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1 Climate variability and parent nesting strategies influence gas exchange across  
2 avian eggshells

3

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14 **Running heading:** Avian eggshell conductance and climate

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26

27 **Abstract**

28

29 Embryo survival in birds depends on a controlled transfer of water vapour and  
30 respiratory gases through the eggshell, and this exchange is critically sensitive to the  
31 surrounding physical environment. As birds breed in most terrestrial habitats  
32 worldwide, we proposed that variation in eggshell conductance has evolved to  
33 optimise embryonic development under different breeding conditions. This is the first  
34 study to take a broad-scale macro-ecological view of avian eggshell conductance,  
35 encompassing all key avian taxonomic groups, to assess how life history and climate  
36 influence the evolution of this trait. Using whole eggs spanning a wide phylogenetic  
37 diversity of birds, we determine that body mass, temperature seasonality and whether  
38 both parents attend the nest are the main determinants of eggshell conductance. Birds  
39 breeding at high latitudes, where seasonal temperature fluctuations are greatest, will  
40 benefit from lower eggshell conductance to combat temporary periods of suspended  
41 embryo growth and prevent dehydration during prolonged incubation. The nest  
42 microclimate is more consistent in species where parents take turns incubating their  
43 clutch, resulting in lower eggshell conductance. This study highlights the remarkable  
44 functional qualities of eggshells and their importance for embryo survival in extreme  
45 climates.

46

47 **Keywords:** avian eggshells, climate, life-history, nest, temperature seasonality, water  
48 vapour conductance

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52 **1. Introduction**

53

54 Adaptive diversification across species typically occurs amidst an array of distinct  
55 ecological niches and environments, and is a key driver in the development of novel  
56 functional traits to enhance the fitness of an organism [1]. The evolution of a new trait  
57 may provide the adaptive potential to exploit a resource that was not previously  
58 possible, or interact with its environment in a new way without a specific change in the  
59 external environment [2]. Close association between certain traits and a species  
60 environment and life-history can therefore point to probable causes of trait divergence  
61 [3]. Traits can evolve rapidly over several generations or slowly over millions of years  
62 in accordance with environmental rates of change [4]. Species persistence during  
63 abrupt climate change will, therefore, depend on their ability to rapidly respond and  
64 adapt to novel environmental conditions [5]. Individual species will either move to more  
65 favourable conditions, tolerate or adapt to their changed environment, or go extinct [6].  
66 Understanding the evolutionary history and diversification of functional traits closely  
67 linked to reproductive success will help predict how species will react to these new  
68 environmental pressures.

69

70 Foremost, the survival of any species is reliant on having a viable embryo. One crucial  
71 step in understanding avian responses to environmental differences over evolutionary  
72 time is a better appreciation of factors shaping avian incubation and their subsequent  
73 influence on the embryo [7]. Birds have evolved multiple functional traits to improve  
74 offspring survival in the nest: arguably one of the most important is the eggshell. Most  
75 bird species lose 10-20% of their fresh egg mass over the incubation period through  
76 the passive diffusion of water vapour through the eggshell to the ambient air [8]. Eggs

77 that lose too much water during incubation frequently do not hatch due to desiccation  
78 [9], while embryos that do not lose enough water from the egg experience respiratory  
79 problems or drown [10]. Maintaining controlled loss of water from inside the egg to the  
80 external environment while allowing sufficient exchange of respiratory gases is,  
81 therefore, essential for normal embryo development and hatching.

82

83 Birds are highly diversified and widely distributed, occupying every continent on Earth  
84 and every terrestrial habitat within it [11]. Some birds breed in extremely inhospitable  
85 environments, such as cold and dry regions [12], deserts [13], moist wetlands [14] and  
86 high altitudes [15]. Among these are ground nesting birds in alpine or Arctic/Antarctic  
87 regions that must cope with unpredictable wind, precipitation, and snow conditions,  
88 with ambient temperatures fluctuating from below freezing to over 45°C [16]. Avian  
89 embryos in such cold regions will freeze to death if left unattended by their parents  
90 [17]. Desert birds that breed in the Sahara, Arabian and Kalahari regions face  
91 extreme physiological challenges to conserve water and avoid dehydration for the  
92 eggs, adults and hatchlings [18]. In contrast to dry, xeric environments, eggs exposed  
93 to high precipitation are prone to rain-induced suffocation [19]. The major challenges  
94 for birds breeding in high altitude regions like the Himalayas is the low barometric  
95 pressure and high solar radiation, which can result in desiccation of egg contents and  
96 overheating of the embryo [15]. Species living at such environmental extremes must  
97 adapt behaviourally or physiologically at each stage of their breeding cycle if they are  
98 to produce viable offspring [20].

99

100 Water vapour conductance through the avian eggshell, herein referred to as  
101 conductance or  $G_{H_2O}$ , is influenced by the properties of the eggshell (e.g., pore length,

102 functional pore area and eggshell cuticle) and humidity and gas composition of the  
103 surrounding environment. Species that incubate their eggs buried [21], in dry [22] or  
104 wet environments [23] or at high altitudes [15] have particularly unusual vapour  
105 pressure gradients, yet are still able to maintain water loss within acceptable limits.  
106  $G_{H_2O}$  may be optimised to suit particular environments through changes in nest-site  
107 preferences, eggshell structures, and incubation behaviours [14], making eggs and  
108 their species-specific conductance ideal model systems for understanding how trait  
109 selection varies over time during diversification.

110

111 Predicting  $G_{H_2O}$  of a species is not straight forward, as multiple ecological factors must  
112 be taken into account. For example, brood-chambers of burrow-nesting birds are often  
113 permanently saturated with water vapour, resulting in a low water-vapour pressure  
114 (favouring enhanced conductance) and longer incubation periods (favouring reduced  
115 conductance) [24]. Inter-species differences in  $G_{H_2O}$  thus can only be untangled by  
116 considering the contribution of multiple life-history traits, and the phylogenetic history  
117 of the lineage. A study across 141 non-passerine species detected differences in  $G_{H_2O}$   
118 between nest types and parental incubation behaviours [25], emphasising the  
119 importance of maintaining a suitable nest microclimate for optimum egg-water loss.  
120 However, it is unknown whether a similar relationship between conductance and  
121 nesting behaviour is expected in the passerines, which comprise over 6,000 species  
122 and represent almost 60% of all living birds [26]. Moreover, previous studies have  
123 typically focused either on (i) one group of birds, e.g., gulls, with the goal to look for  
124 micro-adaptations between closely related species [27], or (ii) eggs of 'extreme  
125 nesters' such as desert-nesting Bedouin fowl (*Gallus domesticus*) [28] and grey gulls  
126 (*Larus modestus*) [29], water-nesting grebes and divers [30] and marsh-nesting black

127 terns (*Chlidonias niger*) [31]. The role of life-history and environmental factors in the  
128 evolution of avian eggshell conductance thus requires a large-scale comparative  
129 analysis encompassing all key taxonomic groups.

130

131 Our aim was to evaluate how climate and life-history influence  $G_{H_2O}$  across a wide  
132 taxonomic distribution of birds spanning across 28 avian orders, after accounting for  
133 the effects of adult body mass and phylogeny. Previous comparative analyses of  
134 eggshell conductance have not corrected for allometric effects of body mass [25],  
135 which can hide potentially important adaptive information relating to the environment  
136 and nesting behaviour of the species. Based on previous findings, we predicted  $G_{H_2O}$   
137 would be primarily explained by body mass. In contrast, we predict that mass-  
138 independent conductance ( $RG_{H_2O}$ ) would be primarily associated with traits known to  
139 affect nest humidity, including climate, nest location and type.

140

## 141 **2. Materials and methods**

142

### 143 **(a) Egg samples and preparation**

144

145 In total, 365 bird species were included in this study. Conductance of whole emptied  
146 eggs at the Natural History Museum, Tring (NHM, UK) was established using the  
147 standard protocol of measuring the decrease in egg mass as a result of water loss  
148 over consecutive days, in eggs kept in constant moisture-free conditions [32].  $G_{H_2O}$   
149 measured using whole eggshells is preferable to eggshell fragments as shell thickness  
150 and porosity varies between different regions of an egg [33]; therefore, we only used  
151 values from whole eggs in this study.

152

153 Eggs were prepared by gently cleaning the surface, filling the egg with water then  
154 sealing the blow hole (see Supporting Information). Eggs were placed in an acrylic  
155 desiccator cabinet (ThermoFisher Scientific, Nalgene™, catalogue number: 5317-  
156 0070) inside a constant-temperature thermocabinet (Porkka, Hertfordshire, UK) at  
157  $30\pm 1^\circ\text{C}$ . Temperature was monitored via a logtag analyser every 10 min (Loggershop,  
158 Bournemouth, Dorset, UK). Self-indicating silica gel (Merck, Honenbrunn, Germany,  
159 catalog number: 101969) were placed in the desiccator to remove all moisture. Any  
160 loss in egg mass was entirely due to the diffusion of water vapour via the shell pores  
161 [34]. The first 24-hrs can give unexpectedly high mass loss values as the outer shell  
162 surface dries out [35]. Therefore, the eggs were left 24-hr before being weighed to the  
163 nearest 0.1 mg (Sartorius, Göttingen, Germany), then were returned to the desiccator.  
164 Eggs were weighed at the same time of day on three successive days to give two  
165 values of 24-hr mass loss ( $M_{\text{H}_2\text{O}}$ ). Species  $G_{\text{H}_2\text{O}}$  was then calculated, as described in  
166 the Supporting Information.

167

168 Species mean  $G_{\text{H}_2\text{O}}$  values of whole eggs reported in the literature ( $n=188$ ) were  
169 incorporated if specimens had been measured under constant conditions  
170 (temperature and humidity), and followed protocols used in the present study.  $G_{\text{H}_2\text{O}}$   
171 measures from whole fresh eggs (unemptied or water-filled) and museum (water-filled)  
172 eggs were combined as  $G_{\text{H}_2\text{O}}$  does not differ significantly between these treatments  
173 [36]. Mean  $G_{\text{H}_2\text{O}}$  values reported in the literature were corrected to adjusted to  
174 standard barometric pressure (1 ATM) at  $30^\circ\text{C}$  (see Supporting Information).

175

176 **(b) Life-history and ecological data**

177

178 We collated data on 18 key life-history traits that have previously been hypothesised  
179 to play a role in the evolution of avian conductance in addition to climate variables  
180 (table 1). This data was extracted from multiple sources detailed in the Figshare  
181 repository (DOI: 10.6084/m9.figshare.12490559). Major sources are detailed in  
182 section (e) of Supplementary Information. Only 13 predictors were included in the  
183 analysis due to collinearity (see Supporting Information). Phylogenetic generalised  
184 least squares (PGLS) method was used to test the evolutionary association between  
185 whole eggshell  $G_{H_2O}$  life-history traits, within a phylogenetic context [37]. In this  
186 procedure, closely related species are assumed to have more similar traits because  
187 of their shared ancestry and consequently will produce more similar residuals from the  
188 least squares regression line. By taking into account the expected covariance structure  
189 of these residuals, modified slope and intercept estimates are generated that account  
190 for interspecific autocorrelation due to phylogeny.

191

192 Prior to updated avian phylogenies based on genomic DNA, near-passerines was a  
193 term given to tree-dwelling birds (within the conventional non-passerines) that were  
194 traditionally believed to be related to Passeriformes due to ecological similarities. In  
195 this study Pterocliiformes (sandgrouse), Columbiformes (pigeons), Cuculiformes  
196 (cuckoos), Caprimulgiformes (nightjars), and Apodiformes (swifts, hummingbirds)  
197 were defined as near-passerines. All passerines and near-passerines are land birds  
198 and have altricial and nidicolous (stay within the nest) chicks, while non-passerine  
199 chicks vary in their mode of development and include water and land birds [38].  
200 Sandgrouse are an exception as they have precocial young and are not tree-dwelling  
201 [39]. In respect to nest architecture, most passerines build open-cup nests, though

202 some build more elaborate dome structures with roofs [40]. Dome nests, however, are  
203 more common among passerines than non-passerines, and are particularly frequent  
204 among very small passerines [41]. Although these groups are no longer recognised  
205 as near-passerines, this definition was used here to distinguish between ecologically  
206 profound differences among birds.

207

208 Avian phylogenetic trees were constructed online (<http://www.birdtree.org>) from the  
209 complete avian phylogeny of Jetz *et al.* [42], and used the primary backbone tree of  
210 Hackett *et al.* [43]. Ten thousand trees were constructed and statistical analyses were  
211 performed in the program R, version 3.6.1 (R Software, Vienna, Austria, [http://www.R-](http://www.R-project.org)  
212 [project.org](http://www.R-project.org)). All quantitative variables (except absolute median latitude, annual  
213 temperature and temperature range) were  $\log_{10}$ -transformed prior to phylogenetic  
214 analysis to reduce skewness [44].

215

216 As body mass affects all aspects of animal biology and ecology [45], our initial set of  
217 phylogenetic analysis account for adult body mass by including this variable as a  
218 predictor of  $\log(G_{H_2O})$ . We repeated our phylogenetic analysis using mass-corrected  
219  $G_{H_2O}$  as the response variable, herein called relative  $G_{H_2O}$  ( $RG_{H_2O}$ ), thereby removing  
220 adult body mass as a predictor.  $RG_{H_2O}$  values were computed as residuals from a  
221 PGLS regression of  $\log(G_{H_2O})$  on  $\log(\text{body mass})$  (slope =  $0.53 \pm 0.03$  s.e.; intercept  
222 =  $-0.69 \pm 0.12$  s.e.;  $\lambda = 0.68$ ; figure S1) for  $G_{H_2O}$ . Using this second series of models,  
223 we can ask how well one or more life-history traits results in higher or lower  $G_{H_2O}$  than  
224 is expected for a given body mass of the adult bird.

225

226 Phylogenetic signal in  $G_{H_2O}$  and  $R_{G_{H_2O}}$  was measured by Pagel's lambda ( $\lambda$ ) [46] using  
227 the *phylosig* function in the package 'phytools' [47] to determine to what the extent  
228 related species were more likely to share similar conductance values than species  
229 drawn randomly from a tree. The *phylosig* function was used to test the hypothesis  
230 that Pagel's  $\lambda$  is different from 0. To test the alternative hypothesis (that Pagel's  $\lambda$  is  
231 less than 1), we computed the difference in the log-likelihood ratio of the lambda model  
232 (*phylosig* function) and Brownian motion model (*brownie.lite* function), then compared  
233 it to a chi-squared ( $\chi^2$ ) distribution with 1 degree of freedom. PGLS models were fitted  
234 using the *phylolm* function in the package 'phylolm' [48]. We ran the full model  
235 containing all traits as predictor variables, then used the 'pdredge' function from the  
236 package MuMIn [49] to fit all possible model combinations with a maximum of five  
237 predictors following protocols by Powney *et al.* [50], in addition to a null model  
238 comprising only the intercept. The best subset of models had an AICc (Akaike's  
239 information criterion adjusted for low sample size) within 2 of the model with the lowest  
240 AICc [51]. Conditional model averaging was then used to identify parameter estimates  
241 and importance for each trait present in at least one of the subset models [52].

242

### 243 **3. Results**

244

245 In total, we used over 2533 eggs from 364 species to assess diversification in  
246 conductance across the avian phylogeny. These species span across 85 families and  
247 represent 28 of the 49 extant avian orders. Overall, bird species in Australia, North  
248 America and South America had higher  $\log(G_{H_2O})$  and  $R_{G_{H_2O}}$  than species in Africa,  
249 Europe and Asia (figure S2).  $G_{H_2O}$  was highest for large flightless birds (e.g., ostriches  
250 (*Struthio camelus*) ( $106.99 \text{ mg day}^{-1} \text{ Torr}^{-1}$ )), nightjars (Caprimulgiformes  $0.55 \pm 0.19$

251 mg day<sup>-1</sup> Torr<sup>-1</sup>) and songbirds (Passeriformes 0.74 ± 0.05 mg day<sup>-1</sup> Torr<sup>-1</sup>). G<sub>H<sub>2</sub>O</sub> was  
252 also high for aquatic birds (e.g., common loons (*Gavia immer*) 98.82 mg day<sup>-1</sup> Torr<sup>-1</sup>),  
253 kiwis (Southern brown kiwi (*Apteryx australis*) 26.22 mg day<sup>-1</sup> Torr<sup>-1</sup>) and penguins  
254 (Sphenisciformes 22.66 ± 5.45 mg day<sup>-1</sup> Torr<sup>-1</sup>). Viewing total phylogenetic variation in  
255 this trait (figure 1a and 2a) revealed that log(G<sub>H<sub>2</sub>O</sub>) and RG<sub>H<sub>2</sub>O</sub> were typically lower in  
256 passerines and near-passerines, than non-passerines (figure 1b).

257

### 258 **(a) Phylogenetic correlation**

259

260 Phylogenetic signal for log(G<sub>H<sub>2</sub>O</sub>) and RG<sub>H<sub>2</sub>O</sub> (see table 2) was significantly different  
261 from 0 (i.e., no phylogenetic signal) ( $P < 0.001$ ) and 1 (i.e., the Brownian explanation)  
262 ( $P < 0.001$ ), meaning that while there is an effect of phylogeny on conductance, it is  
263 influenced by evolutionary process that are weaker than would be seen with a  
264 Brownian motion model of trait evolution. Phylogenetic signal was high for Log(G<sub>H<sub>2</sub>O</sub>)  
265 ( $\lambda = 0.96$ ), showing that closely related species exhibit similar eggshell conductance  
266 prior to accounting for differences in body mass, and this biological similarity  
267 decreases as the evolutionary distance between species increases. Phylogenetic  
268 signal was intermediate for RG<sub>H<sub>2</sub>O</sub> ( $\lambda = 0.55$ ), suggesting that phylogeny and other  
269 selective pressures (e.g., those associated with species life-history or climate) are  
270 important in determining eggshell conductance, after accounting for differences in  
271 species body mass.

272

### 273 **(b) Life-history and climate influence conductance across birds**

274

275 Adult body mass and temperature seasonality were the strongest predictors of  
276  $\log(G_{H_2O})$  across all birds based on conditionally averaged models (Table S1 in  
277 Supporting Information).  $\log(G_{H_2O})$  was significantly higher among heavier species  
278 ( $z=18.40$ ,  $P<0.001$ ; Figure 1c and 3a) since initial egg mass increases with adult body  
279 mass ( $n = 251$ ,  $r^2 = 0.89$ ,  $P<0.001$  [52]).  $\log(G_{H_2O})$  was negatively associated with  
280 increased temperature seasonality across all birds ( $z=2.13$ ,  $P=0.03$ ; figure 2b and 3b).  
281 Temperature seasonality is defined here as the amount of temperature variation over  
282 a given year (or averaged years) based on the standard deviation of monthly  
283 temperature averages [53]. There was also a weaker yet significant effect of dietary  
284 calcium, nest location, mode of development, shared incubation and parental contact  
285 among top-ranked models (table S2).

286

287 Temperature seasonality ( $z=2.20$ ,  $P=0.03$ ) and whether contact incubation was  
288 shared among parents ( $z=2.22$ ,  $P=0.03$ ) were significant in conditional averaged  
289 models after accounting for adult body mass ( $RG_{H_2O}$ ) (table S3 and figure 2).  $RG_{H_2O}$   
290 overall decreased with temperature seasonality (figure 2b and 3b).  $RG_{H_2O}$  was higher  
291 in species where both parents incubate the clutch (figure 2c). Dietary calcium, mode  
292 of development, nest location, and parental contact showed weaker but significant  
293 correlations with  $RG_{H_2O}$  among top-ranked models (table S4).  $RG_{H_2O}$  was higher in  
294 species with calcium-rich diets, precocial young, parents that return to the nest with  
295 wet plumage and ground nesters compared to tree nesters (figure S6). Based on  
296 conditionally averaged models for  $G_{H_2O}$  and  $RG_{H_2O}$ , eggshell conductance across  
297 birds is primarily influenced by adult body mass, temperature seasonality and parent  
298 incubation strategies.

299

#### 300 **4. Discussion**

301

302 This study focused on one performance trait – conductance – of modern avian  
303 eggshells to better understand how birds have achieved high ecological diversity. We  
304 identified the importance of phylogeny, physiology (body mass and mode of  
305 development), behaviour (diet, parental incubation strategies and nest location) and  
306 climate in the evolution of this trait. This study is the first to identify a broad-scale  
307 reduction in eggshell conductance where temperature seasonality increases. Regions  
308 with greater temperature seasonality experience a greater range in temperatures over  
309 the course of a year, and correlates with an organisms' temperature tolerance breadth  
310 [54]. Increased temperature seasonality occurs further from the equator and is  
311 associated with a decline in annual temperature, precipitation and day length [55]. A  
312 comparative study on 139 bird species found that adults inhabiting low and seasonally  
313 variable temperatures had lower basal metabolic rate after removing the effects of  
314 body mass [56]. In light of this, it appears possible that eggshells are already preparing  
315 the embryo for adulthood, with respect to their environment and breeding biology.  
316 Amniotic embryos adjust their metabolic activity and active cell division in response to  
317 varying environmental conditions, and by doing so, alter their period of development  
318 [57]. Reproductive strategies to prolong the egg state are most diverse in reptiles, and  
319 less varied in birds and mammals that provide more parental care [58]. Even so, the  
320 low metabolic rate expected for embryos incubated in highly seasonal climates would  
321 favour a reduction in conductance to prolong their incubation period.

322

323 Broad-scale geographical trends in  $RG_{H_2O}$  identified here may be the result of long-  
324 term evolutionary responses or short-term physiological modifications [59].

325 Evolutionary adaptation would involve changes in  $G_{H_2O}$  over (rather than within)  
326 generations when natural selection acts on genetic variants while acclimatisation  
327 would involve reversible changes to  $G_{H_2O}$  that can happen gradually (>1 day) in  
328 response to recent environment [60]. Intraspecific variation in  $G_{H_2O}$  has been reported  
329 across altitude [61,62] and humidity [63,64] gradients of multiple species, but the  
330 timeframe in which  $G_{H_2O}$  diversification has taken place is unknown. Some studies  
331 propose that rapid evolution of eggshell structure from exposure to novel environments  
332 is unlikely [65,66], and is instead compensated by behavioural modifications of the  
333 parents. Other studies find that incubation behaviour does not significantly modulate  
334 conductance [67], so adaptive responses must be accomplished by changes in  
335 eggshell structure [63].

336

337 Birds are seemingly capable of short-term and instantaneous physiological  
338 adjustments in shell structure in response to environmental variation. Pigeons  
339 (*Columba livia*) bred for several years within an environmentally controlled room  
340 experienced ~30% lower  $G_{H_2O}$  than predicted when exposed to high temperature and  
341 low humidity over a short period [68]. Similarly, domestic chickens (*Gallus domesticus*)  
342 bred at high elevation for multiple generations produced eggshells with a 30% higher  
343  $G_{H_2O}$  within two months of being translocated to low altitudes [69]. In other species,  
344  $G_{H_2O}$  did not change when individuals were transferred to higher altitudes [70] or were  
345 exposed to natural seasonal changes in humidity [71], suggesting there is variation in  
346 the plasticity of a species response. Identifying the speed of the response in eggshell  
347 parameters to novel environments across multiple species will be very informative in  
348 determining climate change effects on bird species and their breeding.

349

350 We found that conductance across birds was also dependant on nest location, whether  
351 parents alternate nest attendance, and whether the parent returns to the nest with wet  
352 plumage, corroborating previous studies [25,72]. Shared incubation between two  
353 parents allows one of them to be relieved from incubation to feed while the other  
354 incubates the egg, thus allowing the eggs to be covered at all times [73]. Clutches that  
355 are incubated by both parents encounter less variation in egg temperature than  
356 clutches that are incubated by a single parent [74], and thus, are expected to have  
357 higher eggshell conductance. Water added to the nest by parents can be many orders  
358 of magnitude higher than water lost by the eggs [75]. Consequently,  $RG_{H_2O}$  is  
359 significantly higher in species where parents return the nest with wet plumage [25].  
360 Eggs laid on the ground, in a burrow, mound or on floating vegetation are subject to  
361 higher humidity than arboreal nesters, leading to eggshell adaptations that promote  
362 water loss. Common loons (*Gavia immer*), for example, had the highest  $RG_{H_2O}$  of the  
363 species investigated. This may be attributed to their high eggshell porosity [76] in  
364 response to building nests on or near the water where transpiration of water is high,  
365 and nest materials can be wet [64]. Nest location and whether parents return to the  
366 nest with wet or dry plumage was significant in most top models where these predictors  
367 were included, but this effect was weak compared to life-history traits retained in  
368 conditionally averaged models. Combined, our results demonstrate that different  
369 behavioural strategies used by parents to alter nest humidity have contributed to the  
370 evolution of conductance among birds.

371

372 Variation in incubation period across the altricial-precocial spectrum reflects a trade-  
373 off between embryo growth rate and degree of maturity when hatched. Precocial  
374 species take up to 2 times longer to incubate an egg of the same size as altricial

375 species, but are far more developed when they hatch [77]. For eggs of the same mass,  
376 precocial species incur a higher total energy cost than altricial species because the  
377 embryo is larger for a longer period during incubation [78]. Consequently, eggs of  
378 species with fast (precocial) growing offspring had significantly higher  $RG_{H_2O}$  than  
379 those of species with slow (altricial) growing offspring based on top-ranked models.  
380 As higher conductance enables greater gas exchange, this may optimise embryo  
381 access to high energy content in precocial eggs [79], thus resulting in a more  
382 developed chick at birth.  $RG_{H_2O}$  in passerines was found here to be particularly low,  
383 likely because they have altricial young, whereas non-passerines consist of precocial  
384 and altricial species.

385

#### 386 **Data accessibility**

387

388 Data is publicly available in the Figshare repository, including specimen and species-  
389 specific water vapour conductance, life histories and sources used in this study (DOI:  
390 10.6084/m9.figshare.12490559). Tables for all PGLS analyses and sources for figure  
391 illustrations are available in Supporting Information.

392

#### 393 **Author's contributions**

394

395 SJP and MRGA developed the project. MRGA collected and analysed the data and  
396 prepared figures. Both authors contributed to the interpretation of the findings. MRGA  
397 wrote the first draft of the manuscript, which both authors then worked on.

398

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405

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411

#### 412 **References**

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- 414 1. Losos JB. 2010 Adaptive radiation, ecological opportunity, and evolutionary  
415 determinism. *Am. Nat.* **175**, 623–639. (doi:10.1086/652433)
- 416 2. Yoder JB *et al.* 2010 Ecological opportunity and the origin of adaptive  
417 radiations. *J. Evol. Biol.* **23**, 1581–1596.
- 418 3. Losos JB, Schoener TW, Langerhans RB, Spiller DA. 2006 Rapid temporal  
419 reversal in predator-driven natural selection. *Science* **314**, 1111.  
420 (doi:10.1126/science.1133584)
- 421 4. Ho W, Zhang J. 2018 Evolutionary adaptations to new environments generally  
422 reverse plastic phenotypic changes. *Nat. Commun.* **9**, 350.  
423 (doi:10.1038/s41467-017-02724-5)
- 424 5. Hendry AP, Gotanda KM, Svensson EI. 2017 Human influences on evolution,

- 425 and the ecological and societal consequences. *Philos. Trans. R. Soc. B Biol.*  
426 *Sci.* **372**, 20160028. (doi:10.1098/rstb.2016.0028)
- 427 6. Hoffmann AA, Hercus MJ. 2000 Environmental stress as an evolutionary force.  
428 *Bioscience* **50**, 217–226. (doi:10.1641/0006-  
429 3568(2000)050[0217:ESAAEF]2.3.CO;2)
- 430 7. Durant SE, Willson JD, Carroll RB. 2019 Parental effects and climate change:  
431 Will avian incubation behavior shield embryos from increasing environmental  
432 temperatures? *Integr. Comp. Biol.* **59**, 1068–1080. (doi:10.1093/icb/icz083)
- 433 8. Ar A, Rahn H. 1980 Water in the avian egg overall budget of incubation. *Integr.*  
434 *Comp. Biol.* **20**, 373–384. (doi:10.1093/icb/20.2.373)
- 435 9. Carey C. 1986 Tolerance of variation in eggshell conductance, water loss, and  
436 water content by red-winged blackbird embryos. *Physiol. Zool.* **59**, 109–122.
- 437 10. Wangenstein OD, Rahn H. 1970 Respiratory gas exchange by the avian  
438 embryo. *Respir. Physiol.* **11**, 31–45. (doi:https://doi.org/10.1016/0034-  
439 5687(70)90100-3)
- 440 11. Konishi M, Emlen ST, Ricklefs RE, Wingfield JC. 1989 Contributions of bird  
441 studies to biology. *Science* **246**, 465–472. (doi:10.1126/science.2683069)
- 442 12. Le Maho Y. 1977 The emperor penguin: A strategy to live and breed in the  
443 cold. *Am. Sci.* **65**, 680–693.
- 444 13. Carey C. 2002 Incubation in Extreme Environments. In *Avian Incubation:*  
445 *Behaviour, Environment, and Evolution* (ed DC Deeming), pp. 238–253.  
446 Oxford University Press: Oxford, UK.
- 447 14. Zicus MC, Rave DP, Riggs MR, Zicus MC, Rave DP, Riggs MR. 2003 Mass  
448 loss from mallard eggs incubated in nest structures. *Wildl. Soc. Bull.* **31**, 270–  
449 278.

- 450 15. Carey C. 1980 Adaptation of the avian egg to high altitude. *Am. Zool.* **20**, 449–  
451 459.
- 452 16. Martin K, Wiebe KL. 2004 Coping mechanisms of alpine and arctic breeding  
453 birds: extreme weather and limitations to reproductive resilience. *Integr. Comp.*  
454 *Biol.* **44**, 177–185. (doi:10.1093/icb/44.2.177)
- 455 17. Spellerberg IF. 1969 Incubation temperatures and thermoregulation in the  
456 McCormick Skua. *Condor* **71**, 59–67. (doi:10.2307/1366049)
- 457 18. McKechnie AE, Wolf BO. 2010 Climate change increases the likelihood of  
458 catastrophic avian mortality events during extreme heat waves. *Biol. Lett.* **6**,  
459 253–256. (doi:10.1098/rsbl.2009.0702)
- 460 19. Öberg M, Arlt D, Pärt T, Laugen AT, Eggers S, Low M. 2015 Rainfall during  
461 parental care reduces reproductive and survival components of fitness in a  
462 passerine bird. *Ecol. Evol.* **5**, 345–356. (doi:10.1002/ece3.1345)
- 463 20. Cloudsley-Thompson JL. 1988 Adaptations to Extreme Environments. In  
464 *Evolution and Adaptation of Terrestrial Arthropods* (ed JL Cloudsley-  
465 Thompson), pp. 80–98. Berlin, Heidelberg: Springer Berlin Heidelberg.  
466 (doi:10.1007/978-3-642-61360-9\_6)
- 467 21. Seymour RS, Ackerman RA. 1980 Adaptations to underground nesting in birds  
468 and reptiles. *Am. Zool.* **20**, 437–447. (doi:10.1093/icb/20.2.437)
- 469 22. Rahn H, Hammel HT. 1982 Incubation water loss, shell conductance, and pore  
470 dimensions in Adelie penguin eggs. *Polar Biol.* **1**, 91–97.  
471 (doi:10.1007/BF00263805)
- 472 23. Adkerman RA, Platter-Rieger M. 1979 Water loss by pied-billed grebe  
473 (*Podilymbus podiceps*) eggs. *Arner. Zool.* **19**, 921.
- 474 24. Lill A, Fell PJ. 2007 Microclimate of nesting burrows of the Rainbow Bee-eater.

- 475 *Emu* **107**, 108–114. (doi:10.1071/MU06046)
- 476 25. Portugal SJ, Maurer G, Thomas GH, Hauber ME, Grim T, Cassey P. 2014  
477 Nesting behaviour influences species-specific gas exchange across avian  
478 eggshells. *J. Exp. Biol.* **217**, 3326–3332. (doi:10.1242/jeb.103291)
- 479 26. Ericson PG, Klopstein S, Irestedt M, Nguyen JM, Nylander JA. 2014 Dating  
480 the diversification of the major lineages of Passeriformes (Aves). *BMC Evol.*  
481 *Biol.* **14**. (doi:10.1186/1471-2148-14-8)
- 482 27. Rahn H, Dawson WR. 1979 Incubation water loss in eggs of Heermann's and  
483 western gulls. *Physiol. Zool.* **52**, 451–460.
- 484 28. Arad Z, Marder J. 1982 Egg-shell water vapour conductance of the domestic  
485 fowl: Comparison between two breeds and their crosses. *Br. Poult. Sci.* **23**,  
486 325–328. (doi:10.1080/00071688208447964)
- 487 29. Guerra C, Aguilar R, Fitzpatrick L. 1988 Water vapor conductance in Gray  
488 gulls (*Larus modestus*) eggs: Adaptation to desert nesting. *Colon. Waterbirds*  
489 **11**, 107–109. (doi:10.2307/1521176)
- 490 30. Sotherland P, Ashen M, Shuman R, Tracy C. 1984 The water balance of bird  
491 eggs incubated in water. *Physiol. Zool.* **57**, 338–348.
- 492 31. Davis TA, Ackerman RA. 1985 Adaptations of black tern (*Chlidonias niger*)  
493 eggs for water loss in a moist nest. *Auk* **102**, 640–643.
- 494 32. Portugal SJ, Hauber ME, Maurer G, Stokke BG, Grim T, Cassey P. 2014  
495 Rapid development of brood-parasitic cuckoo embryos cannot be explained by  
496 increased gas exchange through the eggshell. *J. Zool.* **293**, 219–226.  
497 (doi:10.1111/jzo.12144)
- 498 33. Rokitka MA, Rahn H. 1987 Regional differences in shell conductance and pore  
499 density of avian eggs. *Respir. Physiol.* **68**, 371–376. (doi:10.1016/S0034-

- 500 5687(87)80021-X)
- 501 34. Booth DT, Seymour RS. 1987 Effect of eggshell thinning on water vapor  
502 conductance of malleefowl eggs. *Condor* **89**, 453–459. (doi:10.2307/1368635)
- 503 35. Bamelis FR, De Ketelaere B, Mertens K, Kemps BJ, Decuyper EM, De  
504 Baerdemaeker JG. 2008 Measuring the conductance of eggshells using the  
505 acoustic resonance technique and optical transmission spectra. *Comput.*  
506 *Electron. Agric.* **62**, 35–40. (doi:10.1016/j.compag.2007.08.009)
- 507 36. Portugal SJ, Maurer G, Cassey P. 2010 Eggshell permeability: a standard  
508 technique for determining interspecific rates of water vapor conductance.  
509 *Physiol. Biochem. Zool.* **83**, 1023–1031. (doi:10.1086/656287)
- 510 37. Symonds MRE, Blomberg SP. 2014 A Primer on Phylogenetic Generalised  
511 Least Squares. In *Modern Phylogenetic Comparative Methods and Their*  
512 *Application in Evolutionary Biology* (ed L Garamszegi), pp. 105–130. Berlin,  
513 Heidelberg: Springer.
- 514 38. Carnaby T. 2010 *Beat about the bush, birds*. Johannesburg: Jacana Media.
- 515 39. Temrin H, Tullberg BS. 1995 A phylogenetic analysis of the evolution of avian  
516 mating systems in relation to altricial and precocial young. *Behav. Ecol.* **6**,  
517 296–307. (doi:10.1093/beheco/6.3.296)
- 518 40. Price JJ, Griffith SC. 2017 Open cup nests evolved from roofed nests in the  
519 early passerines. *Proc. R. Soc. B Biol. Sci.* **284**, 20162708.  
520 (doi:10.1098/rspb.2016.2708)
- 521 41. Collias NE. 1997 On the origin and evolution of nest building by passerine  
522 birds. *Condor* **99**, 253–270. (doi:10.2307/1369932)
- 523 42. Jetz W, Thomas GH, Joy JB, Hartmann K, Mooers AO. 2012 The global  
524 diversity of birds in space and time. *Nature* **491**, 444–448.

- 525 (doi:10.1038/nature11631)
- 526 43. Hackett SJ *et al.* 2008 A phylogenomic study of birds reveals their evolutionary  
527 history. *Science* **320**, 1763–1768. (doi:10.1126/science.1157704)
- 528 44. Feng C, Wang H, Lu N, Chen T, He H, Lu Y, Tu XM. 2014 Log-transformation  
529 and its implications for data analysis. *Shanghai Arch. psychiatry* **26**, 105–109.  
530 (doi:10.3969/j.issn.1002-0829.2014.02.009)
- 531 45. Brown JH. 1995 *Macroecology*. Chicago: University of Chicago Press.
- 532 46. Pagel M. 1999 Inferring the historical patterns of biological evolution. *Nature*  
533 **401**, 877–884. (doi:10.1038/44766)
- 534 47. Revell LJ. 2012 phytools: an R package for phylogenetic comparative biology  
535 (and other things). *Methods Ecol. Evol.* **3**, 217–223.
- 536 48. Ho LST, Ané C, Lachlan R, Tarpinian K, Feldman R, Yu Q, Ho MLST. 2014  
537 Phylolm: phylogenetic linear regression. R package version 2.1.
- 538 49. Barton K, Barton MK. 2019 Package ‘MuMIn’. R package. *Version 1*
- 539 50. Powney GD, Rapacciuolo G, Preston CD, Purvis A, Roy DB. 2014 A  
540 phylogenetically-informed trait-based analysis of range change in the vascular  
541 plant flora of Britain. *Biodivers. Conserv.* **23**, 171–185. (doi:10.1007/s10531-  
542 013-0590-5)
- 543 51. Angert AL, Crozier LG, Rissler LJ, Gilman SE, Tewksbury JJ, Chunco AJ.  
544 2011 Do species’ traits predict recent shifts at expanding range edges? *Ecol.*  
545 *Lett.* **14**, 677–689. (doi:10.1111/j.1461-0248.2011.01620.x)
- 546 52. Deeming DC. 2007 Effects of phylogeny and hatchling maturity on allometric  
547 relationships between female body mass and the mass and composition of  
548 bird eggs. *Avian Poult. Biol. Rev.* **18**, 21–37.  
549 (doi:10.3184/147020607X245039)

- 550 53. O'Donnell MS, Ignizio DA. 2012 Bioclimatic predictors for supporting ecological  
551 applications in the conterminous United States. *US Geol. Surv. Data Ser.* **691**.
- 552 54. Sunday J *et al.* 2019 Thermal tolerance patterns across latitude and elevation.  
553 *Philos. Trans. R. Soc. B* **374**, 20190036. (doi:10.1098/rstb.2019.0036)
- 554 55. De Frenne P *et al.* 2013 Latitudinal gradients as natural laboratories to infer  
555 species' responses to temperature. *J. Ecol.* **101**, 784–795. (doi:10.1111/1365-  
556 2745.12074)
- 557 56. White CR, Blackburn TM, Martin GR, Butler PJ. 2007 Basal metabolic rate of  
558 birds is associated with habitat temperature and precipitation, not primary  
559 productivity. *Proc. R. Soc. B Biol. Sci.* **274**, 287–293.  
560 (doi:10.1098/rspb.2006.3727)
- 561 57. Rafferty AR, Reina RD. 2012 Arrested embryonic development: a review of  
562 strategies to delay hatching in egg-laying reptiles. *Proc. R. Soc. B Biol. Sci.*  
563 **279**, 2299–2308. (doi:10.1098/rspb.2012.0100)
- 564 58. Maurer G, Portugal SJ, Cassey P. 2011 Review: an embryo's eye view of  
565 avian eggshell pigmentation. *J. Avian Biol.* **42**, 494–504. (doi:10.1111/j.1600-  
566 048X.2011.05368.x)
- 567 59. Walsberg GE, Voss-Roberts KA. 1983 Incubation in desert-nesting doves:  
568 mechanisms for egg cooling. *Physiol. Zool.* **56**, 88–93.
- 569 60. Llewelyn J, Macdonald SL, Moritz C, Martins F, Hatcher A, Phillips BL. 2018  
570 Adjusting to climate: Acclimation, adaptation and developmental plasticity in  
571 physiological traits of a tropical rainforest lizard. *Integr Zool.* **13**, 411–427.  
572 (doi:10.1111/1749-4877.12309)
- 573 61. Carey C, Garber SD, Thompson EL, James FC. 1983 Avian reproduction over  
574 an altitudinal gradient. II. Physical characteristics and water loss of eggs.

- 575        *Physiol. Zool.* **56**, 340–352. (doi:10.1086/physzool.56.3.30152599)
- 576 62.    Sotherland PR, Packard GC, Taigen TL, Thomas J. 1980 An altitudinal cline in  
577        conductance of cliff swallow (*Petrochelidon pyrrhonota*) eggs to water vapor.  
578        *Auk* **97**, 177–185. (doi:10.1093/auk/97.1.177)
- 579 63.    Stein LR, Badyaev A V. 2011 Evolution of eggshell structure during rapid  
580        range expansion in a passerine bird. *Funct. Ecol.* **25**, 1215–1222.  
581        (doi:10.1111/j.1365-2435.2011.01887.x)
- 582 64.    Davis A, Platter-Reiger MF, Ackerman RA. 1984 Incubation water loss by pied-  
583        billed grebe eggs: adaptation to a hot, wet nest. *Physiol. Zool.* **57**, 384–391.
- 584 65.    Simkiss K. 1980 Eggshell porosity and the water metabolism of the chick  
585        embryo. *J. Zool.* **192**, 1–8. (doi:10.1111/j.1469-7998.1980.tb04213.x)
- 586 66.    Board R. 1982 Properties of avian eggshells and their adaptive value. *Biol.*  
587        *Rev.* **57**, 1–28. (doi:10.1111/j.1469-185X.1982.tb00362.x)
- 588 67.    Walsberg GE. 1983 A test for regulation of nest humidity in two bird species.  
589        *Physiol. Zool.* **56**, 231–235. (doi:10.1086/physzool.56.2.30156054)
- 590 68.    Arad Z, Gavrieli-Levin I, Marder J. 1988 Adaptation of the pigeon egg to  
591        incubation in dry hot environments. *Physiol. Zool.* **61**, 293–300.
- 592 69.    Rahn H, Ledoux T, Paganelli C V., Smith AH. 1982 Changes in eggshell  
593        conductance after transfer of hens from an altitude of 3,800 to 1,200 m. *J.*  
594        *Appl. Physiol.* **53**, 1429–1431. (doi:10.1152/jappl.1982.53.6.1429)
- 595 70.    Carey C, Hoyt DF, Bucher TL, Larson DL. 1984 Eggshell conductances of  
596        avian eggs at different altitudes. In *Respiration and metabolism of embryonic*  
597        *vertebrates*, pp. 259–270. Springer.
- 598 71.    Walsberg GE. 1985 A test for regulation of egg dehydration by control of shell  
599        conductance in Mourning Doves. *Physiol. Zool.* **58**, 473–477.

- 600 72. Vleck CM, Vleck D, Rahn H, Paganelli C V. 1983 Nest microclimate, water-  
601 vapor conductance, and water loss in heron and tern eggs. *Auk* **100**, 76–83.  
602 (doi:10.1093/auk/100.1.76)
- 603 73. Seddon P. 1989 Patterns of nest relief during incubation, and incubation period  
604 variability in the yellow-eyed penguin (*Megadyptes antipodes*). *New Zeal. J.*  
605 *Zool.* **16**, 393–400. (doi:10.1080/03014223.1989.10422905)
- 606 74. Reneerkens J, Grond K, Schekkerman H, Tulp I, Piersma T. 2011 Do  
607 uniparental sanderlings *Calidris alba* increase egg heat input to compensate  
608 for low nest attentiveness? *PLoS One* **6**, 1–9.  
609 (doi:10.1371/journal.pone.0016834)
- 610 75. Grant GS. 1982 *Avian Incubation: Egg Temperature, Nest Humidity, and*  
611 *Behavioral Thermoregulation in a Hot Environment*. Washington, D.C.:  
612 American Ornithologists' Union.
- 613 76. Tullett SG, Board RG. 1977 Determinants of avian eggshell porosity. *J. Zool.*  
614 **183**, 203–211. (doi:10.1111/j.1469-7998.1977.tb04182.x)
- 615 77. Ricklefs RE, Austin SH, Robinson WD. 2017 The adaptive significance of  
616 variation in avian incubation periods. *Auk* **134**, 542–550. (doi:10.1642/AUK-16-  
617 171.1)
- 618 78. Hoyt DF. 1987 A new model of avian embryonic metabolism. *J. Exp. Zool.*  
619 *Suppl* **1**, 127–138.
- 620 79. Sotherland PR, Rahn H. 1987 On the composition of bird eggs. *Condor* **89**,  
621 48–65. (doi:10.2307/1368759)
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625 **Table 1.** Putative predictions and definitions for 13 possible explanations for variation in water vapour conductance ( $G_{H_2O}$ ) in birds.

Predictor	Hypothesis	Definition
Body mass	As adult body mass is correlated to egg mass, heavier birds will have higher $G_{H_2O}$ due to greater egg surface area.	Mean body mass (g) of adult birds.
Clutch size	Evaporation from multiple eggs will create a nest atmosphere of greater humidity and reduced water vapour transfer, so $G_{H_2O}$ should be higher for species with larger clutches.	Number of eggs per brood, measured as geometric mean of the typical minimum and maximum clutch size.
Calcium content	Eggshells of calcium-poor species are expected to be thinner, less dense and more porous than calcium-rich species, and thus facilitate higher $G_{H_2O}$ .	<p><b>(1) Calcium-rich:</b> Species that ingest mollusc shells, fish, shellfish, calcareous grit, calcareous ash or bones.</p> <p><b>(2) Calcium-poor:</b> Species with primarily insectivorous or granivorous diet.</p>

Egg maculation	Maculated eggs are expected to have lower $G_{H_2O}$ than immaculate eggs to reduce the risk of desiccation.	<p><b>(1) Immaculate:</b> No spotting or markings on eggshell surface.</p> <p><b>(2) Maculation:</b> Maculation present on eggshell surface.</p>
Nest type	Fully enclosed nests have less air movement than semi-enclosed and exposed nests, facilitating greater $G_{H_2O}$ .	<p><b>(1) Exposed:</b> Nest is open above and has no side walls (no nest, scrape, saucer, platform, heap).</p> <p><b>(2) Semi-enclosed:</b> Nest is partially open and has side walls (cup, bowl, pendant, sphere, dome, pouch).</p> <p><b>(3) Enclosed:</b> Nest is entirely enclosed (cavity, burrow, crevice).</p>
Nest location	Nests above ground have lower risk of flooding or water accumulation, therefore will have lower $G_{H_2O}$ .	<b>(1) Ground:</b> Nest location in or on the ground, or floating on water.

		<p><b>(2) Tree:</b> Nest located in tree, bush, shrub, wall, cave roof, or attached to reed.</p> <p><b>(3) Cliff:</b> Nest located on cliff.</p>
Nest lining	Incorporation of nest lining will better insulate the egg, therefore will have higher $G_{H_2O}$ .	<p><b>(1) Lined:</b> Nest lining is always or sometimes present.</p> <p><b>(2) Not lined:</b> Nest lining is absent.</p>
Habitat	<p>Among open nesting species, more direct sunlight reaches eggs in open habitats and experience greater air movement around the nest than closed habitats.</p> <p>Open nesting species in open habitats will have lower <math>G_{H_2O}</math> than in closed habitats.</p>	<p><b>(1) Open:</b> Breeds in desert, grassland, open water, open moorland, low shrubs, rocky habitats, seashores and cities.</p> <p><b>(2) Semi-open:</b> Breeds in open shrubland and bushland, scattered bushes, parkland, forest edge.</p> <p><b>(3) Dense:</b> Breeds in forest with a closed canopy, or in the lower vegetation strata of dense thickets, shrubland, mangroves or marshland.</p>

Incubating parent	Nest vapour pressure will decrease when the parent leaves the nest uncovered, which is more likely to occur if incubation is not shared between parents, resulting in lower $G_{H_2O}$ .	<p><b>(1) Shared:</b> Contact incubation of eggs by two adults.</p> <p><b>(2) Not shared:</b> Contact incubation of eggs by single adult.</p>
Mode of development	Higher $G_{H_2O}$ may contribute to improving the use of nutritional support by the embryo of precocial species by removing excess water, thus resulting in increased development at hatching.	<p><b>(1) Altricial:</b> Newly born young are relatively immobile, naked, and usually require care and feeding by the parents.</p> <p><b>(2) Precocial:</b> Newly born young are relatively mobile, covered in feathers, and independent.</p>
Parental care	The eggs of species that provide biparental care are expected to have higher $G_{H_2O}$ as nest humidity and temperature can be better maintained when both parents assist.	<p><b>(1) Uniparental:</b> The brood is provisioned and/or defended by one adult</p> <p><b>(2) Biparental:</b> The brood is provisioned and/or defended by two adults</p>
Parental contact	The wet incubating parent returning to the nest will	<b>(1) Wet plumage:</b> Adults returned habitually to

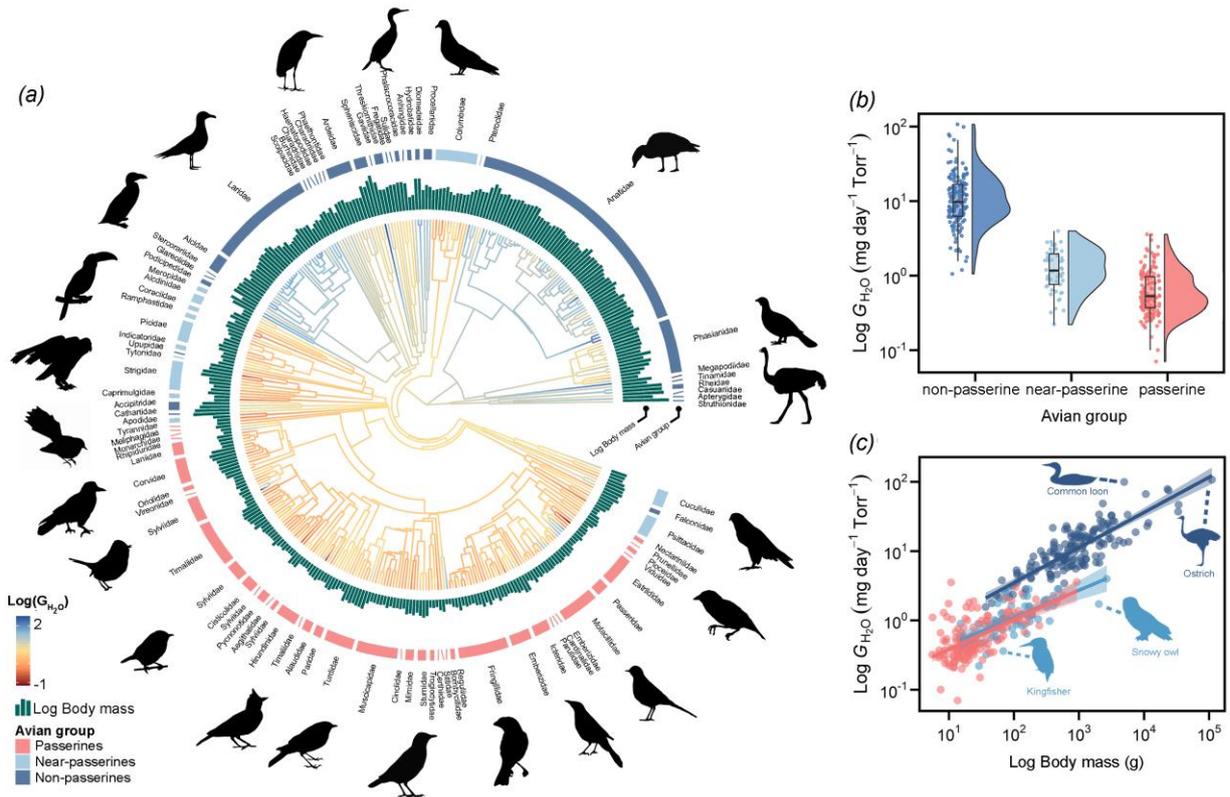
	increase the nest's humidity, thus are expected to have higher $G_{H_2O}$ .	the nest with wet plumage. This included species that feed on freshwater or marine prey or use nests built on water.  <b>(2) Dry plumage:</b> Adults did not return habitually to the nest with wet plumage.
Temperature seasonality	Eggs incubated in environments with highly variable temperature will experience lower $G_{H_2O}$ as high temperature seasonality occurs in cooler environments.	Average temperature seasonality (BIO4) of breeding/resident range, based on WorldClim v1 data.
Precipitation seasonality	Eggs incubated in environments with highly variable precipitation will experience higher $G_{H_2O}$ to combat temporary periods of excessive rain.	Average precipitation seasonality (BIO15) of breeding/resident range, based on WorldClim v1 data.

626

627 **Table 2.** Estimates of phylogenetic signal in eggshell water vapour conductance  
 628 ( $G_{H_2O}$ ) in all birds. Phylogenetic signal was analysed separately for  $\log_{10}$ -transformed  
 629  $G_{H_2O}$  ( $\log(G_{H_2O})$ ) and residual water vapour conductance ( $RG_{H_2O}$ ). The  $p$ -value tests  
 630 the null hypothesis of no phylogenetic signal ( $\lambda = 0$ ) and Brownian motion model ( $\lambda =$   
 631 1) of evolution.

Response variable	Pagel's $\lambda$	Log likelihood	Log likelihood for $\lambda = 0$	Log likelihood for $\lambda = 1$	$P$ for $\lambda = 0$	$P$ for $\lambda = 1$
$\log(G_{H_2O})$	0.96	-74.39	590.76	-125.64	<0.001	<0.001
$RG_{H_2O}$	0.55	27.20	258.50	-92.27	<0.001	<0.001

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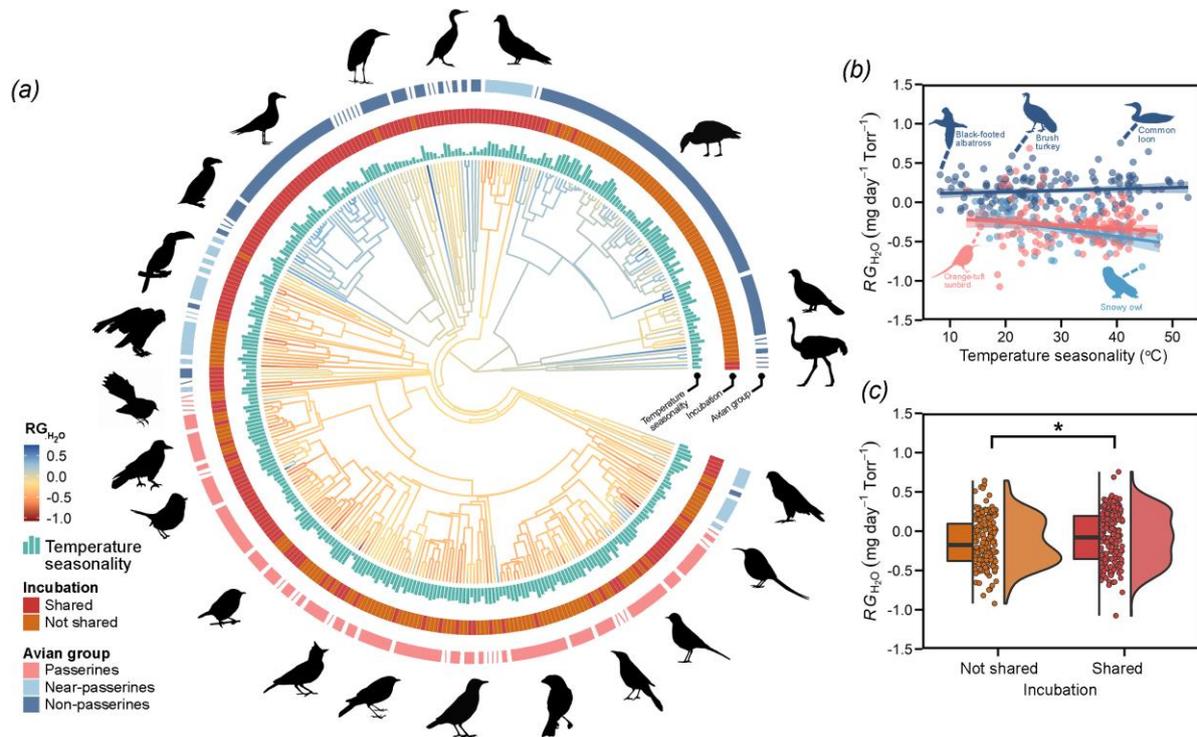


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647 **Figure 1.** Relationship between conductance of whole eggs and ecological variables  
 648 for 364 bird species. (a) Phylogenetic tree from which water vapour conductance  
 649 ( $G_{H_2O}$ ) data were obtained. The bar plots around the phylogeny represents the only  
 650 significant predictors of  $\log(G_{H_2O})$  in conditionally averaged models. Conditional model  
 651 averaging was used to obtain a single average model when more than one PGLS  
 652 model was best ranked (i.e. more than one model with  $\Delta AICc < 2$  from the top ranked  
 653 model). Branch colours show the diversification in  $\log(G_{H_2O})$  across the phylogeny and  
 654 ancestral trait estimates.  $G_{H_2O}$  is plotted as a function of (b) avian group and (c) adult  
 655 body mass (g) within each of the three avian groups. Silhouette illustrations came from  
 656 PhyloPic (<http://phylopic.org>), contributed by various authors under public domain  
 657 license.

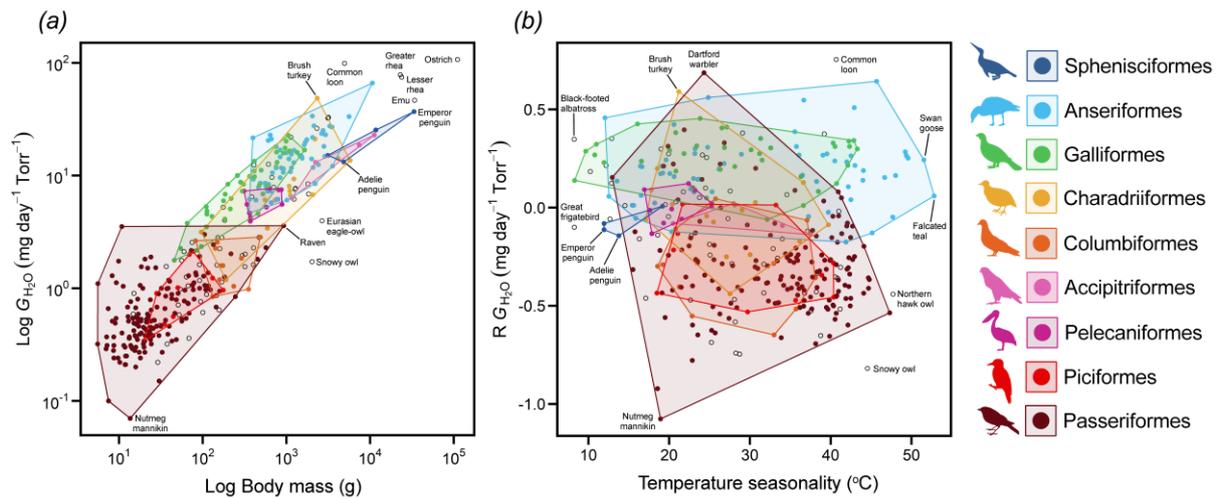
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661 **Figure 2.** Relationship between conductance of whole eggs and ecological variables  
 662 for 364 bird species. (a) Phylogenetic tree of residual water vapour conductance  
 663 ( $RG_{H_2O}$ ). Bar plots and rings around the phylogeny represent significant predictors of  
 664  $RG_{H_2O}$  in conditionally averaged models. Conditional model averaging was used to  
 665 obtain a single average model when more than one PGLS model was best ranked (i.e.  
 666 more than one model with  $\Delta AICc < 2$  from the top ranked model). Branch colours show  
 667 the diversification in  $RG_{H_2O}$  across the phylogeny and ancestral trait estimates.  $RG_{H_2O}$   
 668 is plotted as a function of (b) temperature seasonality within each avian group, and (c)  
 669 whether both parents incubate the eggs. In the hybrid box plot, species  $RG_{H_2O}$  are  
 670 shown as filled circles, vertical lines indicate the median, box shows the interquartile  
 671 range (IQR) and the whiskers are 1.5x IQR (distribution is shown as histograms). *P*-  
 672 values are given in asterisks with \* $<0.05$ , \*\* $<0.01$  and \*\*\* $<0.001$ . Silhouette  
 673 illustrations came from PhyloPic (<http://phylopic.org>), contributed by various authors  
 674 under public domain license.



675

676 **Figure 3.** Partitioning of variation of water vapour conductance ( $G_{H_2O}$ ) among avian  
 677 orders. Dots (one per species) ( $n = 364$ ) show the distribution of  $\log(G_{H_2O})$  as a function  
 678 of (a) adult body mass (g) and (b) residual water vapour conductance ( $R G_{H_2O}$ ) as a  
 679 function of temperature seasonality. Minimum convex hull is plotted for all species  
 680 within a subset of avian orders. Silhouette illustrations came from PhyloPic  
 681 (<http://phylopic.org>), contributed by various authors under public domain license.

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