = 0.80$, $P = 0.004$). To aid interpretation, we show the slopes for ground (solid), cup (dashed) and cavity (dotted) nesting birds whenever significant. For complete statistical output, please refer to Extended Data Fig. 3.

egg heat loss are of great importance^{63,64}. By contrast, in warmer climates birds are less limited by maintaining incubation temperature^{56,65,66}, but must mitigate the risk of predation commonly found

to be greater in the tropics^{22–24,67,68}. These birds limit trips to and from their nests⁶⁹ because parental activity can draw the attention of nest predators^{59,70,71}. However, using a dataset of shorebird nest

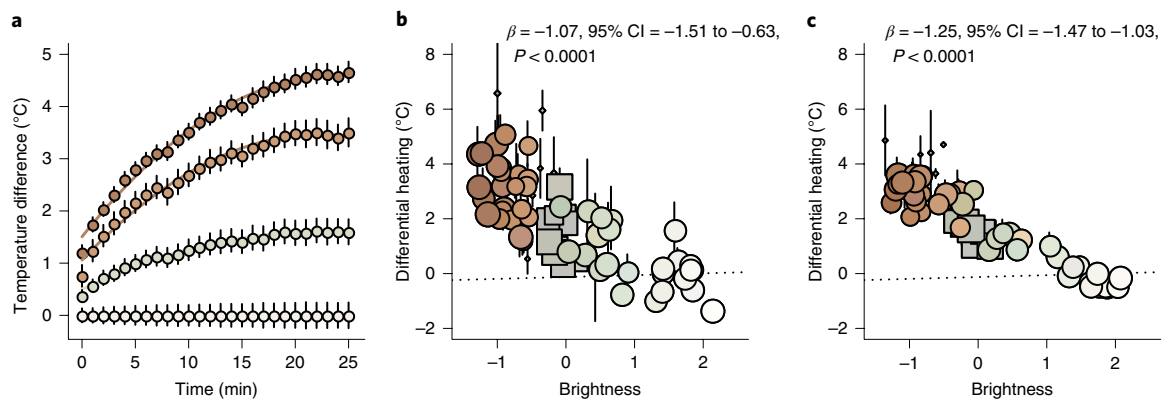


Fig. 5 | Differential heating by eggshell colour. **a**, Heat gained over a 25 min trial (temperature difference) $\pm$ s.e.m. for white, blue, light brown and dark brown (bottom to top) chicken *G. gallus domesticus* eggs ($n = 92$). All temperature differences are calculated relative to the average white chicken eggshell to assess relative differences across multiple trials (see Methods). These eggs were placed in direct sunlight for 25 min. **b**, When chicken eggs (circles; $n = 48$) were maintained at the incubation temperature, their rate of cooling directly related to brightness ($F_{3,44} = 35.8$, $R^2 = 0.71$, $P < 0.0001$). In addition, we have plotted the heat gain in duck (squares; $n = 12$) and Japanese quail (diamonds; $n = 12$) from the same trial that fit this pattern (Extended Data Fig. 5). **c**, Similarly, when chicken eggs ($n = 44$) were maintained at ambient temperature, brightness predicted heat loss ($F_{3,40} = 136.1$, $R^2 = 0.91$, $P < 0.0001$). Similarly, we have plotted duck ($n = 11$) and Japanese quail ($n = 11$) eggs, which also fit the predicted pattern. Trials were conducted at solar noon and in the morning (dashed lines). The points show the averaged values and the s.e.m. represents the error between each egg's morning and solar noon trial. To confirm that these differential heating rates were related to differential solar heating, chicken eggs maintained at the incubation temperature (**b**) were also allowed to cool in a dark room (dotted line) where all eggs cooled at the same rate ($F_{3,44} = 2.07$, $R^2 = 0.12$, $P = 0.12$), regardless of their brightness ($\beta = 0.06$, 95% CI = -0.10 to 0.22, $P = 0.43$). Colour and mass were non-significant in any model (all $P > 0.05$).

predation²³, instead of finding browner or darker eggs in locales with high nest predation, we found that eggs in these regions were brighter (Fig. 2 and Extended Data Fig. 6). Therefore, although nest predation is most probably an important site-specific selection pressure^{72,73}, our results suggest that predation does not explain the global distribution of avian eggshell colour or brightness as well as thermoregulation.

In this study, we provide a robust analysis across a broad phylogenetic diversity of birds at a global scale to consider competing hypotheses on how abiotic factors could have shaped the evolution and distribution of eggshell colour and brightness. We found darker eggshells were associated with colder environments, consistent with a range of findings on the surface colouration of other ectothermic organisms^{2,74}, providing support for Bogert's rule, which is typically supported in ectotherms⁴. However, unlike many of these organisms, the thermal requirements of the avian embryo transition from dependence on external factors to thermoregulate (absorbers) to the eventual ability to thermoregulate themselves (radiators)^{51,75}, making this system a particularly fruitful system for investigating thermoregulation. Our study provides insight into the environmental pressures that have shaped the global distribution of colours and should serve as a call to reinvigorate this line of research^{29,53} and investigate patterns of colour expression in other organisms.

Methods

Colour estimation. We used spectral reflectance data of avian eggshells ($n = 634$) spanning all avian orders excluding Eurypygiformes (2 species: kagu and sunbittern), Leptosomiformes (1 species: cuckoo roller), Mesitornithiformes (3 mesite species) and Pteroclidiformes (16 sandgrouse species)⁷⁶. Of those orders represented in our data, the majority of families within each order were sampled (mean $\pm$ s.e.m. = $89 \pm 3\%$). These reflectance data were smoothed using a locally weighted polynomial function. Because all birds' eggs vary from blue-green to brown¹⁰ due to the admixture of the two avian eggshell pigments, we then quantified 'colour' as the proportion of blue-green reflectance (450–550 nm) relative to the entire spectrum (300–700 nm)⁷⁷. We quantified mean 'brightness' as the mean reflectance from 300 to 700 nm⁷⁷. Both colour variables were centred and scaled and were related to correspond with avian perceived variation in colour and brightness (Extended Data Fig. 7).

Ecogeographical and phylogenetic sampling. We downloaded bird distribution maps from BirdLife International³¹ (available on request at <http://datazone.birdlife.org/species/requestdis>) and buffered entirely oceanic ranges by 10 km using the 'Digital Chart of the World' base map⁷⁸ (available at https://worldmap.harvard.edu/data/geonode/Digital_Chart_of_the_World) to restrict all ranges to land. We chose the 10 km buffer because it was well within one sampling area (see further on). Then, we removed all lakes from all sampled species bird ranges using a 1:10,000,000 map of worldwide lakes from Natural Earth Data v.3.0.0 (ref. ⁷⁹) (available at <https://www.naturalearthdata.com>). We obtained environmental data from WorldClim v.2 (ref. ⁸⁰) (available at <http://worldclim.org/version2>), the National Center for Atmospheric Research⁸¹ (available at <https://www2.acom.ucar.edu/modeling/tuv-download>) and the Atlas of the Biosphere⁸² datasets (<https://nelson.wisc.edu/sage/data-and-models/maps.php>). Next we randomly generated one nest every 10,000 km² within each species' breeding or resident range³¹. Each point ($n = 3,577,243$) was then assigned its species' colour and brightness value. We overlaid an equal-area hexagonal discrete global grid (ISEA aperture 3, resolution 7, $n = 7,158$) and within each hexagon (locales) we averaged both egg and environmental data. We used ArcGIS ArcMap v.10.5 (Esri) for all biogeographical sampling.

We accounted for the phylogenetic relatedness among birds by constructing a phylogenetic hypothesis using a sample of 9,999 fully resolved phylogenetic trees from a recent and complete avian phylogeny^{83,84}. Using these data, we calculated the Bayesian maximum credibility tree, using the mean branch lengths of the candidate set using the Sum Trees program v.4.3.0 with the DendroPy⁸⁵ library v.4.3.0, dropping 10% of trees as burn-in. We then assigned all nodes an ancestral nest type, assuming equal rates, using a maximum likelihood estimation⁸⁶. This phylogeny was used to examine the selection pressures on eggshell colour and brightness using two levels of analysis: assemblage-level and interspecific-level analyses. We classified 'ground' nests as nests laid directly on the ground, while 'cup' nests were nests constructed above the ground, 'domed' nests were those with a distinct covering over the eggs, 'cavity' nests were those made in holes and 'mound' refers to the buried eggs of mound builders.

Assemblage-level analysis. We calculated the mean colour and brightness for all birds at each locale using the 'phyloMean' function in the motmot.2.0 package. This analysis controlled for the phylogenetic relatedness of taxa. When predicting mean colours for each nest type, this analysis considered the nest types of neighbouring, related taxa instead of truncating the data. This is because if phylogenetic dependence of colour is high, one taxon may have an unusual colour for its particular nest, but a very predictable colour for its clade. This generated a mean for all birds living in the locale as well as birds nesting exclusively on the ground, in open nests, dome nests, cavities or in mounds. We removed any locale that prevented phylogenetic (for example, fewer than 3 species) or spatial control (isolated locales and locales with missing information), which reduced our final sample size to 6,692 locales.

To summarize environmental variation at these locales ($n=6,692$), we ran a principal components analysis, using singular value decomposition on normalized values. We include mean annual temperature ($^{\circ}\text{C}$), UVB radiance ($\text{kJ m}^{-2} \text{month}^{-1}$), precipitation (mm) and relative humidity (%) and identified two principal components (with eigenvalues >1) that explained 91% of the variation in this global dataset. Temperature and UVB radiance loaded positively on the first component, while precipitation and humidity loaded negatively on the second component (Extended Data Fig. 1). Thus, high values of PC1 (hereafter, solar intensity) correspond with places that experience a greater solar intensity, and high values of PC2 (hereafter, aridity) represent very arid places (Extended Data Fig. 1). Similarly constructed principal components, including the temperature of the warmest quarter, were highly related to those constructed using mean annual temperature (solar intensity: $r=0.96$, 95% CI = 0.96 to 0.97, $P<0.0001$; aridity: $r=0.98$, 95% CI = 0.97 to 0.99, $P<0.0001$).

Both colour and brightness were spatially autocorrelated (colour: Moran's $I=0.87$, $P<0.0001$; brightness: Moran's $I=0.91$, $P<0.0001$); therefore, we accounted for spatial autocorrelation by using a spatial error model⁸⁷, using first-order Queen's contiguity⁸⁷ (weights were roughly equivalent to approximately 350 km). Specifically, we predicted either brightness or colour by solar intensity and aridity. Because a clear non-linear trend was apparent between both colour variables and solar intensity, we also included a quadratic term for this variable. Then, we used Koenker's studentized version of a Breusch–Pagan test to determine whether our regression model was significantly heteroscedastic; we applied a correction for the s.e.m. estimates whenever significant heteroscedasticity was detected. We calculated and report the effect size, CIs, parameter significance and corrected Akaike information criteria (AIC_c) for each model. Whole-model significance was determined via a likelihood ratio test with a null linear model. All models were weighted by the square root of sampled richness to avoid bias caused by poor sampling. Additionally, all continuous data were centred and scaled so that parameter estimates were comparable standardized β scores. Although total sampled richness and phylogenetic signal varied by geography, we found no quantitative evidence that this would bias our results (Extended Data Fig. 8).

Spatial panel analysis. We expected that exposure to solar radiation should result in a greater potential benefit for dark brown colours, after accounting for geography. Likewise, in the absence of solar radiation there is no thermoregulatory benefit of eggshell colours (see Results). Therefore, in addition to the assemblage-level tests that considered all birds, we investigated these patterns across locales that had ground-nesting ($n=6,542$), open-nesting ($n=6,478$) and cavity-nesting ($n=6,561$) where we assumed exposure to light levels varies from most (ground nests) to least intense (cavity nests). We did not have specific predictions for dome-nesting birds and mound builders, so they were removed from this set of analyses. To address this question, we used a spatial panel error regression model⁸⁸. This analysis allows spatial interaction in the error terms, which is consistent with a model where some determinants of the dependent variable may be unobserved but spatially correlated, or where random shocks, that is, geological or environmental perturbations, may have a spatial pattern⁸⁹. We estimated a spatial pooling model and allowed for the prediction of colour and brightness by environmental variables across three distinct nest types, while controlling for spatial serial correlation. All continuous variables were centred and scaled. However, this requires complete cases for all nest types at each locale. Therefore, to balance the investigation of nest type with using as much of the data as possible, we restricted our sample to only those locales that had all nest types, where we had specific expectations (ground, cup and cavity), leaving only those locales with three observations (nest types) each, and with neighbouring locales ($n=6,209$). We ran this analysis using the *spml* package in R⁹⁰.

Interspecific-level analysis. For each species, we calculated the mean values for both solar intensity scores (see earlier) across their breeding range. We restricted our analysis to species found at locales that were included in our assemblage-level analysis (see earlier), which reduced the total number of species for this test ($n=618$). We then predicted colour and brightness in separate phylogenetic generalized least squares⁹¹ analyses using the package *ape*. These analyses controlled for the maximum likelihood value of Pagel's λ ⁹¹, which transforms the phylogeny so that the data best conform to a model of Brownian motion⁹². Unlike the assemblage-level analysis, there were no visible non-linear trends; therefore, we did not include the polynomial term in these models. Each analysis predicted colour or brightness by solar intensity, aridity, nest type, interaction between solar intensity and nest type and interaction between aridity and nest type. All continuous variables were centred and scaled. If the interaction term was non-significant, we removed the interaction and reran the model.

To consider and accommodate for the possibility that specific birds overly influence these analyses (for example, predominantly ground-nesting larids at the northern end of our sampling range), we performed a jackknife procedure. Specifically, we reran these models, each time removing one species and recording the log-likelihood value for the model. These log-likelihood values were then standardized, so that negative values represent taxa that drive the pattern (that is, their removal makes the model less probable) and positive values represent taxa that detract from the pattern (that is, their removal makes the

model more probable). We considered these values bias estimates and exclusively focused on taxa that may potentially drive (enhance) the reported patterns. For orders that resulted in a log-likelihood reduction of two or more s.d., we removed all the species from those orders and reran the original models. We then compared these 'conservative models' to models using our full dataset, using AIC_c and evidence ratios⁹³.

Thermoregulation and colour. We examined whether variation in avian eggshell brightness could result in differential heating. We measured the surface eggshell temperature of the domestic chicken *G. gallus domesticus* (Carol's Eggs and Stop and Shop), duck *A. platyrhynchos domesticus* (Windy Ridge Farms) and Japanese quail *C. japonica* (Windy Ridge Farms) eggs under natural illumination conditions. The chicken eggs ($n=92$) were either dark brown, light brown, blue-green or white; the duck eggs ($n=23$) were off-white, with some appearing either slightly brown or slightly blue-green; the Japanese quail eggs ($n=23$) were buff-coloured but had variable spotting across their surfaces. Each egg's mass was recorded using a microbalance (Ohaus AV114C Adventurer Pro Analytical Balance, ± 0.0001 g; Ohaus Corporation) to the nearest centigram. In addition, we recorded the upper surfaces of the eggs that were exposed to sunlight using a Jaz Spectrophotometer (Ocean Optics) and quantified each egg's colour and brightness. All tests were conducted on the LIU Post campus (40° 49' 12.8" N, 73° 35' 49.7" W).

First, to assess the rate of cooling under natural conditions, we heated (chicken $n=48$, 12 for each colour; duck $n=12$; Japanese quail $n=12$) eggs overnight to 41 °C in a growth chamber (model DROS33SD; Powers Scientific) on commercially sourced potting soil (Vigoro Organics) coated with locally sourced fallen leaves to approximate a scratch nest. We then placed these eggs outside under direct sunlight in the morning (between 8:37 and 8:58) when the elevation angle of the Sun was approximately 34° (the elevation angle was calculated using <https://www.esrl.noaa.gov/gmd/grad/solcalc/azel.html>). This time and solar angle were selected because they were roughly equivalent to the solar elevation angle at solar noon at 75° N latitude during mid-June, which is a midpoint within the May–August breeding season at such latitudes⁶⁴. We measured egg temperatures every minute for 25 min using a thermal imaging camera (FLIR ONE Pro; FLIR Systems). We chose this time span to approximate the length of an average foraging bout of a wide diversity of birds⁶⁶. After each trial, the eggs were reheated to 41 °C and the experiment was repeated in the afternoon (between 12:37 and 12:48), spanning solar noon, when the elevation angle of the Sun was approximately 68°. These experiments were repeated on the 15, 16 and 18 May 2019. The rate of cooling over a 25 min period was also measured for each chicken egg at room temperature (approximately 24.1 °C) in a dark room without windows (or other light sources).

Second, to assess how rapidly eggs gain heat when exposed to solar radiation, we heated 66 eggs (11 duck, 11 Japanese quail, 11 blue chicken, 11 white chicken, 11 dark brown chicken and 11 light brown chicken) to the outside ambient temperature (16.0 °C morning, 20.5 °C solar noon) in the growth chamber. We then placed them in direct sunlight in the morning (8:43–9:08) and in the afternoon (12:39–13:04) on 22 May 2019, following the same measurement protocol as before (see earlier).

For all trials, we calculated the mean egg temperature for each egg using calibrated thermal images in ImageJ version 1.52n (National Institutes of Health)⁹⁴. Comparisons between trials are complicated because ambient temperatures varied across and between trials. To overcome this and explore relative eggshell pigment heating, we compared each egg to the average white egg from the same trial. We then calculated each egg's change in temperature as the difference between its first and last measurement. Negative values represent eggs that lost more heat than the average white egg, while positive values represent eggs that did not lose as much heat as the average white egg (hereafter, differential heating). Again, we compared each egg's temperature change against that of the mean change experienced by the white chicken eggs during the same trial.

We predicted differential heating of only chicken eggs based on colour, brightness and mass using a general linear model. We ran separate models to predict differential heating in eggs in the absence of light, eggs exposed to direct solar radiation at incubation temperature and eggs exposed to direct solar radiation at ambient temperature. All models produced variance inflation factors <4 (ref. ⁹⁵), suggesting that multicollinearity between brightness and colour did not influence these models.

To explore the potential generality of the role of colour on differential heating, we examined how closely the duck and Japanese quail eggs matched the relationships generated by chicken eggs (see earlier). Our initial experiments suggest that these patterns are driven by extremes in eggshell brightness and colour, but both species were less variable in eggshell brightness or colour than the chicken eggs (Extended Data Fig. 5). Thus, we did not expect differential heating would relate to brightness or colour for either species; however, these species should fall close to the line generated by the chicken eggs if brightness or colour were an important predictor of differential heating. Therefore, to examine the potential applicability to other species, we reran these models including the data for both duck and Japanese quail and examined each egg's residual fit to the regression line. The standardized residuals for these plots should be ≤ 2 if these species correspond with the data presented for the chicken eggs.

Internal and external temperature comparisons. To verify that surface temperatures corresponded with internal temperatures, we inserted a type T thermocouple (SSRTC-TT-T-24-36; Omega) 2.5 cm into a new set of dark brown ($n = 12$) and white eggs ($n = 12$). We recorded the internal temperature using a thermocouple logger (HH506RA; Omega) and the external temperature using a thermal imaging camera (see earlier), and found that the internal and external temperatures for both brown ($r = 0.97$, 95% CI = 0.88 to 0.99, $P < 0.0001$; Extended Data Fig. 9) and white eggs ($r = 0.98$, 95% CI = 0.94 to 1.00, $P < 0.0001$; Extended Data Fig. 9) were highly related.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The BirdLife International species distribution data can be requested at <http://datazone.birdlife.org/species/requestdis>. The Digital Chart of the World basemap can be accessed at https://worldmap.harvard.edu/data/geonode:Digital_Chart_of_the_World. The Natural Earth Data maps can be found at www.naturalearthdata.com/. The Worldclim v.2 temperature data are available at <http://worldclim.org/version2>. The National Center for Atmospheric Research UV data can be found at www2.aom.ucar.edu/modeling/tuv-download. The Atlas of the Biosphere humidity data can be found at <https://nelson.wisc.edu/sage/data-and-models/maps.php>. The ISEA global grid data can be found at <https://cran.r-project.org/web/packages/dggridR/vignettes/dggridR.html> for the R codes or www.arcgis.com/home/item.html?id=38e3e0453e4040c69ff5e9fde306c12c for the shapefiles. Data generated and/or analysed during this study are available from the figshare digital repository⁹⁵ (<https://doi.org/10.6084/m9.figshare.9745109>).

Code availability

The R script used for the statistical analysis is available upon request.

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Author contributions

D.H. developed the concept. D.H. and P.C. collected the data. D.H. and I.R.R. conducted the colour analyses. P.A.W., P.K., M.L.B. and D.H. developed and implemented the ecogeographical models. P.A.W. and D.H. conducted the fieldwork. P.A.W. and D.H. prepared the initial draft and all authors edited the paper.

Competing interests

The authors declare no competing interests.

Additional information

Extended data is available for this paper at <https://doi.org/10.1038/s41559-019-1003-2>.

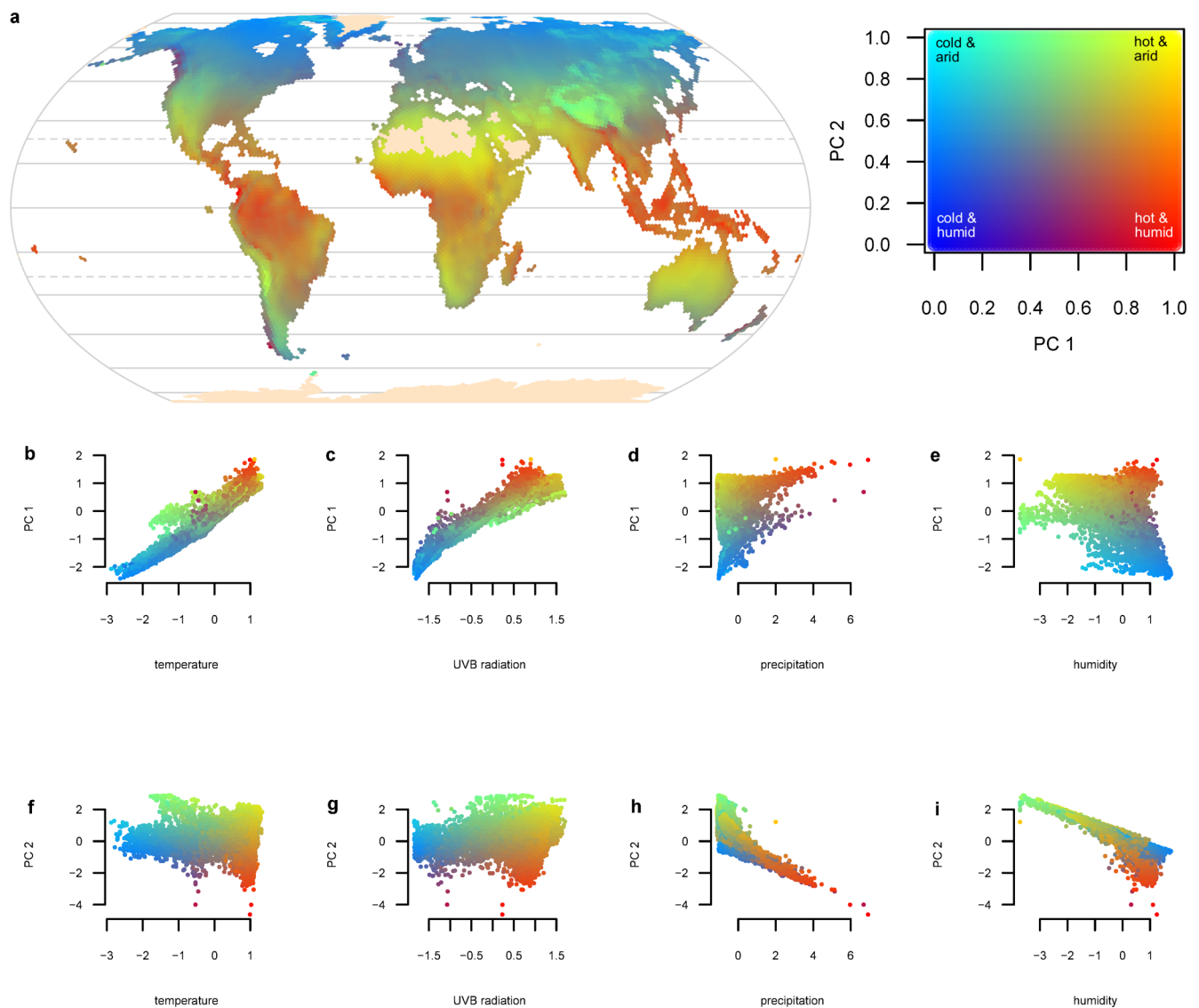
Supplementary information is available for this paper at <https://doi.org/10.1038/s41559-019-1003-2>.

Correspondence and requests for materials should be addressed to D.H.

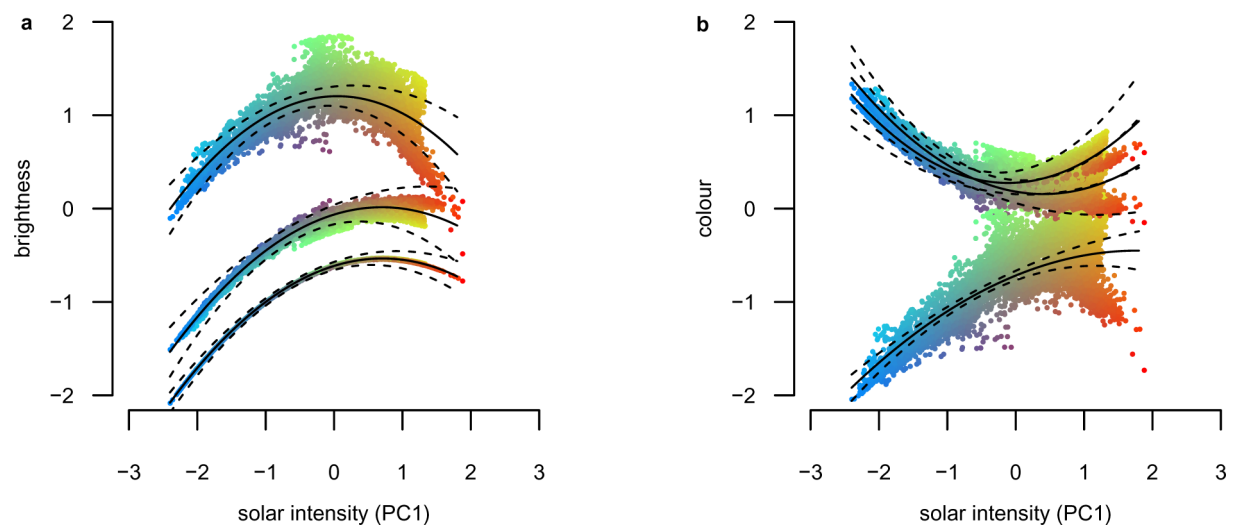
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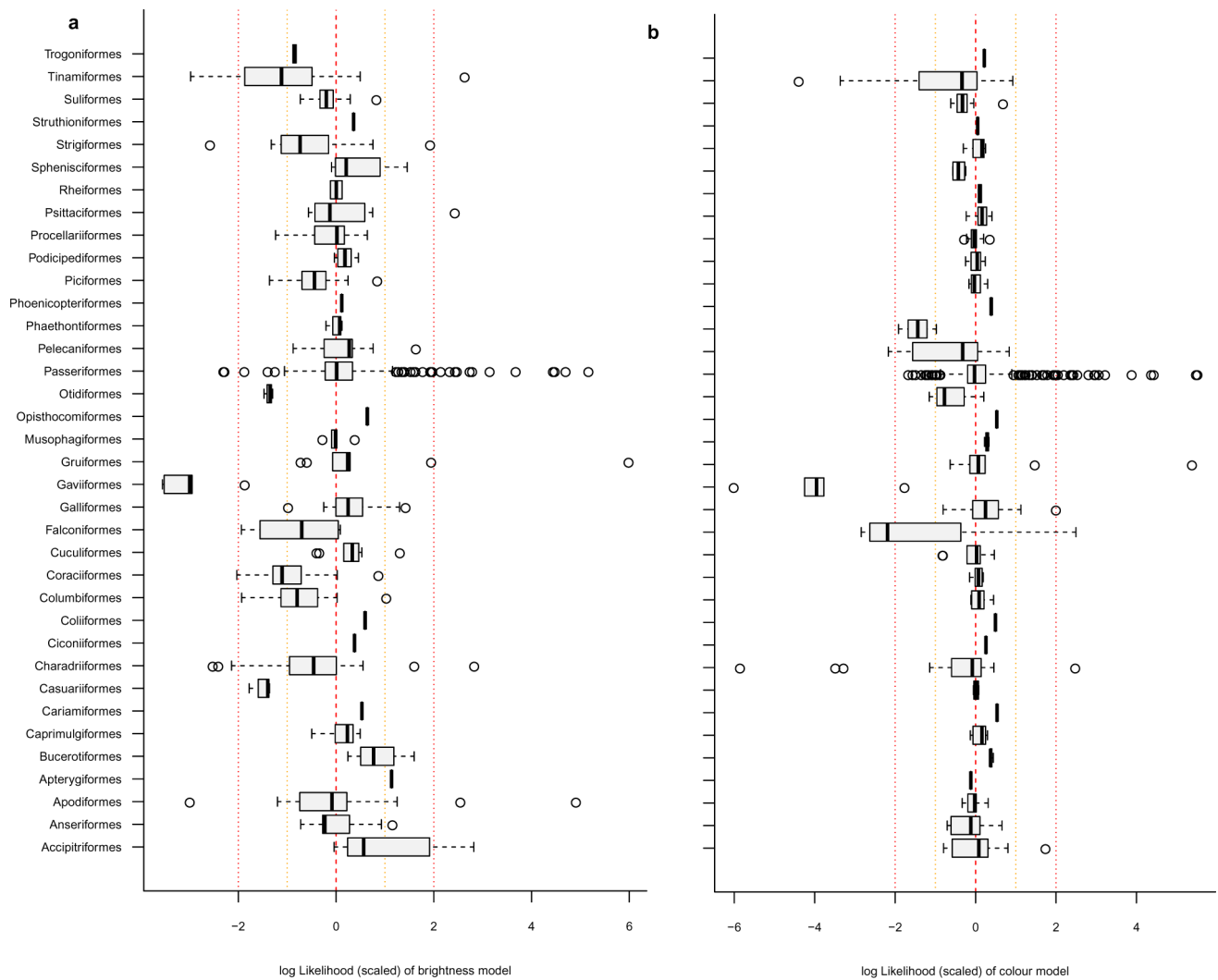
Extended Data Fig. 1 | Global variation in environmental variables. Global variation in the environmental variables across all locales examined in this study ($n = 6,692$). The first two principal components explained 91% of the variance, where **b**, temperature and **c**, UVB radiance loaded positively on PC1 with **d**, precipitation and **e**, humidity loading less strongly (loadings: *temperature* = 0.65, *UVB* = 0.65, *humidity* = -0.22, *precipitation* = -0.32). By contrast, **f**, temperature and **g**, UVB radiance loaded less strongly on PC2 while, **h**, precipitation and **i**, humidity loaded negatively (loadings: *temperature* = -0.04, *UVB* = 0.13, *humidity* = -0.73, *precipitation* = -0.67). Here we illustrate **a**, the discordance and congruence among within PC scores representing environmental variation using a colour scheme (see key). Locales that are relatively cold and humid are shown in darker blue, locales that are relative hot and humid are shown in red, locales that are cold and dry are relatively light blue, and places that are hot and dry (arid) are shown in yellow. We use this colour scheme in Fig. 3 as well as Extended Data Figs. 2 and 6 to aid interpretation.



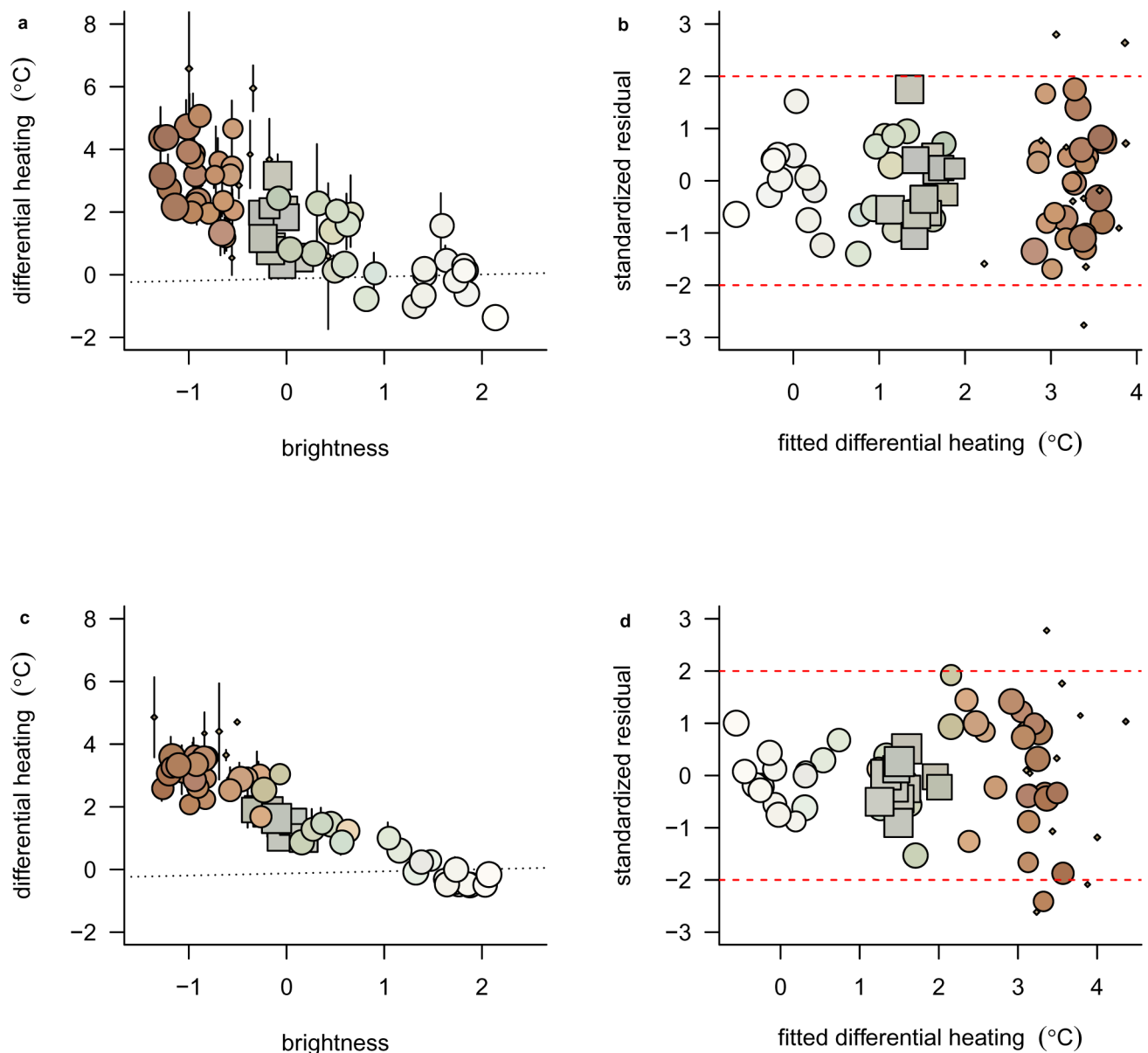
Extended Data Fig. 2 | Variation in eggshell appearance by nest types. The fitted values from linear models (solid lines) predicting **a**, brightness and **b**, colour by solar intensity (PC1), along with their 95% confidence intervals (dashed lines). From top to bottom the fitted lines represent cavity, cup, and ground nesting birds. The colours of dots represent environmental conditions at each locale ($n = 6,992$; see Extended Data Fig. 1 for definition of colour scheme).

	brightness ($\lambda_{ML} = 0.85$, $AIC_c = 1412.1$, $\chi^2 = 74.62$, $p < 0.0001$)				colour ($\lambda_{ML} = 0.80$, $AIC_c = 1559.2$, $\chi^2 = 20.98$, $p = 0.004$)			
Parameter	β	s.e.	t	p	β	s.e.	t	p
(Intercept)	-0.43	0.40	-1.10	0.27	-0.05	0.41	-0.11	0.91
solar intensity	0.10	0.04	2.28	0.02	-0.03	0.05	-0.63	0.53
solar intensity ²	-0.10	0.03	-2.80	0.005	-0.06	0.04	-1.53	0.13
aridity	-0.07	0.07	-0.99	0.32	0.06	0.04	1.40	0.16
nest type (cup)	0.04	0.12	0.32	0.75	0.42	0.13	3.24	0.001
nest type (dome)	0.24	0.14	1.68	0.10	0.44	0.16	2.77	0.006
nest type (cavity)	0.56	0.13	4.20	<0.0001	0.28	0.15	1.90	0.06
nest type (mound)	0.41	1.14	0.34	0.72	0.72	0.52	1.39	0.16
aridity x nest type (cup)	0.24	0.08	2.92	0.004	-	-	-	-
aridity x nest type (dome)	0.03	0.10	0.33	0.74	-	-	-	-
aridity x nest type (cavity)	0.12	0.09	1.28	0.20	-	-	-	-
aridity x nest type (mound)	-0.13	0.64	-0.20	0.84	-	-	-	-

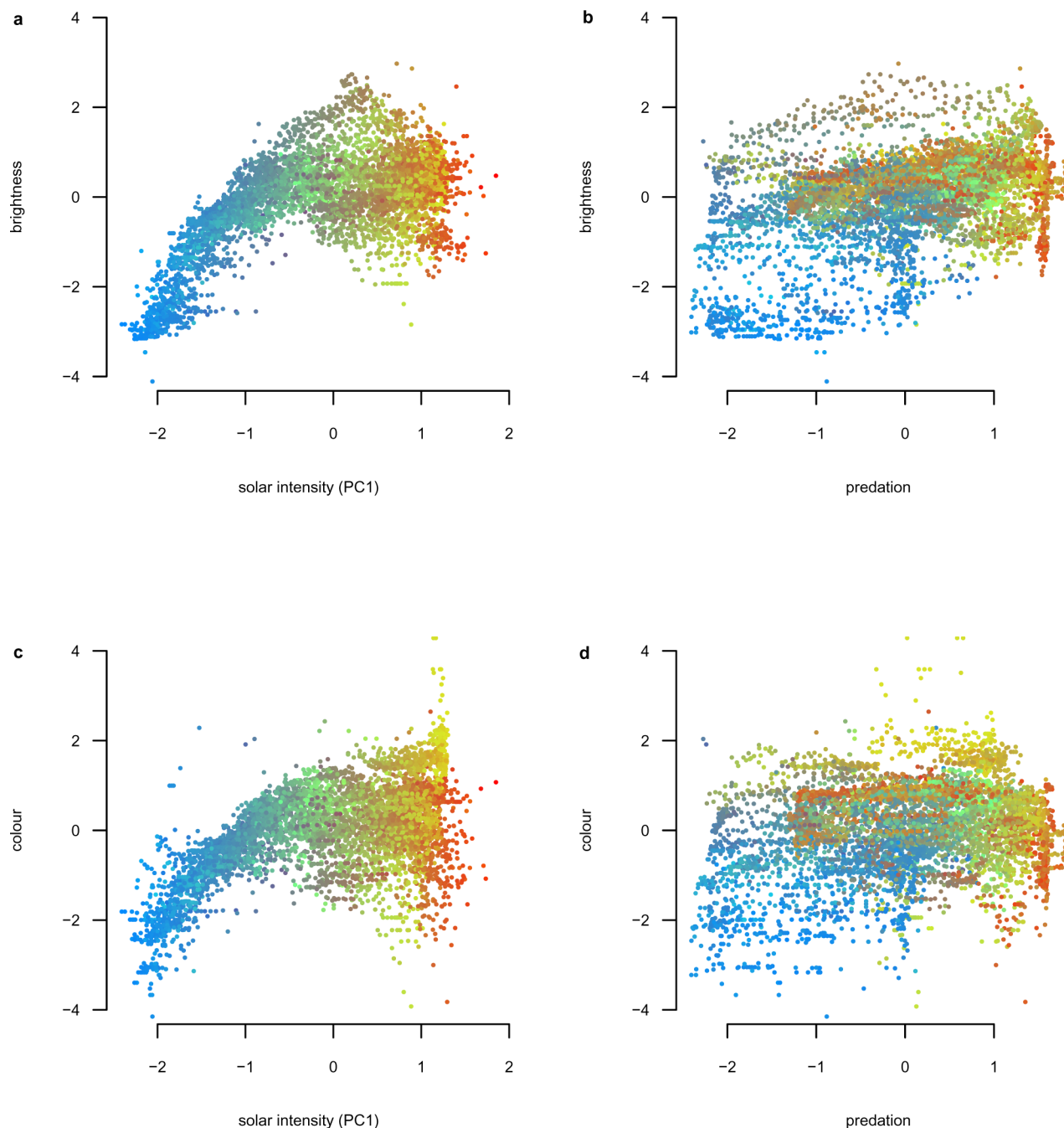
Extended Data Fig. 3 | Parameter estimates from phylogenetic generalized least squares analyses. Models predicting brightness and colour, by solar intensity (PC1), aridity (PC2), nest type, and interactions between nest type and aridity. When interactions were non-significant (for example, interactions with solar intensity) they were removed and the models rerun without the interaction (see Methods). We report Pagel's lambda (λ_{ML}) and AIC_c for each analysis. We report, standardized beta (β), standard error (s.e.m.), a t-value, and significance for each parameter. The effects of all parameters are made with reference to the eggs of ground nesting birds. See Methods for further details.



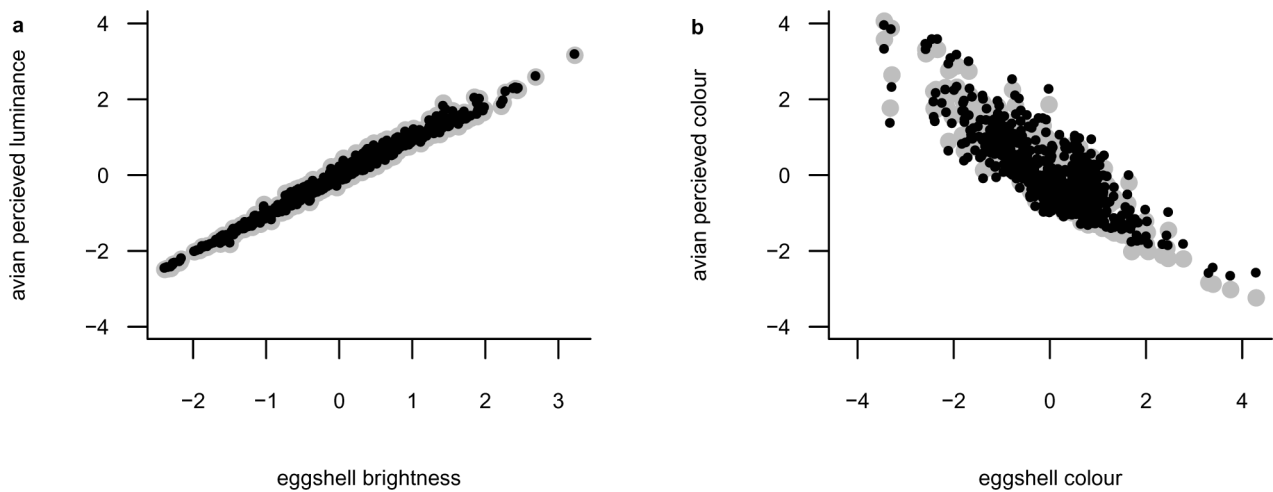
Extended Data Fig. 4 | Influence of avian groups on PGLS analysis. For models predicting **a**, brightness and **b**, colour we explored the influence of individual species by performing a jack-knife procedure, running the each analysis repeatedly, each time removing a different species. We plot the scaled log likelihood values of those analyses by their taxonomic order, and constructed a ‘conservative’ models removing all species from orders that may drive the detected patterns (that is, ≤ 2 s.d. from the mean). The full models explained the data better than the conservative models for both **a**, brightness ($\Delta AIC_c = 7.65$, evidence ratio = 45.74) and **b**, colour ($\Delta AIC_c = 6.75$, evidence ratio = 29.23). For both models the direction and significance for all parameters was the same; therefore, the full models are presented in the Main text. See Methods for further details.



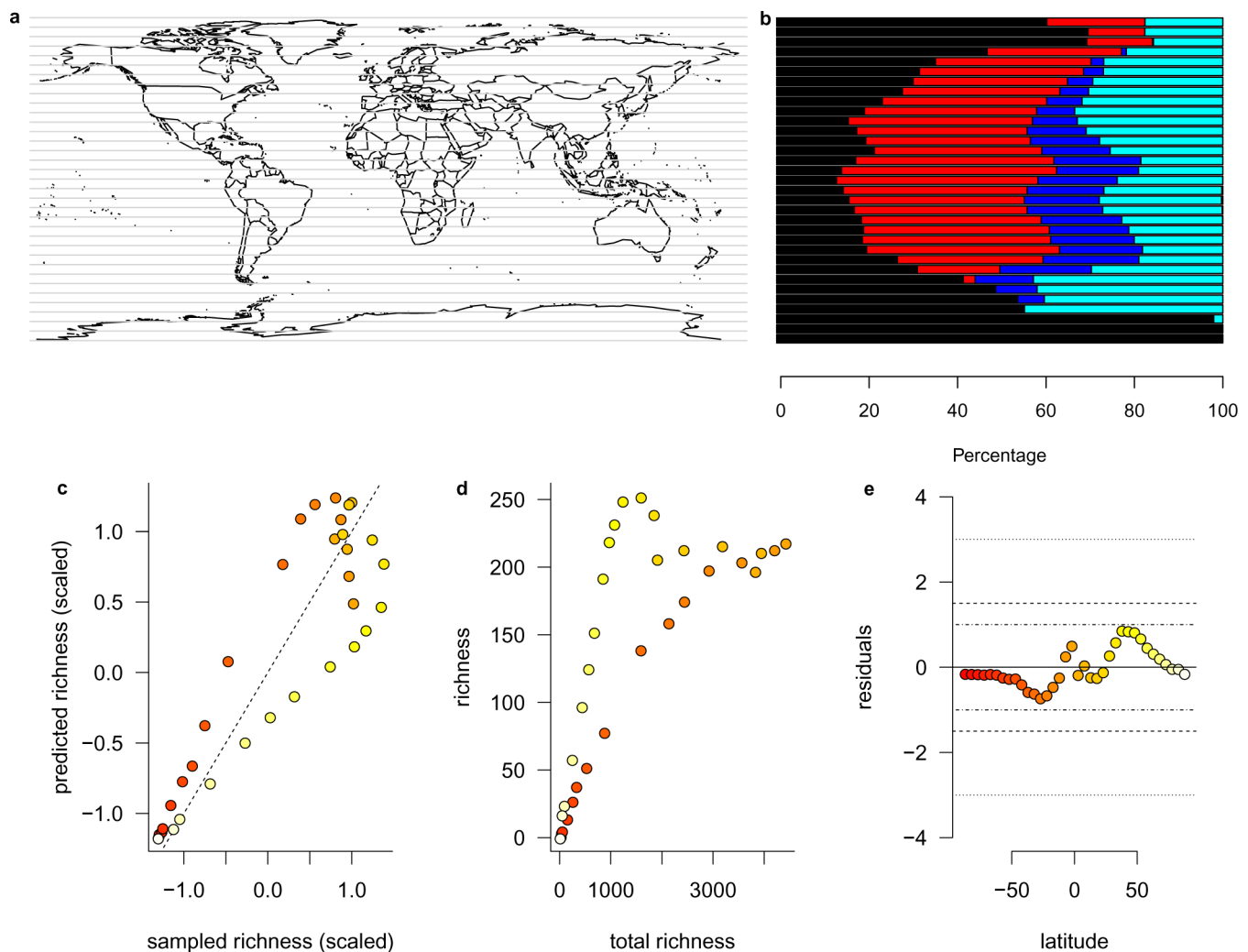
Extended Data Fig. 5 | Heating and cooling rates of eggs. To complement the patterns reported in the main paper, we added mean ($\pm$ s.e.m.) differential heating for duck (square) and quail (small dots) eggs alongside chicken eggs (circles), in a randomized order for each trial. Although neither duck (inter-quartile range = 0.19 s.d.) nor quail (inter-quartile range = 0.48 s.d.) has the variation in eggshell ground brightness corresponding with the chicken (inter-quartile range = 2.22 s.d.) they should fall roughly along the same line as chicken eggs with respect to their rate of heating and cooling, relative to white chicken eggs (see Methods). These represent trials where eggs were **a-b**, exposed to direct solar radiation while at incubation temperature and where eggs were **c-d**, exposed to direct solar radiation while maintained at ambient temperature (see Methods). Here we plot the rates of cooling for these additional species alongside the data plotted in Fig. 5. Including these data within the same model produced nearly identical parameter estimates, significances, and predicted rates of heating and cooling. In addition, we plot the **b,d**, standardized residuals by their fitted values highlighting residuals above or below 2 s.d. The majority of these data fit within these bounds suggesting that despite limit variation in brightness the rate of heating and cooling of these eggs roughly corresponds with those measured for the chicken.



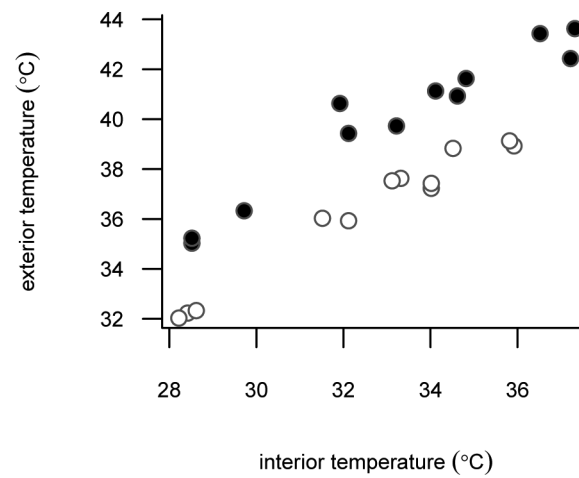
Extended Data Fig. 6 | Comparison between environmental and predation. A plot of eggshell **a**, brightness and colour **c**, versus solar intensity and **b,d**, historic total nest predation rate using publicly available data from a previously published source²³. We show the relationship between brightness and **a**, solar intensity (PC1) and **b**, predation pressure where data were available such that all locales had a spatial neighbour ($n = 6,326$). All variables were centred and scaled to aid interpretation. Additionally, we present the relationship between colour and **c**, solar intensity (PC1) and predation pressure. If predation pressure was driving eggshell phenotypes we would expect the darkest brownest eggshells where predation was highest; however, the opposite pattern is present and significant for predation (*brightness*: $\beta = 0.32$, CI = 0.19 to 0.46, $p < 0.0001$; *colour*: $\beta = 0.21$, CI = 0.08 to 0.34, $p = 0.002$). Please note these predation data apply to shorebirds, and future research should determine if clinal variation in predation applies to all birds. Environmental variables fit data on eggshell brightness ($\Delta AIC_c = 74.8$; evidence ratio $> 1,000$) and colour ($\Delta AIC_c = 111.9$; evidence ratio $> 1,000$) provided a better fit for the data than predation. The colours of the plotted points relate to their relative values of PC1 and PC2 (see Extended Data Fig. 1 for details).



Extended Data Fig. 7 | Relationship between colour and brightness and their avian perceived equivalent scores. The relationship between **a**, eggshell brightness (mean brightness) and egg species' calculated perceived luminance. In addition, present the relationship between **b**, eggshell colour (representing blue-green chroma) and each species' egg colour coordinate within the tetrahedral colour space. All eggs vary from blue-green to brown within this avian perceptual colour space and here we compare variation across the x-axis (corresponding with this axis of variation) within that space and our estimate of colour. These avian visual models corresponding with either the average ultraviolet sensitive receiver (black dots) or the average violet sensitive receiver (grey dots). For the average ultraviolet-sensitive receiver, we used photoreceptor sensitivity estimates for the average ultraviolet-sensitive receiver under idealized illumination, and using the blue-tit *Cyanistes caeruleus* double cone catch to estimate luminance. For the average violet-sensitive receiver, we used photoreceptor sensitivity estimates for the average violet sensitive receiver and double cone catch estimates for the chicken *Gallus gallus* to estimate luminance, under idealized conditions. Avian eggshell brightness was highly correlated with avian perceived eggshell luminance (UVS: $\beta = 0.98$, CI = 0.98 to 0.99, $p < 0.0001$, $\lambda_{ML} = 0.82$; VS: $\beta = 0.98$, CI = 0.971 to 0.99, $p < 0.0001$; $\lambda_{ML} = 0.83$) and eggshell colours were also were highly correlated with avian perceived variation in colour (UVS: $\beta = -0.77$, CI = -0.80 to -0.73 , $p < 0.0001$, $\lambda_{ML} = 0.84$; VS: $\beta = -0.85$, CI = -0.88 to -0.82 , $p < 0.0001$, $\lambda_{ML} = 0.83$).



Extended Data Fig. 8 | Sampling bias. To consider the possibility that our findings are impacted by an overabundance of some nest types in certain places (for example, only ground nesting birds above a certain latitude) or poor sampling overall, we report the relative proportion of birds of a given nest type across space and run an analysis. First, **a**, across 5-degree latitudinal bins, we report the **b**, percentage of birds with each nest type (ground = black, cup = red, dome = dark blue, cavity = light blue, mound = yellow). Values falling above 95% are areas where our ability to infer is limited, in our case we sampled relatively few samples in Antarctica. Therefore, to determine whether our sampled species richness (richness) in each locale was poor relative to total avian richness (that is, species in BirdLife International) within that same locale, we examined **c**, the relationship between richness and total avian richness. Sampling effort **d**, plateaued as a function of specimen availability (that is, limit of samples), but was **c**, predicted by total species richness ($F_{2,33} = 74.67$, $R^2 = 0.82$, $p < 0.0001$). We had relatively **c-e**, higher sampling effort in the northern temperate region (-30 to 60°N) and relatively lower sampling effort in the southern sub-tropical region (approximately -15 to -40°S). This is illustrated in a plot of **c**, of predicted richness and sampled richness as well as **d**, the relationship between total and sampled avian richness. In both plots points are coloured by their latitude (a gradation of colours from South Pole as red to the North Pole as yellow). Although the Northern Hemisphere was well sampled, the **e**, residuals from this model were all less than one standard deviation, indicating that no region was sampled so much, or so little, to be considered an outlier.



Extended Data Fig. 9 | Internal and external relationship. Eggshell external surface temperatures were related to their internal temperatures. Dark brown (filled dots, $n=12$) and white (open dots, $n=12$) eggs were left under natural ambient light conditions (26–30 °C; see Methods for details).

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| ☐ | ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
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Software and code

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Data collection FLIR Tools v 5.13.18031.2002, Image J v 1.52d, Omega HH506RA Thermometer Software v 1.4

Data analysis R v 3.60, R Studio v 1.0.153, ArcMap v 10.5, Sum Trees (with DendroPy) v 4.3.0

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Birdlife Range data can be requested at: <http://datazone.birdlife.org/species/requestdis>. Digital Chart of the World basemap can be found at: https://worldmap.harvard.edu/data/geonode:Digital_Chart_of_the_World. Natural Earth Data maps can be found at: <https://www.naturalearthdata.com/>. Worldclim temperature data is available at: <http://worldclim.org/version2>. National Center for Atmospheric Research UV data can be found at: <https://www2.acom.ucar.edu/modeling/tuv-download>. Atlas of the Biosphere humidity data can be found at: <https://nelson.wisc.edu/sage/data-and-models/atlas/maps.php>. ISEA global grid data can be found at: <https://cran.r-project.org/web/packages/dggridR/vignettes/dggridR.html> for R codes or <https://www.arcgis.com/home/item.html?id=38e3e0453e4040c69ff5e9fde306c12c> for shapefiles. Data generated and/or analysed during this study are available from the Figshare Digital repository: <https://https://doi.org/10.6084/m9.figshare.9745109>.

Field-specific reporting

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Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Here we tested global selection pressures facing avian eggshell colours, by using a published dataset on eggshell reflectance from museum specimens (n = 634 species). We quantified eggshell colour as blue-green chroma, and brightness as mean reflectance and normalized these variables. Then, these colors were associated with the breeding ranges for each species by generating random points within each range (1 point every 10,000km ²). To derive average colors by location, we applied an ISEA hexagon grid, and calculated the phylogenetic mean in each location. We then quantified variation in environmental variables using PCA, and used spatial models to determine whether these environmental variables predicted eggshell colour and brightness. We ran complementary analyses at the inter-specific level using phylogenetic generalized least squares. Finally, to whether eggshell colours do heat differently we measure rates of heating on chicken eggs (n = 96) exposed to direct solar radiation using a FLIR One Pro IR thermometer.
Research sample	Avian eggshell reflectance data was collected from 634 species representing 36 of the extant 40 orders of birds (https://doi.org/10.5061/dryad.2q3r2). Additionally, eggshell surface reflectance as well as surface and internal temperature values collected on domestic chicken eggs (<i>Gallus gallus</i>).
Sampling strategy	The sample size were based on data availability (using published reflectance data) and processing power. We were limited to geospatial analyses based on approximately 3.5 million points. Thus, our sampling density was fixed to accommodate these limitations.
Data collection	Egg color data were collected by D.H. and P.C. using procedures found in Hanley et al. 2015. <i>Gallus gallus domesticus</i> , <i>Anas platyrhynchos domesticus</i> , and <i>Coturnix japonica</i> egg temperature data were collected with a FLIR Pro One, and Omega T-type thermocouple and thermocouple logger by P.W, in addition to <i>Gallus gallus domesticus</i> , <i>Anas platyrhynchos domesticus</i> , and <i>Coturnix japonica</i> egg color data by P.W.
Timing and spatial scale	The spatial scale of the statistical analysis was global. Field tests were run over a 25 minute period with measurements taken at one minute intervals.
Data exclusions	For the ecological study, data was only excluded when one of three conditions were met: 1) Some level of environmental data was missing from a locale (e.g., issues with data resolution), 2) phylogenetic analyses could not run (e.g., if a hexagon had fewer than 3 species sampled), or 3) a locale had no neighboring locales either before or after removal for adjacent locales.
Reproducibility	We ran multiple alternative geospatial models using various spatial weights, compared assemblage vs. inter-specific level analysis, and each time the models produced similar results. Field tests with <i>Gallus gallus</i> eggs were run in 6 trials cooling from incubation temperature and 2 trials heating from ambient temperature over the same timescale, as well as with <i>Coturnix japonica</i> and <i>Anas platyrhynchos</i> eggs (in Supplementary Data 3). These results were very similar to results from the experiments from our first submission (https://doi.org/10.1101/559435), which used different eggs and equipment, and slightly different experimental design.
Randomization	We randomly simulated nesting sites within each species breeding range using the random points tool in ArcMap.
Blinding	No blinding was necessary because we used a random points tool to generate more than 3 million points, thus our analysis was (for all practical purposes) blind to species identity. Egg colours were not visible on FLIR thermal images.
Did the study involve field work?	☒ Yes ☐ No

Field work, collection and transport

Field conditions	Field work was conducted under mostly sunny conditions from May 15th to May 22nd, 2019 at approximately 8:55am and solar noon, and on August 20th, 2019 from 10am to 4pm. Ambient temperature varied from 14 to 21 degrees C in May, and from 26 to 30 degrees C in August.
Location	Field work was completed at the LIU Post campus, in a small open area near the Life Sciences Building.
Access and import/export	All eggs used in this experiment were commercially available.
Disturbance	None

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

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